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Section 1 Neoplastic Disorders

69

Approach to the Patient with Cancer

Dan L. Longo



The application of current treatment techniques (surgery, radiation therapy, chemotherapy, and biologic therapy) results in the cure of nearly two of three patients diagnosed with cancer. Nevertheless, patients experience the diagnosis of cancer as one of the most traumatic and revolutionary events that has ever happened to them. Independent of prognosis, the diagnosis brings with it a change in a person's self-image and in his or her role in the home and workplace. The prognosis of a person who has just been found to have pancreatic cancer is the same as the prognosis of the person with aortic stenosis who develops the first symptoms of congestive heart failure (median survival, ~8 months). However, the patient with heart disease may remain functional and maintain a self-image as a fully intact person with just a malfunctioning part, a diseased organ ("a bum ticker"). By contrast, the patient with pancreatic cancer has a completely altered self-image and is viewed differently by family and anyone who knows the diagnosis. He or she is being attacked and invaded by a disease that could be anywhere in the body. Every ache or pain takes on desperate

significance. Cancer is an exception to the coordinated interaction among cells and organs. In general, the cells of a multicellular organism are programmed for collaboration. Many diseases occur because the specialized cells fail to perform their assigned task. Cancer takes this malfunction one step further. Not only is there a failure of the cancer cell to maintain its specialized function, but it also strikes out on its own; the cancer cell competes to survive using natural mutability and natural selection to seek advantage over normal cells in a recapitulation of evolution. One consequence of the traitorous behavior of cancer cells is that the patient feels betrayed by his or her body. The cancer patient feels that he or she, and not just a body part, is diseased.

THE MAGNITUDE OF THE PROBLEM

No nationwide cancer registry exists; therefore, the incidence of cancer is estimated on the basis of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) database, which tabulates cancer incidence and death figures from 13 sites, accounting for about 10% of the U.S. population, and from population data from the U.S. Census Bureau. In 2021, 1,898 million new cases of invasive cancer (970,250 men and 927,910 women) were diagnosed, and 608,570 persons (319,420 men and 289,150 women) died from cancer. The percent distribution of new cancer cases and cancer deaths by site for men and women is shown in Table 69-1. Cancer incidence has been declining by about 2% each year since 1992. Cancer is the cause of one in four deaths in the United States.

The most significant risk factor for cancer overall is age; two-thirds of all cases were in those aged >65 years. Cancer incidence increases as the third, fourth, or fifth power of age in different sites. For the interval

TABLE 69-1 Distribution of Cancer Incidence and Deaths for 2021

MALE			FEMALE		
SITES	%	NUMBER	SITES	%	NUMBER
Cancer Incidence					
Prostate	26	248,530	Breast	30	281,550
Lung	12	119,100	Lung	13	116,660
Colorectal	8	79,520	Colorectal	8	69,980
Bladder	7	64,280	Endometrial	7	66,570
Melanoma	6	62,260	Melanoma	5	43,850
Kidney	5	48,780	Lymphoma	4	35,930
Lymphoma	5	45,630	Thyroid	3	32,130
Oral cavity	4	38,800	Pancreas	3	28,480
Leukemia	4	35,530	Kidney	3	27,300
Pancreas	3	31,950	Leukemia	3	25,560
All others	20	195,870	All others	21	199,900
All sites	100	970,250	All sites	100	927,910
Cancer Deaths					
Lung	22	69,410	Lung	22	62,470
Prostate	11	34,130	Breast	15	43,600
Colorectal	9	28,520	Colorectal	8	24,460
Pancreas	8	25,270	Pancreas	8	22,950
Liver	6	20,300	Ovary	5	14,460
Leukemia	4	13,900	Endometrial	4	12,940
Esophagus	4	12,410	Liver	3	9,930
Bladder	4	12,260	Leukemia	3	9,760
Lymphoma	4	12,170	Lymphoma	3	8,550
CNS	3	10,500	CNS	3	8,100
All others	25	80,550	All others	25	71,930
All sites	100	319,420	All sites	100	289,150

Source: From Cancer Statistics 2021, RI Seigel et al, © 2021 CA Cancer J Clin. Reproduced with permission of John Wiley & Sons Ltd.

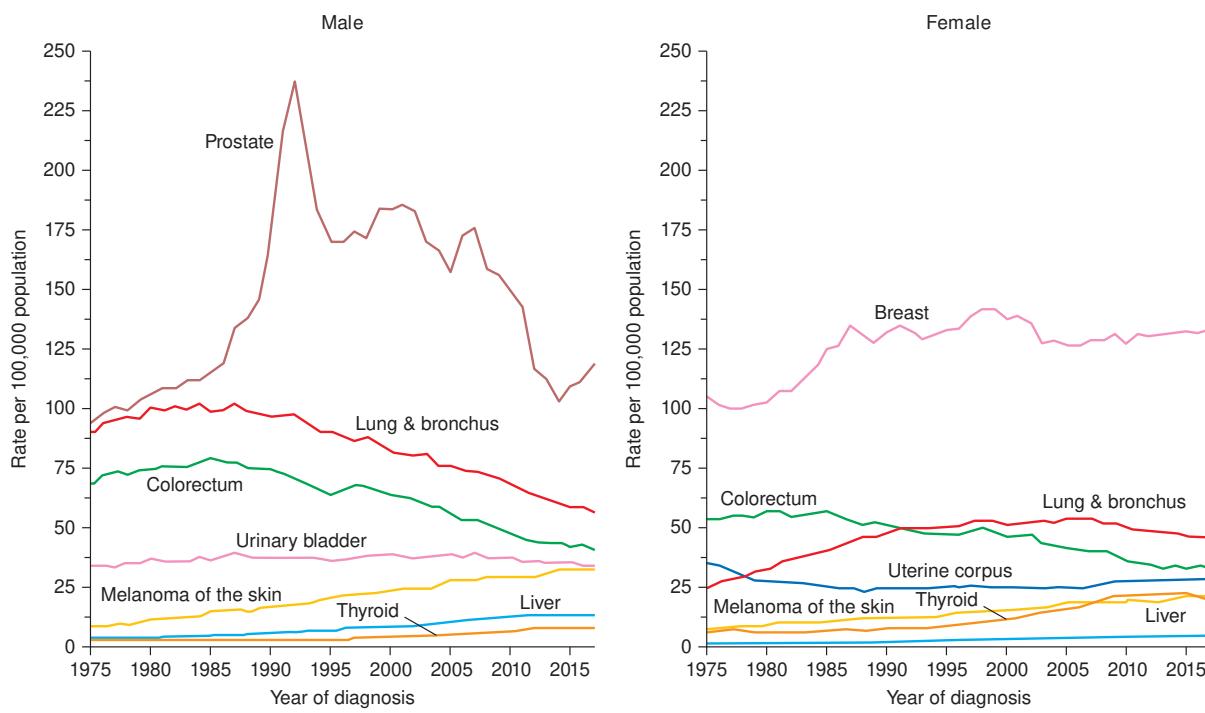


FIGURE 69-1 Trends in cancer incidence, 1975–2017. (From Cancer Statistics 2021, R Siegel et al, © 2021 CA Cancer J Clin. Reproduced with permission of John Wiley & Sons Ltd.)

between birth and age 49 years, 1 in 29 men and 1 in 19 women will develop cancer; for the interval between ages 50 and 59 years, 1 in 15 men and 1 in 17 women will develop cancer; for the interval between ages 60 and 69 years, 1 in 6 men and 1 in 10 women will develop cancer; and for people aged ≥ 70 , 1 in 3 men and 1 in 4 women will develop cancer. Overall, men have a 40.5% risk of developing cancer at some time during their lives; women have a 38.9% lifetime risk.

Cancer is the second leading cause of death behind heart disease. Deaths from heart disease have declined 45% in the United States since 1950 and continue to decline. Cancer has overtaken heart disease as the number one cause of death in persons aged <85 years. Incidence trends over time are shown in Fig. 69-1. After a 70-year period of increase, cancer deaths began to decline in 1990–1991 (Fig. 69-2). Between 1990 and 2010, cancer deaths decreased by 21% among men and 12.3% among women. The incidence has been steady since 2013. The magnitude of the decline is illustrated in Fig. 69-3. The five leading causes of cancer deaths are shown for various populations in Table 69-2. The 5-year survival for white patients was 39% in 1960–1963 and 68% in 2010–2016. Cancers are more often deadly in blacks; the 5-year survival was 63% for the 2010–2016 interval; however, the racial differences are narrowing over time. Incidence and mortality vary among racial and ethnic groups (Table 69-3). The basis for these differences is unclear.

Advances in cancer prevention, diagnosis, and treatment since the early 1990s have averted millions of cancer deaths based on projections from the slopes of the mortality curves leading up to the 1990s (Fig. 69-4).

CANCER AROUND THE WORLD

In 2018, 17 million new cancer cases and 9.5 million cancer deaths were estimated worldwide, according to estimates of GLOBOCAN 2018, developed by the International Agency for Research on Cancer (IARC). Rates are increasing worldwide. When broken down by region of the world, ~45% of cases were in Asia (which has 59.5% of the world's population), 26% in Europe (9.8% of the world's population), 14.5% in North America, 7.1% in Central/South America (the Americas, North and South, account for 13.3% of the world's population), 6% in Africa (16.9% of the world's population), and 1% in Australia/New Zealand

(0.5% of the world's population) (Fig. 69-5). Lung cancer is the most common cancer and the most common cause of cancer death in the world. Its incidence is highly variable, affecting only 2 per 100,000 African women but as many as 61 per 100,000 North American men. Breast cancer is the second most common cancer worldwide; however, it ranks fourth as a cause of death behind lung, stomach, and liver cancer. Among the eight most common forms of cancer, lung (2-fold), breast (3-fold), prostate (2.5-fold), and colorectal (3-fold) cancers are more common in more developed countries than in less developed countries. By contrast, liver (2-fold), cervical (2-fold), and esophageal (2- to 3-fold) cancers are more common in less developed countries. Stomach cancer incidence is similar in more and less developed countries but is much more common in Asia than North America or Africa. The most common cancers in Africa are cervical, breast, and liver cancers. It has been estimated that nine modifiable risk factors are responsible for more than one-third of cancers worldwide. These include smoking, alcohol consumption, obesity, physical inactivity, low fruit and vegetable consumption, unsafe sex, air pollution, indoor smoke from household fuels, and contaminated injections.

PATIENT MANAGEMENT

Important information is obtained from every portion of the routine history and physical examination. The duration of symptoms may reveal the chronicity of disease. The past medical history may alert the physician to the presence of underlying diseases that may affect the choice of therapy or the side effects of treatment. The social history may reveal occupational exposure to carcinogens or habits, such as smoking or alcohol consumption, that may influence the course of disease and its treatment. The family history may suggest an underlying familial cancer predisposition and point out the need to begin surveillance or other preventive therapy for unaffected siblings of the patient. The review of systems may suggest early symptoms of metastatic disease or a paraneoplastic syndrome.

DIAGNOSIS

The diagnosis of cancer relies most heavily on invasive tissue biopsy and should never be made without obtaining tissue; no noninvasive

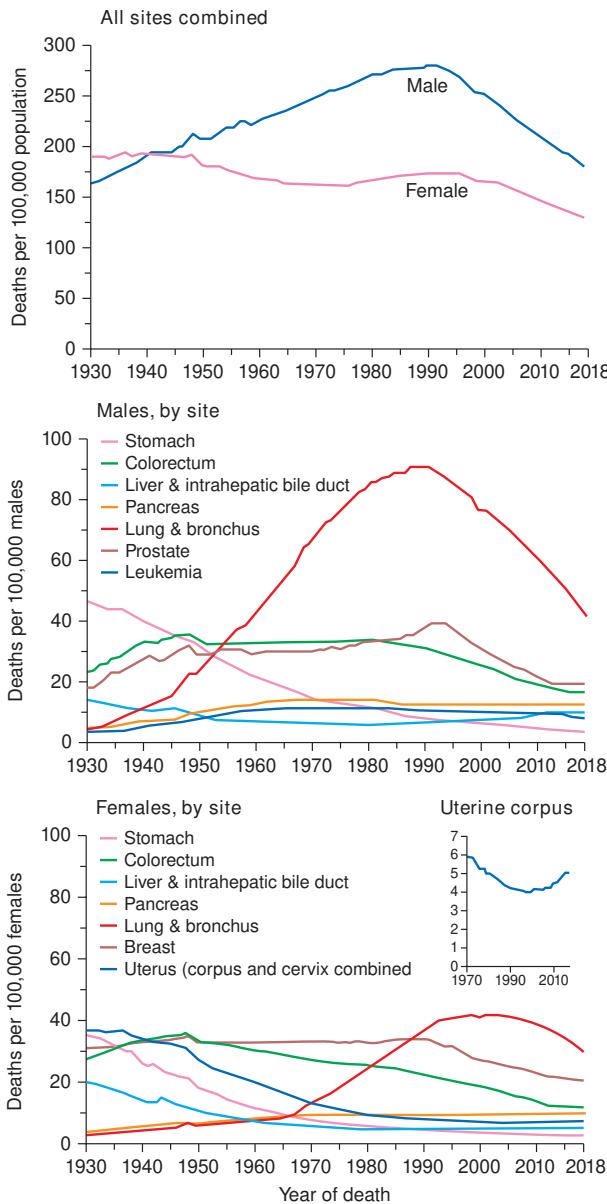


FIGURE 69-2 Trends in cancer mortality rates in men and women, 1930–2018. (From *Cancer Statistics 2021*, R. L. Siegel et al., © 2021 CA Cancer J Clin. Reproduced with permission of John Wiley & Sons Ltd.)

diagnostic test is sufficient to define a disease process such as cancer. Although in rare clinical settings (e.g., thyroid nodules), fine-needle aspiration is an acceptable diagnostic procedure, the diagnosis generally depends on obtaining adequate tissue to permit careful evaluation of the histology of the tumor, its grade, and its invasiveness and to yield further molecular diagnostic information, such as the expression of cell-surface markers or intracellular proteins that typify a particular cancer, or the presence of a molecular marker, such as the t(8;14) translocation of Burkitt's lymphoma. Increasing evidence links the expression of certain genes with the prognosis and response to therapy (Chaps. 71 and 72).

Occasionally, a patient will present with a metastatic disease process that is defined as cancer on biopsy but has no apparent primary site of disease. Efforts should be made to define the primary site based on age, sex, sites of involvement, histology and tumor markers, and personal and family history. Particular attention should be focused on ruling out the most treatable causes (Chap. 92).

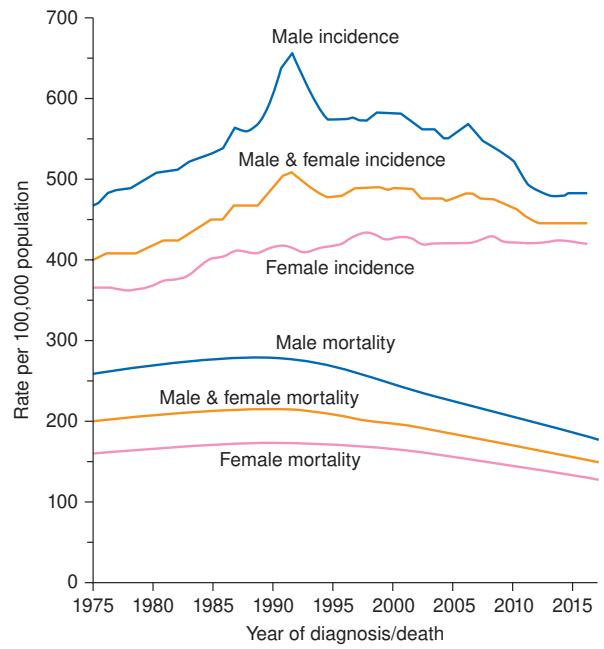


FIGURE 69-3 Trends in cancer incidence and death rates. (From *Cancer Statistics 2021*, R. L. Siegel et al., © 2021 CA Cancer J Clin. Reproduced with permission of John Wiley & Sons Ltd.)

Once the diagnosis of cancer is made, the management of the patient is best undertaken as a multidisciplinary collaboration among the primary care physician, medical oncologists, surgical oncologists, radiation oncologists, oncology nurse specialists, pharmacists, social workers, rehabilitation medicine specialists, and a number of other consulting professionals working closely with each other and with the patient and family.

■ DEFINING THE EXTENT OF DISEASE AND THE PROGNOSIS

The first priority in patient management after the diagnosis of cancer is established and shared with the patient is to determine the extent of disease. The curability of a tumor usually is inversely proportional to the tumor burden. Ideally, the tumor will be diagnosed before symptoms develop or as a consequence of screening efforts (Chap. 70). A very high proportion of such patients can be cured. However, most patients with cancer present with symptoms related to the cancer, caused either by mass effects of the tumor or by alterations associated with the production of cytokines or hormones by the tumor.

For most cancers, the extent of disease is evaluated by a variety of noninvasive and invasive diagnostic tests and procedures. This process is called *staging*. There are two types. *Clinical staging* is based on physical examination, radiographs, isotopic scans, computed tomography (CT) scans, and other imaging procedures; *pathologic staging* takes into account information obtained during a surgical procedure, which might include intraoperative palpation, resection of regional lymph nodes and/or tissue adjacent to the tumor, and inspection and biopsy of organs commonly involved in disease spread. Pathologic staging includes histologic examination of all tissues removed during the surgical procedure. Surgical procedures performed may include a simple lymph node biopsy or more extensive procedures such as thoracotomy, mediastinoscopy, or laparotomy. Surgical staging may occur in a separate procedure or may be done at the time of definitive surgical resection of the primary tumor. A subset of pathologic staging is the examination of tissue obtained at initial surgery that occurs after the delivery of some treatment, which is called neoadjuvant therapy. Stage of disease determined after neoadjuvant therapy is designated with the prefix *y*.

Knowledge of the predilection of particular tumors for spreading to adjacent or distant organs helps direct the staging evaluation.

TABLE 69-2 The Five Leading Primary Tumor Sites for Patients Dying of Cancer Based on Age and Sex in 2018

RANK	SEX	ALL AGES	AGE, YEARS				
			UNDER 20	20–39	40–59	60–79	>80
1	M	Lung	CNS	CNS	Lung	Lung	Lung
	F	Lung	CNS	Breast	Breast	Lung	Lung
2	M	Prostate	Leukemia	Colorectal	Colorectal	Prostate	Prostate
	F	Breast	Leukemia	Cervix	Lung	Breast	Breast
3	M	Colorectal	Bone sarcoma	Leukemia	Liver	Pancreas	Colorectal
	F	Colorectal	Soft tissue sarcoma	Colorectal	Colorectal	Pancreas	Colorectal
4	M	Pancreas	Soft tissue sarcoma	Lymphoma	Pancreas	Colorectal	Bladder
	F	Pancreas	Bone sarcoma	CNS	Ovary	Colorectal	Pancreas
5	M	Liver	Lymphoma	Soft tissue sarcoma	CNS	Liver	Pancreas
	F	Ovary	Kidney	Leukemia	Pancreas	Ovary	Leukemia

Abbreviations: CNS, central nervous system; F, female; M, male.

Source: From RL Siegel et al: Cancer statistics, 2021. CA Cancer J Clin 71:7, 2021.

Information obtained from staging is used to define the extent of disease as localized, as exhibiting spread outside of the organ of origin to regional but not distant sites, or as metastatic to distant sites. The most widely used system of staging is the tumor, node, metastasis (TNM) system codified by the International Union Against Cancer and the American Joint Committee on Cancer. The TNM classification is an anatomically based system that categorizes the tumor on the basis of the size of the primary tumor lesion (T1–4, where a higher number indicates a tumor of larger size), the presence of nodal involvement

(usually N0 and N1 for the absence and presence, respectively, of involved nodes, although some tumors have more elaborate systems of nodal grading), and the presence of metastatic disease (M0 and M1 for the absence and presence, respectively, of metastases). The various permutations of T, N, and M scores (sometimes including tumor histologic grade [G]) are then broken into stages, usually designated by the roman numerals I through IV. Tumor burden increases and curability decreases with increasing stage. Other anatomic staging systems are used for some tumors, e.g., the Dukes classification for

TABLE 69-3 Cancer Incidence and Mortality in Racial and Ethnic Groups, United States, 2013–2018

SITE	SEX	WHITE	BLACK	ASIAN/PACIFIC ISLANDER	AMERICAN INDIAN ^a	HISPANIC
Incidence per 100,000 Population						
All	M	501.4	534.0	294.3	399.8	371.3
	F	442.2	406.6	292.6	388.8	335.5
Breast		131.6	127.3	95.6	94.9	94.8
Colorectal	M	42.6	51.6	34.6	47.2	39.9
	F	31.8	37.9	24.8	38.3	27.6
Kidney	M	23.1	26.1	11.2	31.3	21.9
	F	11.7	13.3	5.3	17.7	12.4
Liver	M	10.7	18.0	19.3	22.9	20.1
	F	3.8	5.5	7.1	9.4	7.9
Lung	M	70.8	79.8	43.2	59.2	37.1
	F	56.4	47.9	27.9	47.9	24.3
Prostate		97.7	171.6	53.8	67.7	85.6
Cervix		7.2	9.0	6.1	8.8	9.5
Deaths per 100,000 Population						
All	M	190.2	227.2	114.6	169.3	134.0
	F	137.8	154.9	84.6	120.1	94.6
Breast		20.1	28.2	11.7	14.8	13.8
Colorectal	M	16.1	23.2	11.2	18.5	14.0
	F	11.5	15.3	7.9	12.4	8.6
Kidney	M	5.5	5.5	2.5	8.3	4.9
	F	2.3	2.3	1.1	3.2	2.2
Liver	M	8.4	13.4	13.1	14.8	13.3
	F	3.6	4.9	5.4	7.0	6.0
Lung	M	49.4	57.0	28.0	38.4	23.0
	F	35.6	30.6	16.3	27.4	12.3
Prostate		17.9	38.3	8.8	18.5	15.6
Cervix		2.0	3.4	1.7	2.4	2.6

^aBased on Indian Health Service delivery areas.

Abbreviations: F, female; M, male.

Source: From Cancer Statistics 2021, RI Seigel et al, © 2021 CA Cancer J Clin. Reproduced with permission of John Wiley & Sons Ltd.

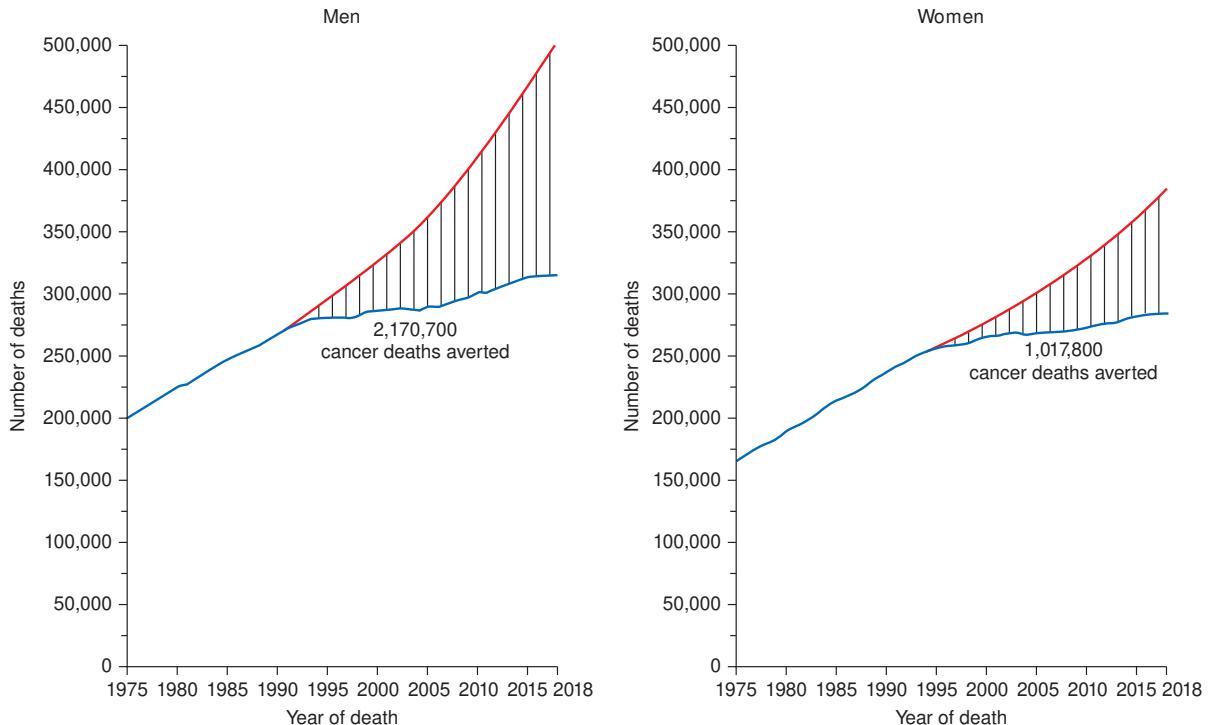


FIGURE 69-4 Cancer deaths averted in men and women since the early 1990s. (From *Cancer Statistics 2021*, RI Seigel et al, © 2021 CA Cancer J Clin. Reproduced with permission of John Wiley & Sons Ltd.)

colorectal cancers, the International Federation of Gynecologists and Obstetricians classification for gynecologic cancers, and the Ann Arbor classification for Hodgkin's disease.

Certain tumors cannot be grouped on the basis of anatomic considerations. For example, hematopoietic tumors such as leukemia, myeloma, and lymphoma are often disseminated at presentation and do not spread like solid tumors. For these tumors, other prognostic factors have been identified (Chaps. 104–111).

In addition to tumor burden, a second major determinant of treatment outcome is the physiologic reserve of the patient. Patients who are bedridden before developing cancer are likely to fare worse, stage for stage, than fully active patients. Physiologic reserve is a determinant of how a patient is likely to cope with the physiologic stresses imposed by the cancer and its treatment. This factor is difficult to assess directly. Instead, surrogate markers for physiologic reserve are used, such as the patient's age or Karnofsky performance status (Table 69-4) or Eastern Cooperative Oncology Group (ECOG) performance status (Table 69-5). Older patients and those with a Karnofsky performance status <70 or ECOG performance status ≥ have a poor prognosis unless the poor performance is a reversible consequence of the tumor.

Increasingly, biologic features of the tumor are being related to prognosis. The expression of particular oncogenes, drug-resistance genes, apoptosis-related genes, and genes involved in metastasis is being found to influence response to therapy and prognosis. The presence of selected cytogenetic abnormalities may influence survival. Tumors with higher growth fractions, as assessed by expression of proliferation-related markers such as proliferating cell nuclear antigen, behave more aggressively than tumors with lower growth fractions. Information obtained from studying the tumor itself will increasingly be used to influence treatment decisions. Host genes involved in drug metabolism can influence the safety and efficacy of particular treatments.

Enormous heterogeneity has been noted by studying tumors; we have learned that morphology is not capable of discerning certain distinct subsets of patients whose tumors have different sets of abnormalities. Tumors that look the same by light microscopy can be very different. Similarly, tumors that look quite different from one another histologically can share genetic lesions that predict responses to

treatments. Furthermore, tumor cells vary enormously within a single patient even though the cells share a common origin.

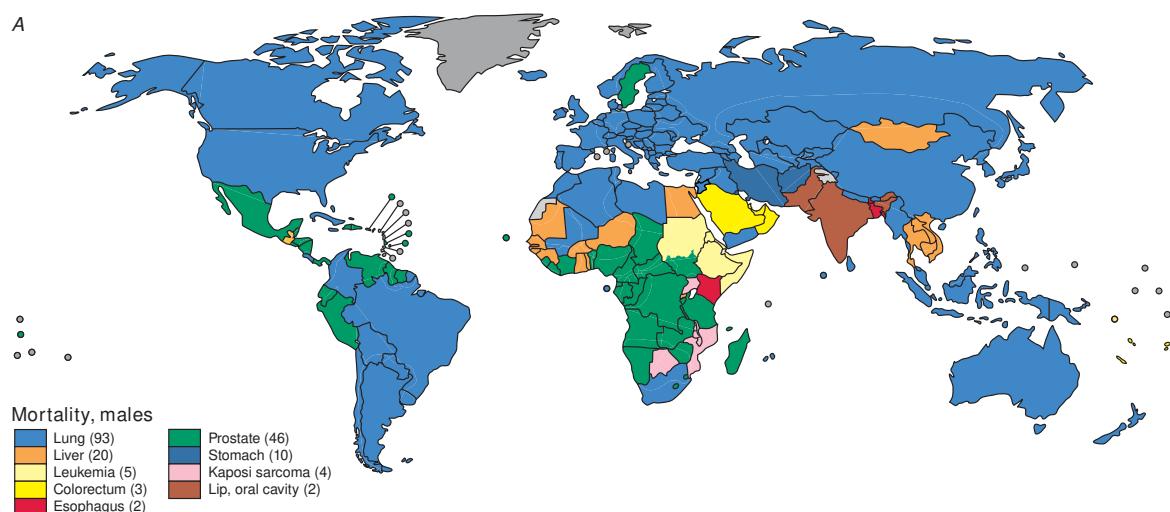
■ MAKING A TREATMENT PLAN

From information on the extent of disease and the prognosis and in conjunction with the patient's wishes, it is determined whether the treatment approach should be curative or palliative in intent. Cooperation among the various professionals involved in cancer treatment is of the utmost importance in treatment planning. For some cancers, chemotherapy or chemotherapy plus radiation therapy delivered before the use of definitive surgical treatment (so-called neoadjuvant therapy) may improve the outcome, as seems to be the case for locally advanced breast cancer and head and neck cancers. In certain settings in which combined-modality therapy is intended, coordination among the medical oncologist, radiation oncologist, and surgeon is crucial to achieving optimal results. Sometimes the chemotherapy and radiation therapy need to be delivered sequentially, and other times concurrently. Surgical procedures may precede or follow other treatment approaches. It is best for the treatment plan either to follow a standard protocol precisely or else to be part of an ongoing clinical research protocol evaluating new treatments. Ad hoc modifications of standard protocols are likely to compromise treatment results.

The choice of treatment approaches was formerly dominated by the local culture in both the university and the practice settings. However, it is now possible to gain access electronically to standard treatment protocols and to every approved clinical research study in North America through a personal computer interface with the Internet.¹

¹The National Cancer Institute maintains a database called PDQ (Physician Data Query) that is accessible on the Internet under the name CancerNet at <https://www.cancer.gov/publications/pdq>. Information can be obtained through a facsimile machine using CancerFax by dialing 301-402-5874. Patient information is also provided by the National Cancer Institute in at least three formats: on the Internet via CancerNet at www.cancer.gov, through the CancerFax number listed above, or by calling 1-800-4-CANCER. The quality control for the information provided through these services is rigorous.

A



B

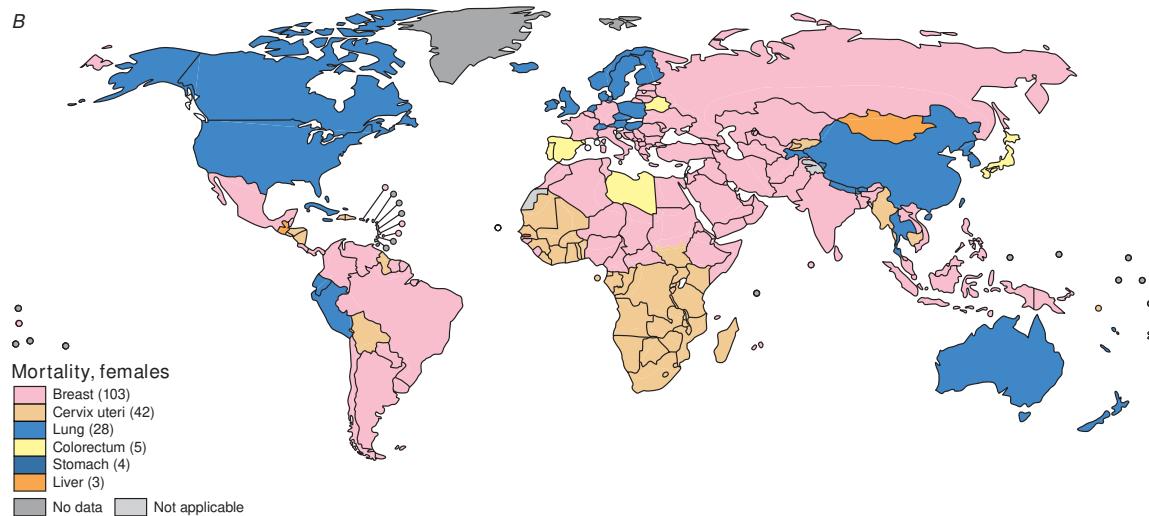


FIGURE 69-5 Global maps showing most common cause of cancer mortality by country in 2018 among (A) men and (B) women. (Reproduced with permission from FBray et al: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394, 2018. Data source: Globocan 2018. Map production: IARC. World Health Organization. © WHO 2018. All rights reserved.)

TABLE 69-4 Karnofsky Performance Index

PERFORMANCE STATUS	FUNCTIONAL CAPABILITY OF THE PATIENT
100	Normal; no complaints; no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance but is able to care for most needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled; hospitalization is indicated, although death is not imminent
20	Very sick; hospitalization is necessary; active supportive treatment is necessary
10	Moribund, fatal processes progressing rapidly
0	Dead

The skilled physician also has much to offer the patient for whom curative therapy is no longer an option. Often a combination of guilt and frustration over the inability to cure the patient and the pressure of a busy schedule greatly limit the time a physician spends with a patient who is receiving only palliative care. Resist these forces. In addition

TABLE 69-5 The Eastern Cooperative Oncology Group (ECOG) Performance Scale

ECOG grade 0: Fully active, able to carry on all predisease performance without restriction
ECOG grade 1: Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
ECOG grade 2: Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about >50% of waking hours
ECOG grade 3: Capable of only limited self-care, confined to bed or chair >50% of waking hours
ECOG grade 4: Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
ECOG grade 5: Dead

Source: Reproduced with permission from MM Oken et al: Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649, 1982.

to the medicines administered to alleviate symptoms (see below), it is important to remember the comfort that is provided by holding the patient's hand, continuing regular examinations, and taking time to talk.

■ MANAGEMENT OF DISEASE AND TREATMENT COMPLICATIONS

Because cancer therapies are toxic (Chap. 73), patient management involves addressing complications of both the disease and its treatment as well as the complex psychosocial problems associated with cancer. In the short term during a course of curative therapy, the patient's functional status may decline. Treatment-induced toxicity is less acceptable if the goal of therapy is palliation. The most common side effects of treatment are nausea and vomiting (see below), febrile neutropenia (Chap. 74), and myelosuppression (Chap. 73). Tools are now available to minimize the acute toxicity of cancer treatment.

New symptoms developing in the course of cancer treatment should always be assumed to be reversible until proven otherwise. The fatalistic attribution of anorexia, weight loss, and jaundice to recurrent or progressive tumor could result in a patient dying from a reversible intercurrent cholecystitis. Intestinal obstruction may be due to reversible adhesions rather than progressive tumor. Systemic infections, sometimes with unusual pathogens, may be a consequence of the immunosuppression associated with cancer therapy. Some drugs used to treat cancer or its complications (e.g., nausea) may produce central nervous system symptoms that look like metastatic disease or may mimic paraneoplastic syndromes such as the syndrome of inappropriate antidiuretic hormone. A definitive diagnosis should be pursued and may even require a repeat biopsy.

A critical component of cancer management is assessing the response to treatment. In addition to a careful physical examination in which all sites of disease are physically measured and recorded in a flow chart by date, response assessment usually requires periodic repeating of imaging tests that were abnormal at the time of staging. If imaging tests have become normal, repeat biopsy of previously involved tissue is performed to document complete response by pathologic criteria. Biopsies are not usually required if there is macroscopic residual disease. A *complete response* is defined as disappearance of all evidence of disease, and a *partial response* as >50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions. The determination of partial response may also be based on a 30% decrease in the sums of the longest diameters of lesions (Response Evaluation Criteria in Solid Tumors [RECIST]). *Progressive disease* is defined as the appearance of any new lesion or an increase of >25% in the sum of the products of the perpendicular diameters of all measurable lesions (or an increase of 20% in the sums of the longest diameters by RECIST). Tumor shrinkage or growth that does not meet any of these criteria is considered *stable disease*. Some sites of involvement (e.g., bone) or patterns of involvement (e.g., lymphangitic lung or diffuse pulmonary infiltrates) are considered unmeasurable. No response is complete without biopsy documentation of their resolution, but partial responses may exclude their assessment unless clear objective progression has occurred.

For some hematologic neoplasms, flow cytometric and genetic assays may determine the presence of residual tumor cells that escape microscopic detection. In general, these techniques can reliably detect as few as 1 tumor cell among 10,000 cells. If such tests do not detect tumor cells, the patient is said to have minimal residual disease negativity, a finding generally associated with more durable remissions. Accumulating data are defining interventions in patients with minimal residual disease positivity that can extend remission duration and survival.

Tumor markers may be useful in patient management in certain tumors. Response to therapy may be difficult to gauge with certainty. However, some tumors produce or elicit the production of markers that can be measured in the serum or urine, and in a particular patient, rising and falling levels of the marker are usually associated with increasing or decreasing tumor burden, respectively. Some clinically useful tumor markers are shown in Table 69-6. Tumor markers are not

TABLE 69-6 Tumor Markers

TUMOR MARKERS	CANCER	NONNEOPLASTIC CONDITIONS
Hormones		
Human chorionic gonadotropin	Gestational trophoblastic disease, gonadal germ cell tumor	Pregnancy
Calcitonin	Medullary cancer of the thyroid	
Catecholamines	Pheochromocytoma	
Oncofetal Antigens		
α Fetoprotein	Hepatocellular carcinoma, gonadal germ cell tumor	Cirrhosis, hepatitis
Carcinoembryonic antigen	Adenocarcinomas of the colon, pancreas, lung, breast, ovary	Pancreatitis, hepatitis, inflammatory bowel disease, smoking
Enzymes		
Prostatic acid phosphatase	Prostate cancer	Prostatitis, prostatic hypertrophy
Neuron-specific enolase	Small-cell cancer of the lung, neuroblastoma	
Lactate dehydrogenase	Lymphoma, Ewing's sarcoma	Hepatitis, hemolytic anemia, many others
Tumor-Associated Proteins		
Prostate-specific antigen	Prostate cancer	Prostatitis, prostatic hypertrophy
Monoclonal immunoglobulin	Myeloma	Infection, MGUS
CA-125	Ovarian cancer, some lymphomas	Menstruation, peritonitis, pregnancy
CA 19-9	Colon, pancreatic, breast cancer	Pancreatitis, ulcerative colitis
CD30	Hodgkin's disease, anaplastic large-cell lymphoma	—
CD25	Hairy cell leukemia, adult T-cell leukemia/lymphoma	Hemophagocytic lymphohistiocytosis

Abbreviation: MGUS, monoclonal gammopathy of uncertain significance.

in themselves specific enough to permit a diagnosis of malignancy to be made, but once a malignancy has been diagnosed and shown to be associated with elevated levels of a tumor marker, the marker can be used to assess response to treatment.

The recognition and treatment of depression are important components of management. The incidence of depression in cancer patients is ~25% overall and may be greater in patients with greater debility. This diagnosis is likely in a patient with a depressed mood (dysphoria) and/or a loss of interest in pleasure (anhedonia) for at least 2 weeks. In addition, three or more of the following symptoms are usually present: appetite change, sleep problems, psychomotor retardation or agitation, fatigue, feelings of guilt or worthlessness, inability to concentrate, and suicidal ideation. Patients with these symptoms should receive therapy. Medical therapy with a serotonin reuptake inhibitor such as fluoxetine (10–20 mg/d), sertraline (50–150 mg/d), or paroxetine (10–20 mg/d) or a tricyclic antidepressant such as amitriptyline (50–100 mg/d) or desipramine (75–150 mg/d) should be tried, allowing 4–6 weeks for response. Effective therapy should be continued at least 6 months after resolution of symptoms. If therapy is unsuccessful, other classes of antidepressants may be used. In addition to medication, psychosocial interventions such as support groups, psychotherapy, and guided imagery may be of benefit.

Many patients opt for unproven or unsound approaches to treatment when it appears that conventional medicine is unlikely to be curative. Those seeking such alternatives are often well educated and

may be early in the course of their disease. Unsound approaches are usually hawked on the basis of unsubstantiated anecdotes and not only cannot help the patient but may be harmful. Physicians should strive to keep communications open and nonjudgmental, so that patients are more likely to discuss with the physician what they are actually doing. The appearance of unexpected toxicity may be an indication that a supplemental therapy is being taken.²

LONG TERM FOLLOW UP/LATE COMPLICATIONS

At the completion of treatment, sites originally involved with tumor are reassessed, usually by radiography or imaging techniques, and any persistent abnormality is biopsied. If disease persists, the multidisciplinary team discusses a new salvage treatment plan. If the patient has been rendered disease-free by the original treatment, the patient is followed regularly for disease recurrence. The optimal guidelines for follow-up care are not known. For many years, a routine practice has been to follow the patient monthly for 6–12 months, then every other month for a year, every 3 months for a year, every 4 months for a year, every 6 months for a year, and then annually. At each visit, a battery of laboratory and radiographic and imaging tests was obtained on the assumption that it is best to detect recurrent disease before it becomes symptomatic. However, where follow-up procedures have been examined, this assumption has been found to be untrue. Studies of breast cancer, melanoma, lung cancer, colon cancer, and lymphoma have all failed to support the notion that asymptomatic relapses are more readily cured by salvage therapy than symptomatic relapses. In view of the enormous cost of a full battery of diagnostic tests and their manifest lack of impact on survival, new guidelines are emerging for less frequent follow-up visits, during which the history and physical examination are the major investigations performed.

As time passes, the likelihood of recurrence of the primary cancer diminishes. For many types of cancer, survival for 5 years without recurrence is tantamount to cure. However, important medical problems can occur in patients treated for cancer and must be examined (Chap. 95). Some problems emerge as a consequence of the disease and some as a consequence of the treatment. An understanding of these disease- and treatment-related problems may help in their detection and management.

Despite these concerns, most patients who are cured of cancer return to normal lives.

SUPPORTIVE CARE

In many ways, the success of cancer therapy depends on the success of the supportive care. Failure to control the symptoms of cancer and its treatment may lead patients to abandon curative therapy. Of equal importance, supportive care is a major determinant of quality of life. Even when life cannot be prolonged, the physician must strive to preserve its quality. Quality-of-life measurements have become common endpoints of clinical research studies. Furthermore, palliative care has been shown to be cost-effective when approached in an organized fashion. A credo for oncology could be to cure sometimes, to extend life often, and to comfort always.

Pain Pain occurs with variable frequency in the cancer patient: 25–50% of patients present with pain at diagnosis, 33% have pain associated with treatment, and 75% have pain with progressive disease. The pain may have several causes. In ~70% of cases, pain is caused by the tumor itself—by invasion of bone, nerves, blood vessels, or mucous membranes or obstruction of a hollow viscus or duct. In ~20% of cases, pain is related to a surgical or invasive medical procedure, to radiation injury (mucositis, enteritis, or plexus, or spinal cord injury), or to chemotherapy injury (mucositis, peripheral neuropathy, phlebitis,

steroid-induced avascular necrosis of the femoral head). In 10% of cases, pain is unrelated to cancer or its treatment.

Assessment of pain requires the methodical investigation of the history of the pain, its location, character, temporal features, provocative and palliative factors, and intensity (Chaps. 12 and 13); a review of the oncologic history and past medical history as well as personal and social history; and a thorough physical examination. The patient should be given a 10-division visual analogue scale on which to indicate the severity of the pain. The clinical condition is often dynamic, making it necessary to reassess the patient frequently. Pain therapy should not be withheld while the cause of pain is being sought.

A variety of tools are available with which to address cancer pain. About 85% of patients will have pain relief from pharmacologic intervention. However, other modalities, including antitumor therapy (such as surgical relief of obstruction, radiation therapy, and strontium-89 or samarium-153 treatment for bone pain), neurostimulatory techniques, regional analgesia, or neuroablative procedures, are effective in an additional 12% or so. Thus, very few patients will have inadequate pain relief if appropriate measures are taken. [A specific approach to pain relief is detailed in Chap. 12.](#)

Nausea Emesis in the cancer patient is usually caused by chemotherapy (Chap. 73). Its severity can be predicted from the drugs used to treat the cancer. Three forms of emesis are recognized on the basis of their timing with regard to the noxious insult. *Acute emesis*, the most common variety, occurs within 24 h of treatment. *Delayed emesis* occurs 1–7 days after treatment; it is rare, but, when present, usually follows cisplatin administration. *Anticipatory emesis* occurs before administration of chemotherapy and represents a conditioned response to visual and olfactory stimuli previously associated with chemotherapy delivery.

Acute emesis is the best understood form. Stimuli that activate signals in the chemoreceptor trigger zone in the medulla, the cerebral cortex, and peripherally in the intestinal tract lead to stimulation of the vomiting center in the medulla, the motor center responsible for coordinating the secretory and muscle contraction activity that leads to emesis. Diverse receptor types participate in the process, including dopamine, serotonin, histamine, opioid, and acetylcholine receptors. The serotonin receptor antagonists ondansetron and granisetron are effective drugs against highly emetogenic agents, as are neurokinin receptor antagonists such as aprepitant and fosaprepitant (see Chap. 73).

As with the analgesia ladder, emesis therapy should be tailored to the situation. For mildly and moderately emetogenic agents, prochlorperazine, 5–10 mg PO or 25 mg PR, is effective. Its efficacy may be enhanced by administering the drug before the chemotherapy is delivered. Dexamethasone, 10–20 mg IV, is also effective and may enhance the efficacy of prochlorperazine. For highly emetogenic agents such as cisplatin, mechlorethamine, dacarbazine, and streptozocin, combinations of agents work best and administration should begin 6–24 h before treatment. Ondansetron, 8 mg PO every 6 h the day before therapy and IV on the day of therapy, plus dexamethasone, 20 mg IV before treatment, is an effective regimen. Addition of oral aprepitant (a substance P/neurokinin 1 receptor antagonist) to this regimen (125 mg on day 1, 80 mg on days 2 and 3) further decreases the risk of both acute and delayed vomiting. Like pain, emesis is easier to prevent than to alleviate.

Delayed emesis may be related to bowel inflammation from the therapy and can be controlled with oral dexamethasone and oral metoclopramide, a dopamine receptor antagonist that also blocks serotonin receptors at high dosages. The best strategy for preventing anticipatory emesis is to control emesis in the early cycles of therapy to prevent the conditioning from taking place. If this is unsuccessful, prophylactic antiemetics the day before treatment may help. Experimental studies are evaluating behavior modification.

Effusions Fluid may accumulate abnormally in the pleural cavity, pericardium, or peritoneum. Asymptomatic malignant effusions may not require treatment. Symptomatic effusions occurring in tumors responsive to systemic therapy usually do not require local treatment

²Information about unsound methods may be obtained from the National Council Against Health Fraud, Box 1276, Loma Linda, CA 92354, or from the Center for Medical Consumers and Health Care Information, 237 Thompson Street, New York, NY 10012.

but respond to the treatment for the underlying tumor. Symptomatic effusions occurring in tumors unresponsive to systemic therapy may require local treatment in patients with a life expectancy of at least 6 months.

Pleural effusions due to tumors may or may not contain malignant cells. Lung cancer, breast cancer, and lymphomas account for ~75% of malignant pleural effusions. Their exudative nature is usually gauged by an effusion/serum protein ratio of ≥ 0.5 or an effusion/serum lactate dehydrogenase ratio of ≥ 0.6 . When the condition is symptomatic, thoracentesis is usually performed first. In most cases, symptomatic improvement occurs for <1 month. Chest tube drainage is required if symptoms recur within 2 weeks. Fluid is aspirated until the flow rate is <100 mL in 24 h. Then either 60 units of bleomycin or 1 g of doxycycline is infused into the chest tube in 50 mL of 5% dextrose in water; the tube is clamped; the patient is rotated on four sides, spending 15 min in each position; and, after 1–2 h, the tube is again attached to suction for another 24 h. The tube is then disconnected from suction and allowed to drain by gravity. If <100 mL drains over the next 24 h, the chest tube is pulled, and a radiograph is taken 24 h later. If the chest tube continues to drain fluid at an unacceptably high rate, sclerosis can be repeated. Bleomycin may be somewhat more effective than doxycycline but is very expensive. Doxycycline is usually the drug of first choice. If neither doxycycline nor bleomycin is effective, talc can be used.

Symptomatic pericardial effusions are usually treated by creating a pericardial window or by stripping the pericardium. If the patient's condition does not permit a surgical procedure, sclerosis can be attempted with doxycycline and/or bleomycin.

Malignant ascites is usually treated with repeated paracentesis of small volumes of fluid. If the underlying malignancy is unresponsive to systemic therapy, peritoneovenous shunts may be inserted. Despite the fear of disseminating tumor cells into the circulation, widespread metastases are an unusual complication. The major complications are occlusion, leakage, and fluid overload. Patients with severe liver disease may develop disseminated intravascular coagulation.

Nutrition Cancer and its treatment may lead to a decrease in nutrient intake of sufficient magnitude to cause weight loss and alteration of intermediary metabolism. The prevalence of this problem is difficult to estimate because of variations in the definition of cancer cachexia, but most patients with advanced cancer experience weight loss and decreased appetite. A variety of both tumor-derived factors (e.g., bombesin, adrenocorticotrophic hormone) and host-derived factors (e.g., tumor necrosis factor, interleukins 1 and 6, growth hormone) contribute to the altered metabolism, and a vicious cycle is established in which protein catabolism, glucose intolerance, and lipolysis cannot be reversed by the provision of calories.

It remains controversial how to assess nutritional status and when and how to intervene. Efforts to make the assessment objective have included the use of a prognostic nutritional index based on albumin levels, triceps skinfold thickness, transferrin levels, and delayed-type hypersensitivity skin testing. However, a simpler approach has been to define the threshold for nutritional intervention as <10% unexplained body weight loss, serum transferrin level <1500 mg/L (150 mg/dL), and serum albumin <34 g/L (3.4 g/dL).

The decision is important, because it appears that cancer therapy is substantially more toxic and less effective in the face of malnutrition. Nevertheless, it remains unclear whether nutritional intervention can alter the natural history. Unless some pathology is affecting the absorptive function of the gastrointestinal tract, enteral nutrition provided orally or by tube feeding is preferred over parenteral supplementation. However, the risks associated with the tube may outweigh the benefits. Megestrol acetate, a progestational agent, has been advocated as a pharmacologic intervention to improve nutritional status. Research in this area may provide more tools in the future as cytokine-mediated mechanisms are further elucidated.

Psychosocial Support The psychosocial needs of patients vary with their situation. Patients undergoing treatment experience fear, anxiety, and depression. Self-image is often seriously compromised

by deforming surgery and loss of hair. Women who receive cosmetic advice that enables them to look better also feel better. Loss of control over how one spends time can contribute to the sense of vulnerability. Juggling the demands of work and family with the demands of treatment may create enormous stresses. Sexual dysfunction is highly prevalent and needs to be discussed openly with the patient. An empathetic health care team is sensitive to the individual patient's needs and permits negotiation where such flexibility will not adversely affect the course of treatment.

Cancer survivors have other sets of difficulties. Patients may have fears associated with the termination of a treatment they associate with their continued survival. Adjustments are required to physical losses and handicaps, real and perceived. Patients may be preoccupied with minor physical problems. They perceive a decline in their job mobility and view themselves as less desirable workers. They may be victims of job and/or insurance discrimination. Patients may experience difficulty reentering their normal past life. They may feel guilty for having survived and may carry a sense of vulnerability to colds and other illnesses. Perhaps the most pervasive and threatening concern is the ever-present fear of relapse (the Damocles syndrome).

Patients in whom therapy has been unsuccessful have other problems related to the end of life.

Death and Dying The most common causes of death in patients with cancer are infection (leading to circulatory failure), respiratory failure, hepatic failure, and renal failure. Intestinal blockage may lead to inanition and starvation. Central nervous system disease may lead to seizures, coma, and central hypoventilation. About 70% of patients develop dyspnea preterminally. However, many months usually pass between the diagnosis of cancer and the occurrence of these complications, and during this period, the patient is severely affected by the possibility of death. The path of unsuccessful cancer treatment usually occurs in three phases. First, there is optimism at the hope of cure; when the tumor recurs, there is the acknowledgment of an incurable disease, and the goal of palliative therapy is embraced in the hope of being able to live with disease; finally, at the disclosure of imminent death, another adjustment in outlook takes place. The patient imagines the worst in preparation for the end of life and may go through stages of adjustment to the diagnosis. These stages include denial, isolation, anger, bargaining, depression, acceptance, and hope. Of course, patients do not all progress through all the stages or proceed through them in the same order or at the same rate. Nevertheless, developing an understanding of how the patient has been affected by the diagnosis and is coping with it is an important goal of patient management.

It is best to speak frankly with the patient and the family regarding the likely course of disease. These discussions can be difficult for the physician as well as for the patient and family. The critical features of the interaction are to reassure the patient and family that everything that can be done to provide comfort will be done. They will not be abandoned. Many patients prefer to be cared for in their homes or in a hospice setting rather than a hospital. The American College of Physicians has published a book called *Home Care Guide for Cancer: How to Care for Family and Friends at Home* that teaches an approach to successful problem-solving in home care. With appropriate planning, it should be possible to provide the patient with the necessary medical care as well as the psychological and spiritual support that will prevent the isolation and depersonalization that can attend in-hospital death.

The care of dying patients may take a toll on the physician. A "burn-out" syndrome has been described that is characterized by fatigue, disengagement from patients and colleagues, and a loss of self-fulfillment. Efforts at stress reduction, maintenance of a balanced life, and setting realistic goals may combat this disorder.

End-of-Life Decisions Unfortunately, a smooth transition in treatment goals from curative to palliative may not be possible in all cases because of the occurrence of serious treatment-related complications or rapid disease progression. Vigorous and invasive medical support for a reversible disease or treatment complication is assumed to be justified. However, if the reversibility of the condition is in doubt,

the patient's wishes determine the level of medical care. These wishes should be elicited before the terminal phase of illness and reviewed periodically. Information about advance directives can be obtained from the American Association of Retired Persons, 601 E Street, NW, Washington, DC 20049, 202-434-2277, or Choice in Dying, 250 West 57th Street, New York, NY 10107, 212-366-5540. Some states allow physicians to assist patients who choose to end their lives. This subject is challenging from an ethical and a medical point of view. Discussions of end-of-life decisions should be candid and involve clear informed consent, waiting periods, second opinions, and documentation. [A full discussion of end-of-life management is provided in Chap. 12.](#)

FURTHER READING

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70

Prevention and Early Detection of Cancer

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Barnett S. Kramer



Improved understanding of carcinogenesis has allowed cancer prevention and early detection to expand beyond identification and avoidance of carcinogens. Specific interventions to reduce cancer mortality by preventing cancer in those at risk and effective screening for early detection of cancer are the goals.

Carcinogenesis is a process that usually extends over years, a continuum of discrete tissue and cellular changes over time resulting in aberrant physiologic processes. Prevention concerns the identification and manipulation of the biologic, environmental, social, and genetic factors in the causal pathway of cancer. Examination of national epidemiologic patterns can provide indicators of the relative contributions of advances in prevention, screening, and therapy in progress against cancer, but randomized trials provide the best evidence to guide practice, especially in the healthy general population.

EDUCATION AND HEALTHFUL HABITS

Public education on the avoidance of identified risk factors for cancer and encouraging healthy habits contributes to cancer prevention. The clinician is a powerful messenger in this process. The patient-provider encounter provides an opportunity to teach patients about the hazards of smoking, influence of a healthy lifestyle and other exposures, and use of proven cancer screening methods.

SMOKING CESSATION

Tobacco smoking is a strong, modifiable risk factor for cardiovascular disease, pulmonary disease, and cancer. Smokers have an 1 in 3 lifetime risk of dying prematurely from a tobacco-related cancer, cardiovascular, or pulmonary disease. Tobacco use causes more deaths from cardiovascular disease than from cancer. Lung cancer and cancers of the larynx, oropharynx, esophagus, kidney, bladder, colon, pancreas, stomach, and uterine cervix are all tobacco related.

The number of cigarettes smoked per day and the level of inhalation of cigarette smoke are correlated with risk of lung cancer mortality. Light- and low-tar cigarettes are not safer because smokers tend to inhale them more frequently and deeply.

Those who stop smoking have a 30–50% lower 10-year lung cancer mortality rate compared to those who continue smoking, despite the fact that some carcinogen-induced gene mutations persist for years after smoking cessation. Smoking cessation and avoidance would save more lives than any other public health activity.

The risk of tobacco smoke is not limited to the smoker. Environmental tobacco smoke, known as secondhand or passive smoke, is carcinogenic and associated with a variety of respiratory illnesses in exposed children.

Tobacco use prevention is a pediatric issue. More than 80% of adult American smokers began smoking before the age of 18 years. Cigarette smoking has been declining in recent years, but in recent surveys, about 8% of high school students reported smoking within the prior month. Electronic cigarettes, on the other hand, are rapidly increasing in use: approximately 28% of high school students and 11% of middle school students are current electronic cigarette users. Counseling of adolescents and young adults is critical to prevent all forms of tobacco use. A clinician's simple advice can be of benefit. Providers should query patients on tobacco use and offer smokers assistance in quitting.

Current approaches to smoking cessation recognize nicotine in tobacco as addicting ([Chap. 454](#)). The smoker who is quitting goes through identifiable stages, including contemplation of quitting, an action phase in which the smoker quits, and a maintenance phase. Smokers who quit completely are more likely to be successful than those who gradually reduce the number of cigarettes smoked or change to lower-tar or lower-nicotine cigarettes. Organized cessation programs may help individual efforts. Heavy smokers may need an intensive broad-based cessation program that includes counseling, behavioral strategies, and pharmacologic adjuncts, such as nicotine replacement (gum, patches, sprays, lozenges, and inhalers), bupropion, and/or varenicline. Electronic cigarettes have been advocated as a tool to achieve smoking cessation in adults, but it is not known how effective electronic cigarettes are for this purpose. The net effects of electronic cigarettes on health are poorly studied. Absence of strict manufacturing controls of vaping material has produced serious injury.

The health risks of cigars are similar to those of cigarettes. Smoking one or two cigars daily doubles the risk for oral and esophageal cancers; smoking three or four cigars daily increases the risk of oral cancers more than eightfold and esophageal cancer fourfold. The risks of occasional use are unknown.

Smokeless tobacco also represents a substantial health risk. Chewing tobacco is a carcinogen linked to dental caries, gingivitis, oral leukoplakia, and oral cancer. The systemic effects of smokeless tobacco (including snuff) may increase risks for other cancers. Esophageal cancer is linked to carcinogens in tobacco dissolved in saliva and swallowed. The net effects of e-cigarettes on health are poorly studied.

PHYSICAL ACTIVITY

Physical activity is associated with a decreased risk of colon and breast cancer. A variety of mechanisms have been proposed. However, such studies are prone to confounding factors such as recall bias, association of exercise with other health-related practices, and effects of preclinical cancers on exercise habits (reverse causality).

DIET MODIFICATION

International epidemiologic studies suggest that diets high in fat are associated with increased risk for cancers of the breast, colon, prostate, and endometrium. Despite correlations, dietary fat has not been proven to cause cancer. Case-control and cohort epidemiologic studies give conflicting results. Diet is a highly complex exposure to many nutrients and chemicals. Low-fat diets are associated with many dietary changes beyond simple subtraction of fat. Other lifestyle factors are also associated with adherence to a low-fat diet.

In some observational studies, dietary fiber has been associated with a reduced risk of colonic polyps and invasive cancer of the colon.

Two large prospective cohort studies of >100,000 health professionals showed no association between fruit and vegetable intake and risk of cancer, however. Cancer-protective effects of increasing fiber and lowering dietary fat have not been shown in the context of a prospective clinical trial. The Polyp Prevention Trial randomly assigned 2000 elderly persons, who had polyps removed, to a low-fat, high-fiber diet versus routine diet for 4 years. No differences were noted in polyp formation.

The U.S. National Institutes of Health Women's Health Initiative, launched in 1994, was a long-term clinical trial enrolling >100,000 women age 45–69 years. It placed women into 22 intervention groups. Participants received calcium/vitamin D supplementation; hormone replacement therapy; and counseling to increase exercise, eat a low-fat diet with increased consumption of fruits, vegetables, and fiber, and cease smoking. The study showed that although dietary fat intake was lower in the diet intervention group, invasive breast cancers were not reduced over an 8-year follow-up period compared to the control group. Additionally, no reduction was seen in the incidence of colorectal cancer in the dietary intervention arm. In the aggregate, cohort studies and randomized trials suggest that reduction of red meat or processed meat consumption has a small (if any) effect on cancer incidence and mortality, although the overall evidence base is weak. Evidence does not currently establish the anticarcinogenic value of vitamin, mineral, or nutritional supplements in amounts greater than those provided by a balanced diet.

■ ENERGY BALANCE

Risk of certain cancers appears to increase modestly (relative risks generally in the 1.0–2.0 range) as body mass index (BMI) increases beyond 25 kg/m². A cohort study of >5 million adults included in the U.K. Clinical Practice Research Datalink (a primary care database) found that each 5 kg/m² increase in BMI was linearly associated with cancers of the uterus, gallbladder, kidney, cervix, thyroid, and leukemia. High BMI appears to have an inverse association with prostate and premenopausal breast cancer.

■ SUN AVOIDANCE

Nonmelanoma skin cancers (basal cell and squamous cell) are induced by cumulative exposure to ultraviolet (UV) radiation. Sunburns, especially in childhood and adolescence, may be associated with an increased risk of melanoma in adulthood. Reduction of sun exposure through use of protective clothing and changing patterns of outdoor activities can reduce skin cancer risk. Sunscreens decrease the risk of actinic keratoses, the precursor to squamous cell skin cancer, but melanoma risk may not be reduced. Sunscreens prevent burning, but they may encourage more prolonged exposure to the sun and may not filter out wavelengths of energy that cause melanoma.

Appearance-focused behavioral interventions in young women can decrease indoor tanning use and other UV exposures and may be more effective than messages about long-term cancer risks. Those who recognize themselves as being at risk tend to be more compliant with sun-avoidance recommendations. Risk factors for melanoma include a propensity to sunburn, a large number of benign melanocytic nevi, and atypical nevi.

CANCER CHEMOPREVENTION

Chemoprevention involves the use of specific natural or synthetic chemical agents to reverse, suppress, or prevent carcinogenesis before the development of invasive malignancy.

Cancer develops through an accumulation of tissue abnormalities associated with genetic and epigenetic changes, and growth regulatory pathways that are potential points of intervention to prevent cancer. The initial changes are termed *initiation*. The alteration can be inherited or acquired through the action of physical, infectious, or chemical carcinogens. Like most human diseases, cancer arises from an interaction between genetics and environmental exposures (Table 70-1). Influences that cause the initiated cell and its surrounding tissue microenvironment to progress through the carcinogenic process and change phenotypically are termed *promoters*. Promoters

TABLE 70-1 Suspected Carcinogens

CARCINOGENS ^a	ASSOCIATED CANCER OR NEOPLASM
Alkylating agents	Acute myeloid leukemia, bladder cancer
Androgens	Prostate cancer
Aromatic amines (dyes)	Bladder cancer
Arsenic	Cancer of the lung, skin
Asbestos	Cancer of the lung, pleura, peritoneum
Benzene	Acute myelocytic leukemia
Chromium	Lung cancer
Diethylstilbestrol (prenatal)	Vaginal cancer (clear cell)
Epstein-Barr virus	Burkitt's lymphoma, nasal T-cell lymphoma
Estrogens	Cancer of the endometrium, liver, breast
Ethyl alcohol	Cancer of the breast, liver, esophagus, head and neck
<i>Helicobacter pylori</i>	Gastric cancer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma
Hepatitis B or C virus	Liver cancer
Human immunodeficiency virus	Non-Hodgkin's lymphoma, Kaposi's sarcoma, squamous cell carcinomas (especially of the urogenital tract)
Human papillomavirus	Cancers of the cervix, anus, oropharynx
Human T-cell lymphotropic virus type 1 (HTLV-1)	Adult T-cell leukemia/lymphoma
Immunosuppressive agents (azathioprine, cyclosporine, glucocorticoids)	Non-Hodgkin's lymphoma
Ionizing radiation (therapeutic or diagnostic)	Breast, bladder, thyroid, soft tissue, bone, hematopoietic, and many more
Nitrogen mustard gas	Cancer of the lung, head and neck, nasal sinuses
Nickel dust	Cancer of the lung, nasal sinuses
Diesel exhaust	Lung cancer (miners)
Phenacetin	Cancer of the renal pelvis and bladder
Polycyclic hydrocarbons	Cancer of the lung, skin (especially squamous cell carcinoma of scrotal skin)
Radon gas	Lung cancer
Schistosomiasis	Bladder cancer (squamous cell)
Sunlight (ultraviolet)	Skin cancer (squamous cell and melanoma)
Tobacco (including smokeless)	Cancer of the upper aerodigestive tract, bladder
Vinyl chloride	Liver cancer (angiosarcoma)

^aAgents that are thought to act as cancer initiators and/or promoters.

include hormones such as androgens, linked to prostate cancer, and estrogen, linked to breast and endometrial cancer. The difference between an initiator and promoter is indistinct; some components of cigarette smoke are “complete carcinogens,” acting as both initiators and promoters. Cancer can be prevented or controlled through interference with the factors that cause cancer initiation, promotion, or progression. Compounds of interest in chemoprevention often have antimutagenic, hormone modulation, anti-inflammatory, antiproliferative, or proapoptotic activity (or a combination).

■ CHEMOPREVENTION OF CANCERS OF THE UPPER AERODIGESTIVE TRACT

Smoking causes diffuse epithelial injury in the oral cavity, neck, esophagus, and lung. Patients cured of squamous cell cancers of the lung, esophagus, oral cavity, and neck are at risk (as high as 5% per year) of developing second cancers of the upper aerodigestive tract. Cessation of cigarette smoking does not markedly decrease the cured cancer patient's risk of second malignancy, even though it does lower the cancer risk in those who have never developed a malignancy. Smoking cessation may halt the early stages of the carcinogenic process (such as metaplasia), but it may have no effect on late stages of carcinogenesis.

This “field carcinogenesis” hypothesis for upper aerodigestive tract cancer has made “cured” patients an important population for chemoprevention of second malignancies.

Persistent oral human papillomavirus (HPV) infection, particularly HPV-16, increases the risk for cancers of the oropharynx. This association exists even in the absence of other risk factors such as smoking or alcohol use (although the magnitude of increased risk appears greater than additive when HPV infection and smoking are both present). Oral HPV infection is believed to be largely sexually acquired. Although the evidence is not definitive, the use of the HPV vaccine is associated with a reduction in prevalence of oropharyngeal infection rates and may eventually reduce oropharyngeal cancer rates (unlike cancers of the cervix, no precursor lesion for oropharyngeal tumors is known).

Oral leukoplakia, a premalignant lesion commonly found in smokers, has been used as an intermediate marker of chemopreventive activity in smaller shorter-duration, randomized, placebo-controlled trials. Although therapy with high, relatively toxic doses of isotretinoin (*13-cis*-retinoic acid) causes regression of oral leukoplakia, more tolerable doses of isotretinoin have not shown benefit in the prevention of head and neck cancer.

Several large-scale trials have assessed agents in the chemoprevention of lung cancer in patients at high risk. In the α -tocopherol/ β -carotene (ATBC) Lung Cancer Prevention Trial, participants were male smokers, age 50–69 years at entry. Participants had smoked an average of one pack of cigarettes per day for nearly 36 years. Participants received α -tocopherol, β -carotene, and/or placebo in a randomized, two-by-two factorial design. After median follow-up of 6 years, lung cancer incidence and mortality were statistically significantly increased in those receiving β -carotene. α -Tocopherol had no effect on lung cancer mortality. However, patients receiving α -tocopherol had a higher incidence of hemorrhagic stroke.

The β -Carotene and Retinol Efficacy Trial (CARET) involved 17,000 American smokers and workers with asbestos exposure. Entrants were randomly assigned to one of four arms and received β -carotene, retinol, and/or placebo in a two-by-two factorial design. This trial also demonstrated harm from β -carotene: a lung cancer rate of 5 per 1000 subjects per year for those taking placebo versus 6 per 1000 subjects per year for those taking β -carotene.

The ATBC and CARET results demonstrate the importance of testing chemoprevention hypotheses thoroughly before widespread implementation because the results contradict a number of observational studies.

CHEMOPREVENTION OF COLON CANCER

Many colon cancer prevention trials are based on the premise that most colorectal cancers develop from adenomatous polyps. These trials use adenoma recurrence or disappearance as a surrogate endpoint (not yet validated) for colon cancer prevention. Clinical trial results suggest that nonsteroidal anti-inflammatory drugs (NSAIDs), such as piroxicam, sulindac, and aspirin, may prevent adenoma formation or cause regression of adenomatous polyps. The mechanism of action of NSAIDs is unknown, but they are presumed to work through the cyclooxygenase pathway. A meta-analysis of four randomized controlled trials (albeit primarily designed to examine aspirin’s effects on cardiovascular events) found that aspirin at doses of at least 75 mg/d resulted in a 33% relative reduction in colorectal cancer incidence after 20 years, with no clear increase in efficacy at higher doses. Based on a systematic review of evidence from randomized trials for primary prevention of cardiovascular disease, the U.S. Preventive Services Task Force concluded that the balance of benefits and harms favored initiating low-dose aspirin for colorectal cancer and cardiovascular disease prevention in adults age 50–59 if they have a 10% or greater 10-year risk of cardiovascular disease. Low-dose aspirin does not appear to benefit the elderly, however. The ASPREE trial, which compared 100 mg of daily aspirin to placebo for improvement in the composite endpoint of death, dementia, or survival in the healthy elderly, was stopped because of a lack of benefit, including cancer. Cyclooxygenase-2 (COX-2) inhibitors have been considered for colorectal cancer and polyp prevention. Trials with COX-2 inhibitors were initiated, but an increased risk of cardiovascular events

in those taking the COX-2 inhibitors was noted, suggesting that these agents are not suitable for chemoprevention in the general population.

The Women’s Health Initiative demonstrated that postmenopausal women taking estrogen plus progestin have a 44% lower relative risk of colorectal cancer compared to women taking placebo. Of >16,600 women randomized and followed for a median of 5.6 years, 43 invasive colorectal cancers occurred in the hormone group and 72 in the placebo group. The positive effect on colon cancer is mitigated by the modest increase in cardiovascular and breast cancer risks associated with combined estrogen plus progestin therapy.

Most case-control and cohort studies have not confirmed early reports of an association between regular statin use and a reduced risk of colorectal cancer. No randomized controlled trials have addressed this hypothesis. A meta-analysis of statin use showed no protective effect of statins on overall cancer incidence or death.

CHEMOPREVENTION OF BREAST CANCER

Tamoxifen is an antiestrogen with partial estrogen agonistic activity in some tissues, such as endometrium and bone. One of its actions is to upregulate transforming growth factor β , which decreases breast cell proliferation. In a randomized placebo-controlled prevention trial involving >13,000 pre- and postmenopausal women at high risk, tamoxifen decreased the risk of developing breast cancer by 49% (from 43.4 to 22 per 1000 women) after a median follow-up of nearly 6 years. Tamoxifen also reduced bone fractures; a small increase in risk of endometrial cancer, stroke, pulmonary emboli, and deep vein thrombosis was noted. The International Breast Cancer Intervention Study (IBIS-I) and the Italian Randomized Tamoxifen Prevention Trial also demonstrated a reduction in breast cancer incidence with tamoxifen use. A trial comparing tamoxifen with another selective estrogen receptor modulator, raloxifene, performed in postmenopausal women showed that raloxifene is comparable to tamoxifen in cancer prevention, but without the risk of endometrial cancer. Raloxifene was associated with a smaller reduction in invasive breast cancers and a trend toward more noninvasive breast cancers, but fewer thromboembolic events than tamoxifen; the drugs are similar in risks of other cancers, fractures, ischemic heart disease, and stroke. Both tamoxifen and raloxifene (the latter for postmenopausal women only) have been approved by the U.S. Food and Drug Administration (FDA) for reduction of breast cancer in women at high risk for the disease (1.66% risk at 5 years based on the Gail risk model: <http://www.cancer.gov/bcrisktool/>).

Because the aromatase inhibitors are even more effective than tamoxifen in adjuvant breast cancer therapy, it has been hypothesized that they would be more effective in breast cancer prevention. A randomized, placebo-controlled trial of exemestane reported a 65% relative reduction (from 5.5 to 1.9 per 1000 women) in the incidence of invasive breast cancer in women at elevated risk after a median follow-up of about 3 years. Common adverse effects included arthralgias, hot flashes, fatigue, and insomnia. No trial has directly compared aromatase inhibitors with selective estrogen receptor modulators for breast cancer chemoprevention.

CHEMOPREVENTION OF PROSTATE CANCER

Finasteride and dutasteride are 5- α -reductase inhibitors. They inhibit conversion of testosterone to dihydrotestosterone (DHT), a potent stimulator of prostate cell proliferation. The Prostate Cancer Prevention Trial (PCPT) randomly assigned men age 55 years or older at average risk of prostate cancer to finasteride or placebo. All men in the trial were being regularly screened with prostate-specific antigen (PSA) levels and digital rectal examination. After 7 years of therapy, the incidence of prostate cancer was 18.4% in the finasteride arm, compared with 24.4% in the placebo arm, a statistically significant difference. However, the finasteride group had more patients with tumors of Gleason score 7 and higher compared with the placebo arm (6.4 vs 5.1%). Long-term (10–15 years) follow-up did not reveal any statistically significant differences in overall or prostate cancer-specific mortality between all men in the finasteride and placebo arms or in men diagnosed with prostate cancer, but the power to detect a difference was limited.

Dutasteride has also been evaluated as a preventive agent for prostate cancer. The Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial was a randomized double-blind trial in which 8200 men with an elevated PSA (2.5–10 ng/mL for men age 50–60 years and 3–10 ng/mL for men age 60 years or older) and negative prostate biopsy on enrollment received daily 0.5 mg of dutasteride or placebo. The trial found a statistically significant 23% relative risk reduction in the incidence of biopsy-detected prostate cancer in the dutasteride arm at 4 years of treatment (659 cases vs 858 cases, respectively). Overall, across years 1 through 4, no difference was seen between the arms in the number of tumors with a Gleason score of 7 to 10; however, during years 3 and 4, there was a statistically significant difference in tumors with Gleason score of 8 to 10 in the dutasteride arm (12 tumors vs 1 tumor, respectively).

The clinical importance of the apparent increased incidence of higher-grade tumors in the 5- α -reductase inhibitor arms of these trials likely represents an increased sensitivity of PSA and digital rectal exam for high-grade tumors in men receiving these agents due to a decrease in prostatic volume. Although the FDA acknowledged that detection bias may have accounted for the finding, a causative role for 5- α -reductase inhibitors could not be conclusively dismissed. These agents are therefore not FDA-approved for prostate cancer prevention.

Because all men in both the PCPT and REDUCE trials were being screened and because screening approximately doubles the rate of prostate cancer, it is not known if finasteride or dutasteride decreases the risk of prostate cancer in men who are not being screened or simply reduces the risk of non-life-threatening cancers detectable by screening.

Several favorable laboratory and observational studies led to the formal evaluation of selenium and α -tocopherol (vitamin E) as potential prostate cancer preventives. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) assigned 35,533 men to receive 200 μ g/d selenium, 400 IU/d α -tocopherol, selenium plus vitamin E, or placebo. After a median follow-up of 7 years, a trend toward an increased risk of developing prostate cancer was observed for men taking vitamin E alone as compared to the placebo arm (hazard ratio 1.17; 95% confidence interval, 1.004–1.36).

VACCINES AND CANCER PREVENTION

A number of infectious agents cause cancer. Hepatitis B and C are linked to liver cancer; some HPV strains are linked to cervical, anal, and head and neck cancer; and *Helicobacter pylori* is associated with gastric adenocarcinoma and gastric lymphoma. Vaccines to protect against these agents may therefore reduce the risk of their associated cancers.

The hepatitis B vaccine is effective in preventing hepatitis and hepatomas due to chronic hepatitis B infection.

A nonavalent vaccine (covering HPV strains 6, 11, 16, 18, 31, 33, 45, 52, and 58) is available for use in the United States. HPV types 6 and 11 cause genital papillomas. The remaining HPV types cause cervical and anal cancer; reduction in HPV types 16 and 18 alone could prevent >70% of cervical cancers worldwide. For individuals not previously infected with these HPV strains, the vaccine demonstrates high efficacy in preventing persistent strain-specific HPV infections. Studies also confirm the vaccine's ability to prevent preneoplastic lesions (cervical or anal intraepithelial neoplasia [CIN/AIN] I, II, and III). The durability of the immune response beyond 10–12 years is not currently known. The vaccine does not appear to impact preexisting infections. A two-dose schedule is currently recommended in the United States for females and males age 9–14 years; teens and young adults who start the series between 15 and 26 years are recommended to receive three doses of the vaccine. However, observational studies suggest similar efficacy with a single dose in young girls, and a large randomized trial is currently comparing one to two doses.

SURGICAL PREVENTION OF CANCER

Some organs in some individuals are at such high risk of developing cancer that surgical removal of the organ at risk may be considered. Women with severe cervical dysplasia are treated with laser or loop

electrosurgical excision or conization. Colectomy may be used to prevent colon cancer in patients with familial polyposis or ulcerative colitis.

Prophylactic bilateral mastectomy may be chosen for breast cancer prevention among women with genetic predisposition to breast cancer. In a prospective series of 139 women with *BRCA1* and *BRCA2* mutations, 76 chose to undergo prophylactic mastectomy, and 63 chose close surveillance. At 3 years, no cases of breast cancer had been diagnosed in those opting for surgery, but eight patients in the surveillance group had developed breast cancer. A larger ($n = 639$) retrospective cohort study reported that three patients developed breast cancer after prophylactic mastectomy compared with an expected incidence of 30–53 cases. Postmastectomy breast cancer-related deaths were 81–94% lower in high-risk women compared with sister controls and 100% lower in moderate-risk women when compared with expected rates.

Prophylactic salpingo-oophorectomy may also be employed for the prevention of ovarian and breast cancers among high-risk women. A prospective cohort study evaluating the outcomes of *BRCA* mutation carriers demonstrated a statistically significant association between prophylactic salpingo-oophorectomy and a reduced incidence of ovarian or primary peritoneal cancer (36% relative risk reduction, or a 4.5% absolute difference). Studies of prophylactic oophorectomy for prevention of breast cancer in women with genetic mutations have shown relative risks of approximately 0.50; the risk reduction may be greatest for women having the procedure at younger (i.e., <50 years) ages. The observation that most high-grade serous "ovarian cancers" actually arise in the fallopian tube fimbria raises the possibility that this lethal subtype may be prevented by ovary-sparing salpingectomy.

All of the evidence concerning the use of prophylactic mastectomy and salpingo-oophorectomy for prevention of breast and ovarian cancer in high-risk women has been observational in nature; such studies are prone to a variety of biases, including case selection bias, family relationships between patients and controls, and inadequate information about hormone use. Thus, they may give an overestimate of the magnitude of benefit.

CANCER SCREENING

Screening is a means of early detection in asymptomatic individuals, with the goal of decreasing morbidity and mortality. While screening can potentially reduce disease-specific deaths and has been shown to do so in cervical, colon, lung, and breast cancer, it is also subject to a number of biases that can suggest a benefit when actually there is none. Biases can even mask net harm. Early detection does not in itself confer benefit. Cause-specific mortality, rather than survival after diagnosis, is the preferred endpoint (see below).

Because screening is done on asymptomatic, healthy persons, it should offer substantial likelihood of benefit that outweighs harm. Screening tests and their appropriate use should be carefully evaluated before their use is widely encouraged in screening programs.

A large and increasing number of genetic mutations and nucleotide polymorphisms have been associated with an increased risk of cancer. Testing for these genetic mutations could in theory define a high-risk population. However, most of the identified mutations have very low penetrance and individually provide limited predictive accuracy. The ability to predict the development of a particular cancer may someday present therapeutic options as well as ethical dilemmas. It may eventually allow for early intervention to prevent a cancer or limit its severity. People at high risk may be ideal candidates for chemoprevention and screening; however, efficacy of these interventions in the high-risk population should be investigated. Currently, persons at high risk for a particular cancer can engage in intensive screening. While this course is clinically reasonable, it is not known if it reduces mortality in these populations.

The Accuracy of Screening A screening test's accuracy or ability to discriminate disease is described by four indices: sensitivity, specificity, positive predictive value, and negative predictive value (Table 70-2). Sensitivity, also called the true-positive rate, is the proportion of persons with the disease who test positive in the screen

TABLE 70-2 Assessment of the Value of a Diagnostic Test^a

	CONDITION PRESENT	CONDITION ABSENT		
Positive test	<i>a</i>	<i>b</i>		
Negative test	<i>c</i>	<i>d</i>		
<i>a</i> = true positive				
<i>b</i> = false positive				
<i>c</i> = false negative				
<i>d</i> = true negative				
Sensitivity	The proportion of persons with the condition who test positive: $a/(a+c)$			
Specificity	The proportion of persons without the condition who test negative: $d/(b+d)$			
Positive predictive value (PPV)	The proportion of persons with a positive test who have the condition: $a/(a+b)$			
Negative predictive value	The proportion of persons with a negative test who do not have the condition: $d/(c+d)$			
Prevalence, sensitivity, and specificity determine PPV				
$\text{PPV} = \frac{\text{prevalence} \times \text{sensitivity}}{(\text{prevalence} \times \text{sensitivity}) + (1-\text{prevalence})(1-\text{specificity})}$				

^aFor diseases of low prevalence, such as cancer, poor specificity has a dramatic adverse effect on PPV such that only a small fraction of positive tests are true positives.

(i.e., the ability of the test to detect disease when it is present). *Specificity*, or 1 minus the false-positive rate, is the proportion of persons who do not have the disease who test negative in the screening test (i.e., the ability of a test to correctly indicate that the disease is not present). The *positive predictive value* is the proportion of persons who test positive and who actually have the disease. Similarly, *negative predictive value* is the proportion testing negative who do not have the disease. The sensitivity and specificity of a test are independent of the underlying prevalence (or risk) of the disease in the population screened, but the predictive values depend strongly on the prevalence of the disease.

Screening is most beneficial, efficient, and economical when the target disease is common in the population being screened. Specificity is at least as important to the ultimate feasibility and success of a screening test as sensitivity.

Potential Biases of Screening Tests Common biases of screening are lead time, length-biased sampling, and selection. These biases can make a screening test seem beneficial when actually it is not (or even causes net harm). Whether beneficial or not, screening can create the false impression of an epidemic by increasing the number of cancers diagnosed. It can also produce a shift in the *proportion* of patients diagnosed at an early stage (even without a reduction in absolute incidence of late-stage disease) and inflate survival statistics without reducing mortality (i.e., the number of deaths from a given cancer relative to the number of those at risk for the cancer). In such a case, the *apparent* duration of survival (measured from date of diagnosis) increases without lives being saved or life expectancy changed.

Lead-time bias occurs whether or not a test influences the natural history of the disease; the patient is merely diagnosed at an earlier date. Survival *appears* increased even if life is not prolonged. The screening test only prolongs the time the subject is aware of the disease and spends as a cancer patient.

Length-biased sampling occurs because screening tests generally can more easily detect slow-growing, less aggressive cancers than fast-growing cancers. Cancers diagnosed due to the onset of symptoms between scheduled screenings are on average more aggressive, and treatment outcomes are not as favorable. An extreme form of length bias sampling is termed *overdiagnosis*, the detection of “pseudo disease.” The reservoir of some undetected slow-growing tumors is large. Many of these tumors fulfill the histologic criteria of cancer but will never become clinically significant or cause death during the patient’s remaining life span. This problem is compounded by the fact that the

most common cancers appear most frequently at ages when competing causes of death are more frequent.

Selection bias occurs because the population most likely to seek screening often differs from the general population to which the screening test might be applied. In general, volunteers for studies are more health conscious and likely to have a better prognosis or lower mortality rate, irrespective of the screening result. This is termed the *healthy volunteer effect*.

Potential Drawbacks of Screening Risks associated with screening include harm caused by the screening intervention itself, harm due to the further investigation of persons with positive tests (both true and false positives), and harm from the treatment of persons with a true-positive result, whether or not life is extended by treatment (e.g., even if a screening test reduces relative cause-specific mortality by 15–30%, 70–85% of those diagnosed still go on to die of the target cancer). The diagnosis and treatment of cancers that would never have caused medical problems can lead to the harm of unnecessary treatment and give patients the anxiety of a cancer diagnosis. The psychosocial impact of cancer screening can be substantial when applied to the entire population.

Assessment of Screening Tests Good clinical trial design can offset some biases of screening and demonstrate the relative risks and benefits of a screening test. A randomized controlled screening trial with cause-specific mortality as the endpoint provides the strongest support for a screening intervention. Overall mortality should also be reported to detect an adverse effect of screening and treatment on other disease outcomes (e.g., cardiovascular disease, treatment-induced cancers). In a randomized trial, two like populations are randomly established. One is given the usual standard of care (which may be no screening at all) and the other receives the screening intervention being assessed. Efficacy for the population studied is established when the group receiving the screening test has a better cause-specific mortality rate than the control group. Studies showing a reduction in the incidence of advanced-stage disease, improved survival, or a stage shift are weaker (and possibly misleading) evidence of benefit. These latter criteria are early indicators but not sufficient to establish the value of a screening test.

Although a randomized, controlled screening trial provides the strongest evidence to support a screening test, it is not perfect. Unless the trial is population-based, it does not remove the question of generalizability to the target population. Screening trials generally involve thousands of persons and last for years. Less definitive study designs are therefore often used to estimate the effectiveness of screening practices. However, every nonrandomized study design is subject to strong confounders. In descending order of strength, evidence may also be derived from the findings of internally controlled trials using intervention allocation methods other than randomization (e.g., allocation by birth date, date of clinic visit); the findings of analytic observational studies; or the results of multiple time series studies with or without the intervention.

Screening for Specific Cancers Screening for cervical, colon, and breast cancer has the potential to be beneficial for certain age groups. Depending on age and smoking history, lung cancer screening can also be beneficial in specific settings. Special surveillance of those at high risk for a specific cancer because of a family history or a genetic risk factor may be prudent, but few studies have assessed the effect on mortality. A number of organizations have considered whether or not to endorse routine use of certain screening tests. Because criteria have varied, they have arrived at different recommendations. The American Cancer Society (ACS) and the U.S. Preventive Services Task Force (USPSTF) publish screening guidelines (Table 70-3); the American Academy of Family Practitioners (AAFP) often follows/endorses the USPSTF recommendations; and the American College of Physicians (ACP) develops recommendations based on structured reviews of other organizations’ guidelines.

TABLE 70-3 Screening Recommendations for Asymptomatic Subjects Not Known to Be at Increased Risk for the Target Condition^a

CANCER TYPE	TEST OR PROCEDURE	USPSTF	ACS
Breast	Self-examination	"D" ^b (Not in current recommendations; from 2009)	Women, all ages: No specific recommendation
	Clinical examination	Women ≥40 years: "I" (as a stand-alone without mammography) (Not in current recommendations; from 2009)	Women, all ages: Do not recommend
	Mammography	Women 40–49 years: The decision to start screening mammography in women prior to age 50 years should be an individual one. Women who place a higher value on the potential benefit than the potential harms may choose to begin biennial screening between the ages of 40 and 49 years. ("C") Women 50–74 years: Every 2 years ("B") Women ≥75 years: "I"	Women 40–44 years: Provide the opportunity to begin annual screening Women 45–54 years: Screen annually Women ≥55 years: Transition to biennial screening or have the opportunity to continue annual screening Women ≥40 should continue screening mammography as long as their overall health is good and they have a life expectancy of 10 years or longer
	Magnetic resonance imaging (MRI)	"I" (Not in current recommendations; from 2009)	Women with >20% lifetime risk of breast cancer: Screen with MRI plus mammography annually Women with 15–20% lifetime risk of breast cancer: Discuss option of MRI plus mammography annually Women with <15% lifetime risk of breast cancer: Do not screen annually with MRI
	Tomosynthesis	Women, all ages: "I"	No specific recommendation
Cervical	Pap test (cytology)	Women <21 years: "D" Women 21–29 years: Screen with cytology alone every 3 years ("A") Women 30–65 years: Screen with cytology alone every 3 years, or with co-testing (HPV testing + cytology) every 5 years (two of three options, see HPV test below) ("A") Women >65 years, with adequate, normal prior Pap screenings: "D" Women after total hysterectomy for noncancerous causes: "D"	Women <21 years: No screening Women 21–29 years: Screen every 3 years Women 30–65 years: Screen with co-testing (HPV testing + cytology) every 5 years or cytology alone every 3 years (see HPV test below) Women >65 years: No screening following adequate negative prior screening Women after total hysterectomy for noncancerous causes: Do not screen
	HPV test	Women <30 years: Do not use HPV testing for cervical cancer screening Women 30–65 years: Screen with HPV testing alone or in combination with cytology every 5 years (two of three options, see Pap test above) ("A") Women >65 years, with adequate, normal prior Pap screenings: "D" Women after total hysterectomy for noncancerous causes: "D"	Women <30 years: Do not use HPV testing for cervical cancer screening Women 30–65 years: Preferred approach to screen with HPV and cytology co-testing every 5 years (see Pap test above) Women >65 years: No screening following adequate negative prior screening Women after total hysterectomy for noncancerous causes: Do not screen
Colorectal	Overall	Adults 50–75 years: "A" Screen for colorectal cancer; the risks and benefits of the different screening methods vary Adults 76–85 years: "C" The decision to screen should be an individual one, taking into account the patient's overall health and prior screening history	Adults ≥45–75 years: Screen for colorectal cancer with either a high-sensitivity stool-based test or a structural (visual) examination (≥45 years, qualified recommendation; ≥50 years, strong recommendation). Adults 76–85 years: Individualize screening based on patient preferences, life expectancy, health status, and prior screening history (qualified recommendation). Adults >85 years: Discourage screening (qualified recommendation). Every 5 years
	Sigmoidoscopy	Every 5 years; modeling suggests improved benefit if performed every 10 years in combination with annual FIT	Adults ≥45 years: Every 5 years
	Fecal occult blood testing (FOBT)	Every year	Adults ≥45 years: Every year
	Colonoscopy	Every 10 years	Adults ≥45 years: Every 10 years
	Fecal DNA testing	At least every 3 years	Adults ≥45 years: Every 3 years
	Fecal immunochemical testing (FIT)	Every year	Adults ≥45 years: Every year
	Computed tomography (CT) colonography	Every 5 years	Adults ≥45 years: Every 5 years

(Continued)

TABLE 70-3 Screening Recommendations for Asymptomatic Subjects Not Known to Be at Increased Risk for the Target Condition^a (Continued)

CANCER TYPE	TEST OR PROCEDURE	USPSTF	ACS
Lung	Low-dose CT scan	Adults 55–80 years, with a ≥30 pack-year smoking history, still smoking or have quit within past 15 years: “B” Discontinue once a person has not smoked for 15 years or develops a health problem that substantially limits life expectancy or the ability to have curative lung surgery	Men and women, 55–74 years, with ≥30 pack-year smoking history, still smoking or have quit within past 15 years: Discuss benefits, limitations, and potential harms of screening; offer smoking cessation counseling where relevant; only perform screening in high-volume, high-quality lung cancer screening and treatment centers.
Ovarian	CA-125 Transvaginal ultrasound	Women, all ages: “D” Women with a high-risk hereditary cancer syndrome: No recommendation	Currently, there are no reliable screening tests for the early detection of ovarian cancer. For women at high risk of ovarian cancer, it has not been proven that using transvaginal ultrasound or serum CA-125 lowers their chances of dying from ovarian cancer.
Prostate	Prostate-specific antigen (PSA)	Men 55–69 years: The decision to undergo periodic PSA-based screening should be an individual one. Men should have an opportunity to discuss the potential benefits and harms of screening with their clinician. Clinicians should not screen men who do not express a preference for screening (“C”) Men ≥70 years: “D”	Starting at age 50, men at average risk and with a life expectancy of ≥10 years should talk to a doctor about the uncertainties, risks, and potential benefits of screening. If African American or have a father or brother who had prostate cancer before age 65, men should have this talk starting at age 45. For men with more than one first-degree relative with prostate cancer diagnosed before age 65, have this talk starting at age 40. How often they are screened will depend on their PSA level.
	Digital rectal examination (DRE)	No individual recommendation	As for PSA; if men decide to be tested, they should have the PSA blood test with or without a rectal exam.
Skin	Complete skin examination by clinician or patient	Adults, all ages: “I”	No guidelines

^aSummary of the screening procedures recommended for the general population by the USPSTF and the ACS. These recommendations refer to asymptomatic persons who are not known to have risk factors, other than age or gender, for the targeted condition. ^aUSPSTF lettered recommendations are defined as follows: “A”: The USPSTF recommends the service because there is high certainty that the net benefit is substantial; “B”: The USPSTF recommends the service because there is high certainty that the net benefit is moderate or moderate certainty that the net benefit is moderate to substantial; “C”: The USPSTF recommends selectively offering or providing this service to individual patients based on professional judgment and patient preferences; there is at least moderate certainty that the net benefit is small; “D”: The USPSTF recommends against the service because there is moderate or high certainty that the service has no net benefit or that the harms outweigh the benefits; “I”: The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of the service.

Abbreviations: ACS, American Cancer Society; USPSTF, U.S. Preventive Services Task Force.

BREAST CANCER Breast self-examination, clinical breast examination by a caregiver, mammography, and magnetic resonance imaging (MRI) have all been variably advocated as useful screening tools.

A number of trials have suggested that annual or biennial screening with mammography in normal-risk women older than age 50 years decreases breast cancer mortality. Each trial has been criticized for design flaws. In most trials, breast cancer-related mortality rates were decreased by 15–30%. Experts disagree on whether average-risk women age 40–49 years should receive regular screening (Table 70-3). The U.K. Age Trial, the only randomized trial of breast cancer screening to specifically evaluate the impact of mammography in women age 40–49 years, found no statistically significant difference in breast cancer mortality for screened women versus controls after about 11 years of follow-up (relative risk 0.83; 95% confidence interval 0.66–1.04); however, <70% of women received screening in the intervention arm, potentially diluting the observed effect. A meta-analysis of nine large randomized trials showed an 8% relative reduction in mortality (relative risk 0.92; 95% confidence interval 0.75–1.02) from mammography screening for women age 39–49 years after 11–20 years of follow-up. This is equivalent to 3 breast cancer deaths prevented per 10,000 women >10 years (although the result is not statistically significant). At the same time, nearly half of women age 40–49 years screened annually will have false-positive mammograms necessitating further evaluation, often including biopsy. Estimates of overdiagnosis range from 10 to 50% of diagnosed invasive cancers. In the United States, widespread screening over the past several decades has not been accompanied by a reduction in incidence of metastatic breast cancer despite a large increase in early-stage disease, suggesting a substantial amount of overdiagnosis at the population level. In addition, the substantial improvements in systemic therapy have likely decreased the impact of mammography and early detection on falling breast cancer mortality rates.

Digital breast tomosynthesis is a newer method of breast cancer screening that reconstructs multiple x-ray images of the breast into superimposed “three-dimensional” slices. Although some evidence is available concerning the test characteristics of this modality, there are currently no data on its effects on health outcomes such as breast cancer-related morbidity, mortality, or overdiagnosis rates. A large randomized trial comparing standard digital mammography to tomosynthesis is in progress.

No study of breast self-examination has shown it to decrease mortality. A randomized controlled trial of approximately 266,000 women in China demonstrated no difference in breast cancer mortality between a group that received intensive breast self-exam instruction and reinforcement/reminders and controls at 10 years of follow-up. However, more benign breast lesions were discovered and more breast biopsies were performed in the self-examination arm.

Genetic screening for *BRCA1* and *BRCA2* mutations and other markers of breast cancer risk has identified a group of women at high risk for breast cancer. Unfortunately, when to begin and the optimal frequency of screening have not been defined. Mammography is less sensitive at detecting breast cancers in women carrying *BRCA1* and *BRCA2* mutations, possibly because such cancers occur in younger women, in whom mammography is known to be less sensitive. MRI screening may be more sensitive than mammography in women at high risk due to genetic predisposition or in women with very dense breast tissue, but specificity may be lower. An increase in overdiagnosis may accompany the higher sensitivity. The impact of MRI on breast cancer mortality with or without concomitant use of mammography has not been evaluated in a randomized controlled trial.

CERVICAL CANCER Screening with Papanicolaou (Pap) smears decreases cervical cancer mortality. The cervical cancer mortality rate has fallen substantially since the widespread use of the Pap smear. With

the onset of sexual activity comes the risk of sexual transmission of HPV, the fundamental etiologic factor for cervical cancer. Screening guidelines recommend regular Pap testing for all women who have reached the age of 21 (before this age, even in individuals that have begun sexual activity, screening may cause more harm than benefit). The recommended interval for Pap screening is 3 years. In all cases, screening more frequently adds little benefit but leads to important harms, including unnecessary procedures and overtreatment of transient lesions. Beginning at age 30, guidelines also include HPV testing with or without Pap smear. The screening interval for women who test normal using this approach may be lengthened to 5 years.

An upper age limit at which screening ceases to be effective is not known, but women age 65 years with no abnormal results in the previous 10 years may choose to stop screening. Screening should be discontinued in women who have undergone a hysterectomy with cervical excision for noncancerous reasons.

Although the efficacy of the Pap smear in reducing cervical cancer mortality has never been directly confirmed in a randomized, controlled setting, a clustered randomized trial in India evaluated the impact of one-time cervical visual inspection and immediate colposcopy, biopsy, and/or cryotherapy (where indicated) versus counseling on cervical cancer deaths in women age 30–59 years. After 7 years of follow-up, the age-standardized rate of death due to cervical cancer was 39.6 per 100,000 person-years in the intervention group versus 56.7 per 100,000 person-years in controls.

COLORECTAL CANCER Fecal occult blood testing (FOBT), digital rectal examination (DRE), rigid and flexible sigmoidoscopy, colonoscopy, and computed tomography (CT) colonography have been considered for colorectal cancer screening. A meta-analysis of five randomized controlled trials demonstrated a 22% relative reduction in colorectal cancer mortality after two to nine rounds of biennial FOBT at 30 years of follow-up; annual screening was shown to result in a greater colorectal cancer mortality reduction in a single trial (a 32% relative reduction). However, only 2–10% of those with occult blood in the stool have cancer. The high false-positive rate of FOBT substantially increases the number of colonoscopies performed.

Fecal immunochemical tests (FITs) have higher sensitivity for colorectal cancer than FOBT tests. Limited observational evidence suggests FITs may have lower ability to detect proximal versus distal colonic tumors. Multitargeted stool DNA testing combines FIT with testing for altered DNA biomarkers in cells that are shed into the stool. Although limited evidence demonstrates that it can have a higher single-test sensitivity for colorectal cancer than FIT alone, its specificity is lower, resulting in a higher number of false-positive tests and follow-up colonoscopies. There are no studies evaluating its effects on colorectal cancer incidence, morbidity, or mortality.

A blood test for the methylated *SEPT9* gene associated with colorectal cancer is available. However, its sensitivity is low, no longitudinal data have been collected on its performance or efficacy, and it is not recommended as a first-line screening test.

Two meta-analyses of five randomized controlled trials of sigmoidoscopy found an 18% relative reduction in colorectal cancer incidence and a 28% relative reduction in colorectal cancer mortality. Participant ages ranged from 50 to 74 years, with follow-up ranging from 6 to 13 years. Diagnosis of adenomatous polyps by sigmoidoscopy should lead to evaluation of the entire colon with colonoscopy. The most efficient interval for screening sigmoidoscopy is unknown, but an interval of 5 years is often recommended. Case-control studies suggest that intervals of up to 15 years may confer benefit; the randomized U.K. trial demonstrated colorectal cancer mortality reduction with one-time screening.

One-time colonoscopy detects 25% more advanced lesions (polyps >10 mm, villous adenomas, adenomatous polyps with high-grade dysplasia, invasive cancer) than one-time FOBT with sigmoidoscopy; comparative *programmatic* performance of the two modalities over time is not known. Perforation rates are about 4/10,000 for colonoscopy and 1/10,000 for sigmoidoscopy. Debate continues on whether colonoscopy is too expensive and invasive and whether sufficient provider capacity exists to be recommended as the preferred screening

tool in standard-risk populations. Some observational studies suggest that efficacy of colonoscopy to decrease colorectal cancer mortality is primarily limited to the left side of the colon.

CT colonography, if done at expert centers, appears to have a sensitivity for polyps ≥5 mm, comparable to colonoscopy. However, the rate of extracolonic findings of abnormalities of uncertain significance that must nevertheless be worked up is high (~5–37%); the long-term cumulative radiation risk of repeated colonography screenings is also a concern.

LUNG CANCER Chest x-ray and sputum cytology have been evaluated in several randomized lung cancer screening trials. The most recent and largest ($n = 154,901$) of these, a component of the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial, found that, compared with usual care, annual chest x-ray did not reduce the risk of dying from lung cancer (relative risk 0.99; 95% confidence interval 0.87–1.22) after 13 years. However, it showed evidence of overdiagnosis associated with chest x-ray. Low-dose CT has also been evaluated in several randomized trials. The largest and longest of these, the National Lung Screening Trial (NLST), was a randomized controlled trial of screening for lung cancer in ~53,000 persons age 55–74 years with a 30+ pack-year smoking history. It demonstrated a statistically significant reduction of about 3 fewer deaths per 1000 people screened with CT compared to chest x-ray after 12 years. However, the harms include the potential radiation risks associated with multiple scans, the discovery of incidental findings of unclear significance, and a high rate of false-positive test results. Both incidental findings and false-positive tests can lead to invasive diagnostic procedures associated with anxiety, expense, and complications (e.g., pneumo- or hemothorax after lung biopsy). The NLST was performed at experienced screening centers, and the balance of benefits and harms may differ in the community setting at less experienced centers.

OVARIAN CANCER Adnexal palpation, transvaginal ultrasound (TVUS), and serum CA-125 assay have been considered for ovarian cancer screening. A large randomized, controlled trial has shown that an annual screening program of TVUS and CA-125 in average-risk women does not reduce deaths from ovarian cancer (relative risk 1.21; 95% confidence interval 0.99–1.48). Adnexal palpation was dropped early in the study because it did not detect any ovarian cancers that were not detected by either TVUS or CA-125. A second large randomized trial used a two-stage screening approach incorporating a risk of ovarian cancer algorithm that determined whether additional testing with CA-125 or TVUS was required. At 14 years of follow-up, there was no statistically significant reduction in ovarian cancer deaths. The risks and costs associated with the high number of false-positive results are impediments to routine use of these modalities for screening. In the PLCO trial, 10% of participants had a false-positive result from TVUS or CA-125, and one-third of these women underwent a major surgical procedure; the ratio of surgeries to screen-detected ovarian cancer was approximately 20:1. In September 2016, the FDA issued a safety communication recommending against using any screening test, including the risk of ovarian cancer algorithm, for ovarian cancer.

PROSTATE CANCER The most common prostate cancer screening modalities are digital rectal exam (DRE) and serum PSA assay. An emphasis on PSA screening has caused prostate cancer to become the most common nonskin cancer diagnosed in American males. This disease is prone to lead-time bias, length bias, and overdiagnosis, and substantial debate continues among experts as to whether screening should be offered unless the patient specifically asks to be screened. Virtually all organizations stress the importance of informing men about the uncertainty regarding screening efficacy and the associated harms. Prostate cancer screening clearly detects many asymptomatic cancers, but the ability to distinguish tumors that are lethal but still curable from those that pose little or no threat to health is limited, and randomized trials indicate that the effect of PSA screening on prostate cancer mortality across a population is, at best, small. Men older than age 50 years have a high prevalence of indolent, clinically insignificant prostate cancers (about 30–50% of men, increasing further as men age).

Two major randomized controlled trials of the impact of PSA screening on prostate cancer mortality have been published. The PLCO Cancer Screening Trial was a multicenter U.S. trial that randomized almost 77,000 men age 55–74 years to receive either annual PSA testing for 6 years or usual care. At 13 years of follow-up, no statistically significant difference in the number of prostate cancer deaths was noted between the arms (rate ratio 1.09; 95% confidence interval 0.87–1.36). More than half of men in the control arm received at least one PSA test during the trial, which may have diluted a small effect.

The European Randomized Study of Screening for Prostate Cancer (ERSPC) was a multinational study that randomized 182,000 men between age 50 and 74 years (with a predefined “core” screening group of men age 55–69 years) to receive PSA testing or no screening. Recruitment and randomization procedures, as well as actual frequency of PSA testing, varied by country. After a median follow-up of 15.5 years, a 20% relative reduction in the risk of prostate cancer death in the screened arm was noted in the “core” screening group. The trial found that 570 men (95% confidence interval 380–1137 men) would need to be invited to screening, and 18 cases of prostate cancer detected, to avert 1 death from prostate cancer. There was an unexplained imbalance in treatment between the two study arms, with a higher proportion of men with clinically localized cancer receiving radical prostatectomy in the screening arm and receiving it at experienced referral centers.

Screening must be linked to effective therapy in order to have any benefit. Two trials conducted after the initiation of widespread PSA testing did not find a substantial decrease in prostate cancer deaths in control arms of “watchful waiting” or monitoring (i.e., no curative treatment) compared to radical prostatectomy or radiation therapy. Prostate cancer-specific survival was very good (about 99%) and nearly identical at a median follow-up of 10 years. Treatments for low-stage prostate cancer, such as surgery and radiation therapy, can cause substantial morbidity, including impotence and urinary incontinence.

SKIN CANCER Visual examination of all skin surfaces by the patient or by a health care provider is used in screening for basal and squamous cell cancers and melanoma. No prospective randomized study has been performed to look for a mortality decrease. Unfortunately, screening is associated with a substantial rate of overdiagnosis.

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CANCER IS A GENETIC DISEASE

Cancer arises through a series of somatic alterations in DNA that result in unrestrained cellular proliferation. Most of these alterations involve subtle sequence changes in DNA (i.e., mutations). The somatic mutations may originate as a consequence of random replication errors or exposure to carcinogens (e.g., radiation) and can be exacerbated by faulty DNA repair processes. While most cancers arise sporadically, clustering of cancers occurs in families that carry a germline mutation in a cancer gene.

HISTORICAL PERSPECTIVE

The idea that cancer progression is driven by sequential somatic mutations in specific genes has only gained general acceptance in the past 30 years. Before the advent of the microscope, cancer was believed to be composed of aggregates of mucus or other noncellular matter. By the middle of the nineteenth century, it became clear that tumors were masses of cells and that these cells arose from the normal cells of the tissue from which the cancer originated. The molecular basis for the uncontrolled proliferation of cancer cells was to remain a mystery for another century. During that time, a number of theories for the origin of cancer were postulated. The great biochemist Otto Warburg proposed the combustion theory of cancer, which stipulated that cancer was due to abnormal oxygen metabolism. Others believed that all cancers were caused by viruses and that cancer was in fact a contagious disease.

In the end, observations of cancer occurring in chimney sweeps, studies of x-rays, and the overwhelming data demonstrating cigarette smoke as a causative agent in lung cancer, together with Ames's work on chemical mutagenesis, were consistent with the idea that cancer originated through changes in DNA. However, it was not until the somatic mutations responsible for cancer were identified at the molecular level that the genetic basis of cancer was definitively established. Although the viral theory of cancer did not prove to be generally accurate (with exceptions such as human papillomaviruses, which can cause cervical and other cancers), the study of retroviruses led to the discovery of the first human *oncogenes* in the late 1970s. Oncogenes are one of the two major classes of cancer driver genes. The study of families with genetic predisposition to cancer was instrumental to the discovery of the other major class of cancer driver genes, called *tumor-suppressor genes*. Current technologies permit the sequence analysis of entire cancer genomes and provide a comprehensive view of the genetic changes that cause tumors to arise and become malignant. The field that studies the various types of mutations, as well as the consequences of these mutations in tumor cells, is now known as *cancer genetics*.

THE CLONAL ORIGIN AND MULTISTEP NATURE OF CANCER

Nearly all cancers originate from a single cell; this clonal origin is a critical discriminating feature between neoplasia and hyperplasia. Multiple cumulative mutational events are invariably required for the progression of a tumor from normal to fully malignant phenotype. The process can be seen as Darwinian microevolution in which, at each successive step, the mutated cells gain a growth advantage resulting in the expansion of a neoplastic clone (Fig. 71-1). Based on observations of cancer frequency increases during aging, the epidemiologists Armitage and Doll and Nordling independently proposed that cancer is a result of three discrete cellular changes. Remarkably, this early model has been validated by extensive sequencing of cancer genomes. These studies revealed that just three causal mutations are required for the development of several of the most common cancers. Overall, it is currently believed that most common solid tumors require a minimum of three mutated cancer driver genes (either oncogenes or tumor-suppressor genes) for their development. One or two mutations are

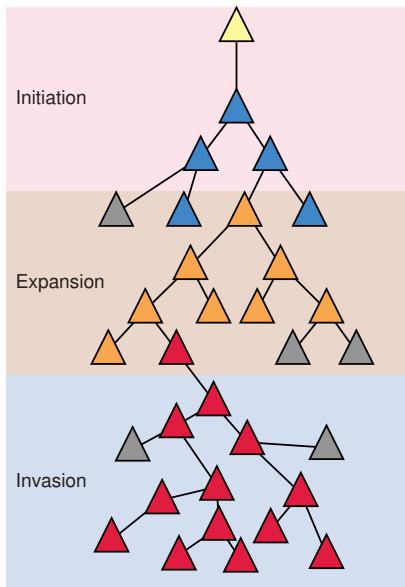


FIGURE 71-1 Multistep clonal development of malignancy. In this diagram, a series of three cumulative mutations, each with a modest growth advantage acting alone, eventually results in a malignant tumor. Note that not all such alterations result in progression. The actual number of cumulative mutations necessary to transform from the normal to the malignant state has been estimated to be three for several of the most common types of cancer. (Adapted and modified from PC Nowell: *The clonal evolution of tumor cell populations*. *Science* 194:23, 1976.)

sufficient for benign tumorigenesis, but not for the invasive capacity that distinguishes cancers from benign tumors. Less common tumors, such as liquid tumors (leukemias or lymphomas), sarcomas, and childhood tumors, appear to require only two driver gene alterations for malignancy. Note that a cancer driver gene is best defined as one containing a mutation that increases the selective growth advantage of the cell containing it. Normally, cell birth and cell death are in perfect equilibrium; every time a cell is born, another in the same lineage dies. Cancer driver gene mutations alter this equilibrium, so that more cells are born than die. The imbalance is often slight, so that the difference between cell birth and cell death can be less than 1%. This explains, in combination with the low rate of mutation, why tumorigenesis—the journey from a normal cell to a typical malignant, solid tumor—often takes decades.

We now know the precise nature of the genetic alterations responsible for nearly all malignancies and are beginning to understand how these alterations promote the distinct stages of tumor growth. The prototypical example is colon cancer, in which analyses of genomes from the entire spectrum of neoplastic growths—from normal colon epithelium through adenoma to carcinoma—have identified mutations that are highly characteristic of each type of lesion (Fig. 71-2).

TWO TYPES OF CANCER GENES: ONCOGENES AND TUMOR SUPPRESSOR GENES

Oncogenes and tumor-suppressor genes exert their effects on tumor growth through their ability to determine cell fates, influence cell survival, and contribute to genome maintenance. The underlying molecular mechanisms can be extremely complex. While tightly regulated in normal cells, oncogenes acquire mutations that typically relieve this control and lead to increased activity of the

gene products. This activating mutational event occurs in a single allele. In contrast, the normal function of tumor-suppressor genes is usually to restrain cell growth, and this function is lost in cancer. Because of the diploid nature of mammalian cells, both alleles must be inactivated for a cell to completely lose the function of a tumor-suppressor gene. Thus, it requires two genetic events to inactivate a tumor-suppressor gene mutation, while only one genetic event is required to activate an oncogene.

A subset of tumor-suppressor genes controls the ability of the cell to maintain the integrity of its genome. Cells with a deficiency in these genes acquire an increased number of mutations throughout their genomes, including those in oncogenes and tumor-suppressor genes. This “mutator” phenotype was first hypothesized by Loeb to explain how the multiple rare mutational events required for tumorigenesis can occur in the lifetime of an individual. A mutator phenotype underlies several forms of cancer, such as those associated with deficiencies in DNA mismatch repair. The great majority of cancers do not harbor repair deficiencies, and their rate of mutation is similar to that observed in normal cells. Many of these cancers, however, appear to harbor a different kind of genetic instability, affecting the loss or gains of whole chromosomes or large parts thereof (as explained in more detail below).

ONCOGENES IN HUMAN CANCER

Work by Peyton Rous in the early 1900s revealed that a chicken sarcoma could be transmitted from animal to animal in cell-free extracts, suggesting that cancer could be induced by an agent acting positively to promote tumor formation. The agent responsible for the transmission of the cancer was a retrovirus (Rous sarcoma virus [RSV]), and the oncogene responsible was identified 75 years later as *V-SRC*. Other oncogenes were also discovered through their presence in the genomes of retroviruses that are capable of causing cancers in chickens, mice, and rats. The nonmutated cellular homologues of these viral genes are called proto-oncogenes and are often targets of mutation or aberrant regulation in human cancer. Whereas many oncogenes were discovered on the basis of their presence in retroviruses, other oncogenes, particularly those involved in translocations characteristic of particular leukemias and lymphomas, were identified through genomic approaches. Investigators cloned the sequences surrounding the chromosomal translocations observed cytogenetically and identified the genes activated at the breakpoints (see below). Some of these were oncogenes previously found in retroviruses (like *ABL*, involved in chronic myeloid leukemia [CML]), whereas others were new (like *BCL2*, involved in B-cell lymphoma). In the normal cellular environment, proto-oncogenes have crucial roles in cell proliferation and

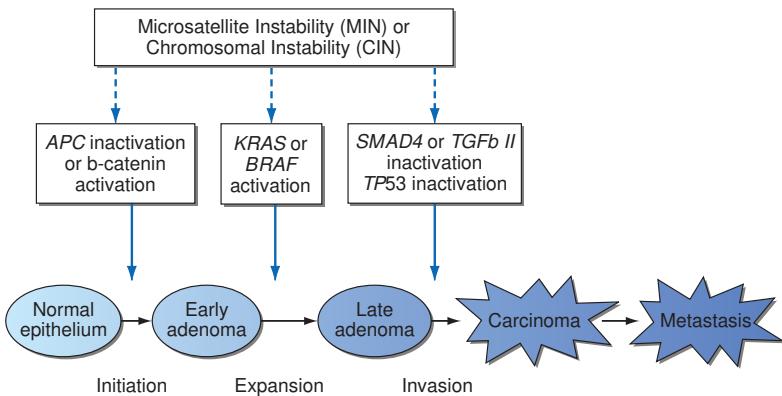


FIGURE 71-2 Progressive somatic mutational steps in the development of colon carcinoma. The accumulation of alterations in a number of different genes results in the progression from normal epithelium through adenoma to full-blown carcinoma. Genetic instability (microsatellite or chromosomal) accelerates the progression by increasing the likelihood of mutation at each step. Patients with familial polyposis are already one step into this pathway, because they inherit a germline alteration of the *APC* gene. TGF, transforming growth factor.

TABLE 71-1 Oncogenes Commonly Altered in Human Cancers

ONCOGENE	FUNCTION	ALTERATION IN CANCER	NEOPLASM
<i>AKT1</i>	Serine/threonine kinase	Point mutation	Skin
<i>BRAF</i>	Serine/threonine kinase	Point mutation	Melanoma, thyroid, colorectal
<i>CCND1</i>	Cell cycle progression	Amplification	Esophageal, head and neck
<i>CTNNB1</i>	Signal transduction	Point mutation	Colon, liver, uterine, melanoma
<i>EGFR</i>	Signal transduction	Point mutation	Lung
<i>FLT3</i>	Signal transduction	Point mutation	AML
<i>IDH1</i>	Chromatin modification	Point mutation	Glioma
<i>MDM2</i>	Inhibitor of p53	Amplification	Sarcoma, glioma
<i>MDM4</i>	Inhibitor of p53	Amplification	Breast
<i>MYC</i>	Transcription factor	Amplification	Prostate, ovarian, breast, liver, pancreatic
<i>MYCL1</i>	Transcription factor	Amplification	Ovarian, bladder
<i>MYCN</i>	Transcription factor	Amplification	Neuroblastoma
<i>PIK3CA</i>	Phosphoinositol-3-kinase	Point mutation	Multiple cancers
<i>KRAS</i>	GTPase	Point mutation	Pancreatic, colorectal, lung
<i>NRAS</i>	GTPase	Point mutation	Melanoma

Abbreviation: AML, acute myeloid leukemia.

differentiation. Table 71-1 is a partial list of oncogenes known to be involved in human cancer.

The normal growth and differentiation of cells is controlled by growth factors that bind to receptors on the surface of the cell. The signals generated by the membrane receptors are transmitted inside the cells through signaling cascades involving kinases, G proteins, and other regulatory proteins. Ultimately, these signals affect the activity of transcription factors in the nucleus, which regulate the expression of genes crucial in cell proliferation, cell differentiation, and cell death. Oncogene products function at critical steps in these signaling pathways (Chap. 72). Inappropriate activation of these pathways can lead to tumorigenesis.

MECHANISMS OF ONCOGENE ACTIVATION

POINT MUTATION

Point mutation (alternatively known as single nucleotide substitution) is a common mechanism of oncogene activation. For example, mutations in *KRAS* are present in >95% of pancreatic cancers and 40% of colon cancers. Activating *KRAS* mutations are less common in other cancer types, although they can occur at significant frequencies in leukemia, lung, and thyroid cancers. Remarkably—and in contrast to the diversity of mutations found in tumor-suppressor genes—most of the activated *KRAS* alleles contain point mutations in codons 12, 13, or 61. These mutations lead to constitutive activation of the mutant RAS protein. The restricted pattern of mutations observed in oncogenes compared to that of tumor-suppressor genes reflects the fact that gain-of-function mutations must occur at specific sites, while a broad variety of mutations can lead to loss of activity. Indeed, inactivation of a gene can in theory be accomplished through the introduction of a stop codon anywhere in the coding sequence, whereas activations require precise substitutions at residues that can somehow lead to an increase in the activity of the encoded protein under particular circumstances within the cell.

DNA AMPLIFICATION

The second mechanism for activation of oncogenes is DNA sequence amplification, leading to overexpression of the gene product. This increase in DNA copy number may cause cytologically recognizable chromosome alterations referred to as *homogeneous staining regions* (HSRs) if integrated within chromosomes, or *double minutes* (dmins) if extrachromosomal. With microarray and DNA sequencing technologies, the entire genome can be surveyed for gains and losses of DNA sequences, thus pinpointing chromosomal regions likely to contain genes important in the development or progression of cancer.

Numerous genes have been reported to be amplified in cancer. Several of these genes, including *NMYC* and *LMYC*, were identified

through their presence within the amplified DNA sequences of a tumor and their homology to known oncogenes. Because amplified regions often include hundreds of thousands of base pairs, multiple oncogenes may be amplified in a single amplicon in some cancers. For example, *MDM2*, *GLI1*, *CDK4*, and *TPSPAN31* at chromosomal location 12q13-15 have been shown to be co-amplified in several types of sarcomas and other tumors; which of these genes play the causal role in the neoplastic process is still an active area of research. Amplification of a cellular gene is often a predictor of poor prognosis; for example, *ERBB2/HER2* and *NMYC* are often amplified in aggressive breast cancers and neuroblastoma, respectively.

CHROMOSOMAL REARRANGEMENT

Chromosomal alterations provide important clues to the genetic changes in cancer. The chromosomal alterations in human solid tumors such as carcinomas are heterogeneous

and complex and occur as a result of the frequent chromosomal instability observed in these tumors (see below). In contrast, the chromosome alterations in myeloid and lymphoid tumors are often simple translocations, that is, reciprocal transfers of chromosome arms from one chromosome to another. The breakpoints of recurring chromosome abnormalities usually occur at the site of cellular oncogenes. Table 71-2 lists representative examples of recurring chromosome alterations in malignancy and the associated gene(s) rearranged or deregulated by the chromosomal rearrangement. Translocations are often observed in liquid tumors in general and are particularly common in lymphoid tumors, probably because these cell types have the capability to rearrange their DNA to generate antigen receptors. Indeed, antigen receptor genes are commonly involved in the translocations, implying that an imperfect regulation of receptor gene rearrangement may be involved in their pathogenesis. In addition to transcription factors and signal transduction molecules, translocation may result in the overexpression of cell cycle regulatory proteins or proteins such as cyclins and of proteins that regulate cell death. Recurrent translocations have more recently been identified in solid tumors such as prostate cancers. For example, fusions between *TMPRSS2* and *ERG*, which are normally located in tandem on chromosome 21, contribute to more than one-third of all prostate cancers.

The first reproducible chromosome abnormality detected in human malignancy was the Philadelphia chromosome detected in CML.

TABLE 71-2 Representative Oncogenes at Chromosomal Translocations

GENE (CHROMOSOME)	TRANSLOCATION	MALIGNANCY
<i>BCR-ABL</i>	(9;22)(q34;q11)	Chronic myeloid leukemia
<i>BCL1</i> (11q13.3)- <i>IgH</i> (14q32)	(11;14)(q13;q32)	Mantle cell lymphoma
<i>BCL2</i> (18q21.3)- <i>IgH</i> (14q32)	(14;18)(q32;q21)	Follicular lymphoma
<i>FLI-EWSR1</i>	(11;22)(q24;q12)	Ewing's sarcoma
<i>LCK-TORB</i>	(1;7)(p34;q35)	T-cell acute lymphocytic leukemia
<i>PAX3-FOXO1</i>	(2;13)(q35;q14)	Rhabdomyosarcoma
<i>PAX8-PPARG</i>	(2;3)(q13;p25)	Thyroid
<i>IL21R-BCL6</i>	(3;16)(q27;p11)	Non-Hodgkin's lymphoma
<i>TAL1-TCTA</i>	(1;3)(p34;p21)	Acute T-cell leukemia
<i>TMPRSS2-ERG</i>	Peararrangement on Chr21q22	Prostate

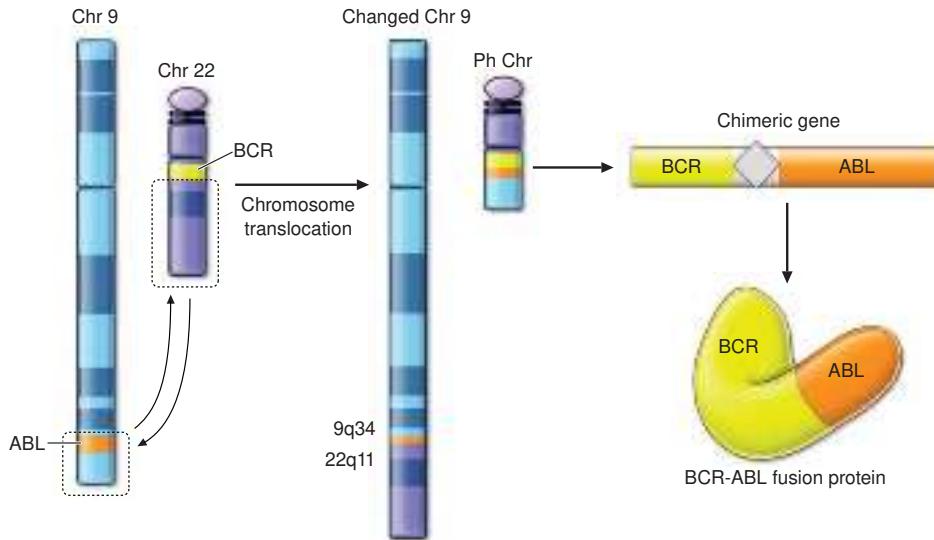


FIGURE 71-3 Specific translocation seen in chronic myeloid leukemia (CML). The Philadelphia chromosome (Ph) is derived from a reciprocal translocation between chromosomes 9 and 22 with the breakpoint joining the sequences of the *ABL* oncogene with the *BCR* gene. The fusion of these DNA sequences allows the generation of an entirely novel fusion protein with modified function.

This cytogenetic abnormality is generated by reciprocal translocation involving the *ABL* oncogene on chromosome 9, encoding a tyrosine kinase, being placed in proximity to the breakpoint cluster region (*BCR*) gene on chromosome 22. Figure 71-3 illustrates the generation of the translocation and its protein product. The consequence of expression of the *BCR-ABL* gene product is the activation of signal transduction pathways leading to cell growth independent of normal external signals. Imatinib (marketed as Gleevec), a drug that specifically blocks the activity of Abl tyrosine kinase, has shown remarkable efficacy with little toxicity in patients with CML. The successful targeting of *BCR-ABL* by imatinib is the paradigm for molecularly targeted anticancer therapies.

CHROMOSOMAL INSTABILITY IN SOLID TUMORS

Solid tumors generally contain an abnormal number of chromosomes, a state known as aneuploidy; chromosomes from aneuploid tumors exhibit structural alterations such as translocations, deletions, and amplifications. These abnormalities reflect an underlying defect in cancer cells known as chromosomal instability. While aneuploidy is a striking cellular phenotype, chromosomal instability is manifest as only a small increase in the tendency of cells to gain, lose, or rearrange chromosomes during any given cell cycle. This intrinsically low rate of chromosome aberration implies that cancer cells become aneuploid only after many generations of clonal expansion. The molecular basis of aneuploidy remains incompletely understood. It is widely believed that defects in checkpoints, the quality-control mechanisms that halt the cell cycle if chromosomes are damaged or misaligned, contribute to chromosomal instability. This hypothesis emerged from experimental observations that the tumor suppressor p53 controls checkpoints that regulate the initiation of DNA replication and the onset of mitosis. These processes are therefore defective in many cancer cells. The mitotic spindle checkpoint, which ensures proper chromosome attachment to the mitotic spindle before allowing the sister chromatids to separate, is also altered in some cancers, irrespective of p53 status. The precise relationship between checkpoint deficiency, p53, and chromosomal instability remains unclear, but it is believed that even a subtle perturbation of the highly orchestrated process of cell division can impact the ability of a cell to faithfully replicate and segregate its complement of chromosomes. From a therapeutic standpoint, the checkpoint defects that are prevalent in cancers have been proposed as

vulnerabilities that may be exploited by novel agents and combinatorial strategies.

In contrast to the genome-wide cytogenetic changes that are typical indications of an underlying chromosomal instability, more focal patterns of chromosomal rearrangement have been recurrently detected in several cancer types. A curious phenomenon known as *chromothripsis* causes dozens of distinct breakpoints that are localized on one or several chromosomes. These striking structural alterations are thought to reflect a single event in which a chromosome is fragmented and then imprecisely reassembled. While the exact process that underlies chromothripsis remains obscure and its effects on driver genes are not yet clear, a transient period of extreme instability stands in contrast to the gradual loss, gain, and rearrangement of chromosomes that are typically observed in serially cultured cancer cells.

TUMOR SUPPRESSOR GENE INACTIVATION IN CANCER

The first functional evidence for tumor-suppressor genes came from experiments showing that fusion of mouse cancer cells with normal mouse fibroblasts led to a nontumorigenic phenotype in the fused cells. The normal role of tumor-suppressor genes is to restrain cell growth, and the function of these genes is inactivated in cancer. The three major types of somatic lesions observed in tumor-suppressor genes during tumor development are *point mutations*, small insertions and/or deletions known as *indels*, and *large deletions*. Point mutations or indels in the coding region of tumor-suppressor genes will frequently lead to truncated protein products or allele-specific loss of RNA expression by the process of *nonsense-mediated decay*. Unlike the highly recurrent point mutations that are found in critical positions of activated oncogenes, known as mutational *hotspots*, the point mutations that cause tumor-suppressor gene inactivation tend to be distributed throughout the open reading frame. Large deletions lead to the loss of a functional product and sometimes encompass the entire gene or even the entire chromosome arm, leading to loss of heterozygosity (LOH) in the tumor DNA compared to the corresponding normal tissue DNA (Fig. 71-4). LOH in tumor DNA often indicates the presence of a tumor-suppressor gene at a particular chromosomal location, and LOH studies have been useful in the positional cloning of many tumor-suppressor genes. The rate of LOH is increased in the presence of chromosomal instability, a relationship that would explain the selective forces leading to the high prevalence of aneuploidy in late-stage cancers.

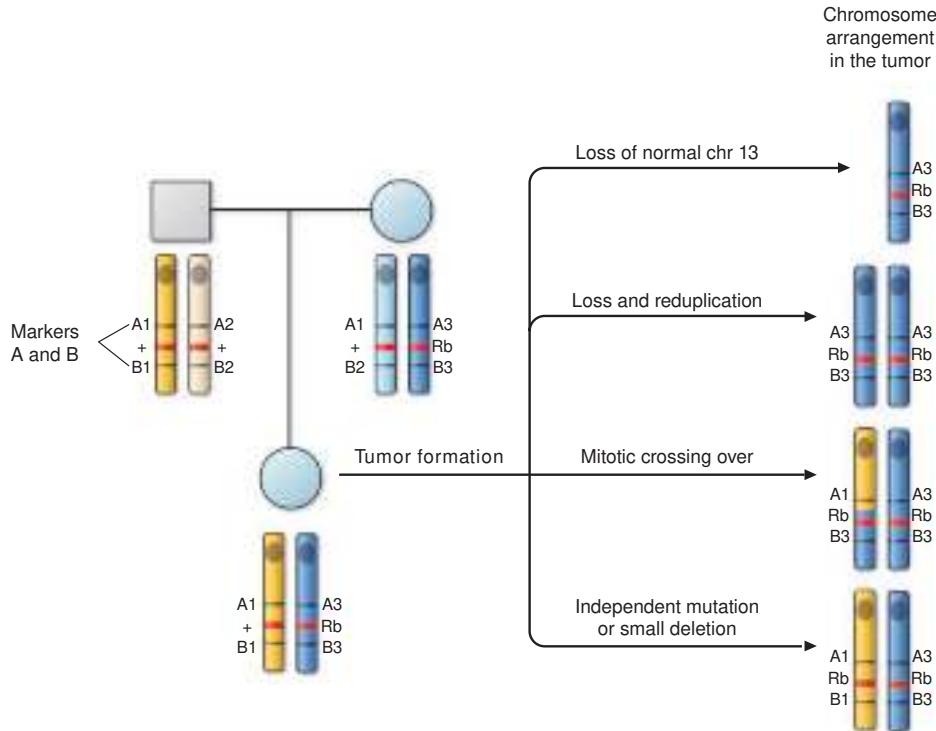


FIGURE 71-4 Diagram of possible mechanisms for tumor formation in an individual with hereditary (familial) retinoblastoma. On the left is shown the pedigree of an affected individual who has inherited the abnormal (*Rb*) allele from her affected mother. The normal allele is shown as a (+). The four chromosomes of her two parents are drawn to indicate their origin. Flanking the retinoblastoma locus are genetic markers (A and B) also analyzed in this family. Markers A3 and B3 are on the chromosome carrying the retinoblastoma disease gene. Tumor formation results when the normal allele, which this patient inherited from her father, is inactivated. On the right are shown four possible ways in which this could occur. In each case, the resulting chromosome 13 arrangement is shown. Note that in the first three situations, the normal allele (*B1*) has been lost in the tumor tissue, which is referred to as loss of heterozygosity (LOH) at this locus.

Gene silencing, an epigenetic change that leads to the loss of gene expression, occurs in conjunction with hypermethylation of the promoter and histone deacetylation, and is another mechanism of tumor-suppressor gene inactivation. An *epigenetic modification* refers to a covalent modification of chromatin, heritable by cell progeny that may involve DNA but does not involve a change in the DNA sequence. The inactivation of the second X chromosome in female cells is a physiologic example of an epigenetic silencing that prevents gene expression from the inactivated chromosome. Genomic regions of hypermethylated and hypomethylated DNA can be detected by specialized techniques, and a subset of these regional modifications has consequences on the cell's behavior.

FAMILIAL CANCER SYNDROMES

A small fraction of cancers occurs in patients with a genetic predisposition. Based on studies of inherited and sporadic forms of retinoblastoma, Knudson and others formulated a hypothesis that explains the differences between sporadic and inherited forms of the same tumor type. In inherited forms of cancer, called *cancer predisposition syndromes*, one allele of a particular tumor-suppressor gene is inherited in mutant form. This germline mutation is not sufficient to initiate a tumor, however; the other allele, inherited from the unaffected parent, must become somatically inactivated in a normal stem cell for tumorigenesis to be initiated. In sporadic (noninherited) forms of the same disease, all cells in the body start out with two normal copies of the tumor-suppressor gene. A single cell must then sequentially acquire mutations in both alleles of the tumor-suppressor gene to initiate a tumor. Thus, biallelic mutations of the same tumor-suppressor gene are required for both inherited and noninherited forms of the disease; the only difference is that individuals with the inherited form have a "head start": they already have one allele mutated, from conception, and only need one additional mutation to initiate the process (Fig. 71-4).

This distinction explains why those with inherited forms of the disease develop more cancers, at an earlier age, than the general population. It also explains why, even though every cell in an individual with a cancer predisposition syndrome has a mutant gene, only a relatively small number of tumors arise during his or her lifetime. The reason is that the vast majority of cells within such individuals are functionally normal because one of the two alleles of the tumor-suppressor gene is normal. Mutations are uncommon events, and only the rare cells that develop a mutation in the remaining normal allele will exhibit uncontrolled proliferation. The same principle applies to virtually all types of cancer predisposition syndromes, though the particular genes differ. For example, inherited mutations in *RBL*, *WT1*, *VHL*, *APC*, and *BRCA1* lead to predispositions to retinoblastomas, Wilms' tumors, renal cell carcinomas, colorectal carcinomas, and breast carcinomas, respectively (Table 71-3). Also note that the biallelic inactivation of any of these genes is not sufficient to develop cancer; it requires other, additional somatic alterations in other genes for the initiating cells to evolve to malignancy, as noted above.

Roughly 100 familial cancer syndromes have been reported; the great majority are very rare. Most of these syndromes exhibit an autosomal dominant pattern of inheritance, although some of those associated with DNA repair abnormalities (xeroderma pigmentosum, Fanconi's anemia, ataxia telangiectasia) are inherited in an autosomal recessive fashion. Table 71-3 shows a number of cancer predisposition syndromes and the responsible genes.

The next section examines inherited colon cancer predispositions in detail because several lessons of general importance have been derived from the study of these syndromes.

Familial adenomatous polyposis (FAP) is a dominantly inherited colon cancer syndrome caused by germline mutations in the adenomatous polyposis coli (*APC*) tumor-suppressor gene on chromosome 5. Affected individuals develop hundreds to thousands of adenomas in

TABLE 71-3 Cancer Predisposition Syndromes and Associated Genes

SYNDROME	GENE	CHROMOSOME	INHERITANCE	TUMORS
Ataxia telangiectasia	ATM	11q22-q23	AR	Breast
Autoimmune lymphoproliferative syndrome	FAS FASL	10q24 1q23	AD	Lymphomas
Bloom's syndrome	BLM	15q26.1	AR	Various
Cowden's syndrome	PTEN	10q23	AD	Breast, thyroid
Familial adenomatous polyposis	APC MUTYH	5q21 1p34.1	AD AR	Colorectal (early onset)
Familial melanoma	CDKN2A	9p21	AD	Melanoma, pancreatic
Familial Wilms' tumor	WT1	11p13	AD	Kidney (pediatric)
Heredity breast/ovarian cancer	BRCA1 BRCA2	17q21 13q12.3	AD	Breast, ovarian, prostate
Heredity diffuse gastric cancer	CDH1	16q22	AD	Stomach
Heredity multiple exostoses	EXT1 EXT2	8q24 11p11-12	AD	Exostoses, chondrosarcoma
Heredity retinoblastoma	RB1	13q14.2	AD	Retinoblastoma, osteosarcoma
Heredity nonpolyposis colon cancer (HNPCC)	MSH2 MLH1 MSH6 PMS2	2p16 3p21.3 2p16 7p22	AD	Colon, endometrial, ovarian, stomach, small bowel, ureter carcinoma
Heredity papillary renal carcinoma	MET	7q31	AD	Papillary kidney
Juvenile polyposis syndrome	SMAD4 BMPR1A	18q21	AD	Gastrointestinal, pancreatic
Li-Fraumeni syndrome	TP53	17p13.1	AD	Sarcoma, breast
Multiple endocrine neoplasia type 1	MEN1	11q13	AD	Parathyroid, endocrine, pancreas, and pituitary
Multiple endocrine neoplasia type 2a	RET	10q11.2	AD	Medullary thyroid carcinoma, pheochromocytoma
Neurofibromatosis type 1	NF1	17q11.2	AD	Neurofibroma, neurofibrosarcoma, brain
Neurofibromatosis type 2	NF2	22q12.2	AD	Vestibular schwannoma, meningioma, spine
Nevoid basal cell carcinoma syndrome (Gorlin's syndrome)	PTCH1	9q22.3	AD	Basal cell carcinoma, medulloblastoma, jaw cysts
Tuberous sclerosis	TSC1 TSC2	9q34 16p13.3	AD	Angiofibroma, renal angiomyolipoma
von Hippel–Lindau disease	VHL	3p25-26	AD	Kidney, cerebellum, pheochromocytoma

Abbreviations: AD, autosomal dominant; AR, autosomal recessive.

the colon. In each of these adenomas, the *APC* allele inherited from the affected parent has been inactivated by virtue of a somatic mutation (Fig. 71-2). This inactivation usually occurs through a gross chromosomal event resulting in loss of all or a large part of the long arm of chromosome 5, where *APC* resides. In other cases, the remaining allele is inactivated by a subtle intragenic mutation of *APC*, which is typically a single base substitution resulting in a nonsense codon. Gross chromosomal losses occur more commonly than point mutations in normal cells, explaining why chromosomal loss rather than point mutation is the predominant mechanism underlying the inactivation of the normal allele of *APC*. The same is true for other cancer predisposition syndromes caused by other inherited tumor suppressor gene mutations; gross chromosomal events are generally responsible for inactivation of the tumor-suppressor gene allele inherited from the nonaffected parent. Several thousand adenomas form in FAP patients, and a small subset of the millions of cells within an adenoma will acquire a second mutation, leading to tumor progression, that is, a larger adenoma. A third mutation in such a larger adenoma may convert it to a carcinoma. If untreated (by colectomy), at least one of the adenomas will progress to cancer by the time patients are in their mid-40s. *APC* can be considered to be a gatekeeper for colon tumorigenesis in that in the absence of mutation of this gatekeeper (or a gene acting within the same pathway), a colorectal tumor simply cannot be initiated. Figure 71-5 shows the germline and somatic mutations found in the *APC* gene. A negative regulator of a signaling pathway that determines cell fate during development, the *APC* protein provides differentiation and apoptotic cues

to colonic epithelial cells as they migrate up the crypt. Defects in this process can lead to abnormal accumulation of cells that would otherwise differentiate and eventually undergo apoptosis.

In contrast to patients with FAP, patients with hereditary nonpolyposis colon cancer (HNPCC, or Lynch's syndrome) do not develop polyposis, but instead develop only one or a small number of adenomas that rapidly progress to cancer. HNPCC is due to inherited mutations in one of four DNA mismatch repair genes (Table 71-3) that are components of a repair system responsible for correcting errors in newly replicated DNA. Germline mutations in *MSH2* and *MLH1* account for more than 90% of HNPCC cases, and mutations in *MSH6* and *PMS2* account for the remainder. When a somatic mutation inactivates the remaining wild-type allele of a mismatch repair gene, the cell develops a hypermutable phenotype characterized by profound genomic instability that is most readily apparent in short repeated sequences called *microsatellites* and is sometimes called microsatellite instability (MSI). The high rate of mutation in such cells impacts all genes, including oncogenes and tumor suppressor genes, and thereby accelerates the activation of the former and the inactivation of the latter (Fig. 71-2). HNPCC can be considered a disease of tumor progression; once tumors are initiated (by an inactivating mutation of *APC* or by some other gene in the *APC* pathway), tumors rapidly progress because of the accelerated mutation rate. Progression from a tiny adenoma to carcinoma takes only a few years in HNPCC patients instead of the two or three decades this progression takes in patients with FAP (or in patients with sporadic colorectal tumors). Approximately half of

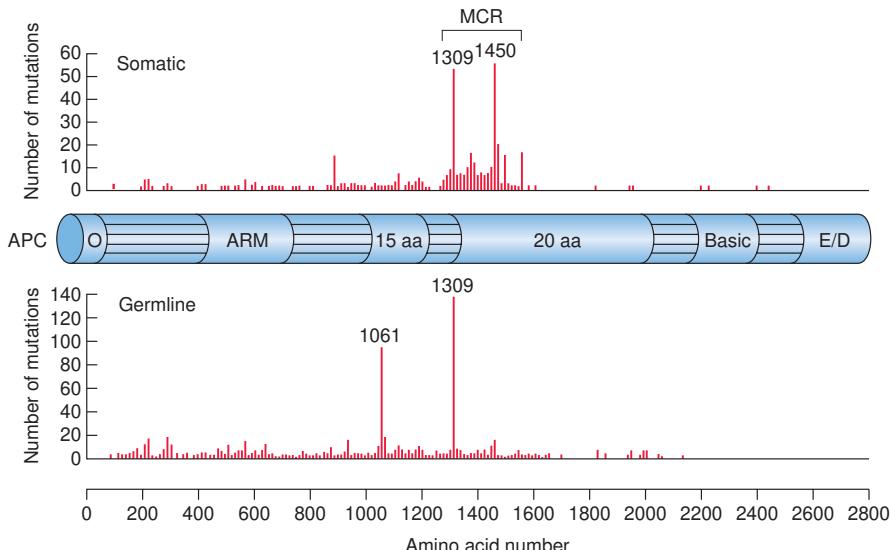


FIGURE 71-5 Germline and somatic mutations in the tumor-suppressor gene adenomatous polyposis coli (*APC*). *APC* encodes a 2843-amino-acid protein with six major domains: an oligomerization region (O), armadillo repeats (ARM), 15-amino-acid repeats (15 aa), 20-amino-acid repeats (20 aa), a basic region, and a domain involved in binding EB1 and the *Drosophila* discs large homologue (E/D). Shown are 650 somatic and 826 germline mutations representative of the mutations that occur within the *APC* gene (from the *APC* database at www.umd.be/APC). All known pathogenic mutations of *APC* result in the truncation of the *APC* protein. Germline mutations are found to be relatively evenly distributed up to codon 1600 except for two mutation hotspots surrounding amino acids 1061 and 1309, which together account for one-third of the mutations found in familial adenomatous polyposis (FAP) families.

HNPCC patients develop colorectal cancers by the time they are in their mid-40s—similar to that of FAP patients. This coincidence in age of onset emphasizes that both tumor initiation (abnormal in FAP patients) and tumor progression (abnormal in HNPCC patients) are the two pillars of cancer development and are equally important for cancer development.

Another general principle is apparent from the comparison between FAP and HNPCC patients. The tumors in FAP patients, like those in patients without hereditary predisposition to cancers, exhibit chromosomal instability rather than MSI. Indeed, MSI and chromosomal instability tend to be mutually exclusive in colon cancers, suggesting that they represent alternative mechanisms for the generation of genomic instability (Fig. 71-2). Other cancer types rarely exhibit MSI. Chromosomal instability is far more prevalent than MSI among all cancer types, perhaps explaining why nearly all cancers are aneuploid.

Although most autosomal dominant inherited cancer syndromes are due to mutations in tumor-suppressor genes (Table 71-3), there are a few interesting exceptions. Multiple endocrine neoplasia type 2, a dominant disorder characterized by pituitary adenomas, medullary carcinoma of the thyroid, and (in some pedigrees) pheochromocytoma, is due to gain-of-function mutations in the proto-oncogene *RET* on chromosome 10. Similarly, gain-of-function mutations in the tyrosine kinase domain of the *MET* oncogene lead to hereditary papillary renal carcinoma. Interestingly, loss-of-function mutations in the *RET* gene cause a completely different disease, Hirschsprung's disease (aganglionic megacolon [Chaps. 328 and 388]).

Although the heritable forms of cancer have taught us much about the mechanisms of growth control, most forms of cancer do not follow simple Mendelian patterns of inheritance. The majority of human cancers arise in a sporadic fashion, solely as a result of somatic mutation, and in the absence of any mutations in cancer-predisposing genes in their germlines.

GENETIC TESTING FOR FAMILIAL CANCER

The discovery of cancer susceptibility genes raises the possibility of DNA testing to predict the risk of cancer in individuals of affected families. An algorithm for cancer risk assessment and decision making

in high-risk families using genetic testing is shown in Fig. 71-6. Once a mutation is discovered in a family, subsequent testing of asymptomatic family members can be crucial in patient management. A negative gene test in these individuals can prevent years of anxiety, providing comfort in the knowledge that their cancer risk is no higher than that of the general population. On the other hand, a positive test may lead to alteration of clinical management, such as increased frequency of cancer screening and, when feasible and appropriate, prophylactic surgery. Potential negative consequences of a positive test result include psychological distress (anxiety, depression) and discrimination, although the Genetic Information Nondiscrimination Act (GINA) makes it illegal for predictive genetic information to be used to discriminate in health insurance or employment. Testing should therefore not be conducted without counseling before testing is administered and during and after disclosure of the test result.

It is now feasible to obtain high-quality sequence of all of the protein-coding DNA sequences, and even of the entire genome, in any given individual. In such studies, numerous variants in DNA sequences will inevitably be identified in every subject, but the significance of the vast majority of these DNA sequence findings will be unclear. Even mutations in tumor-suppressor genes will be difficult to interpret unless there is an obvious functional implication, such as the truncation of the open reading frame, or that particular mutation has previously been correlated with cancer in other individuals. Germline mutations associated with cancer predisposition are uncommon in individuals without a family history of cancer, though they do occur. Much more common are *variants of unknown significance* (VUS). VUS that are found during genetic testing cannot be used to evaluate the relative risk of cancer but may nonetheless cause anxiety because they represent a deviation from the reference allele that is established as “normal.” Because of the low yield of informative mutations that modify cancer risk and the frequent identification of VUS, it is generally not appropriate to use DNA sequencing to assess cancer risk in individuals without a family history of cancer. However, there are exceptions: *testing may be appropriate in some subpopulations with a known increased risk, even without a personal family history*. For example, two mutations in the breast cancer susceptibility gene *BRCA1*, 185delAG and 5382insC, exhibit a sufficiently high frequency in the Ashkenazi

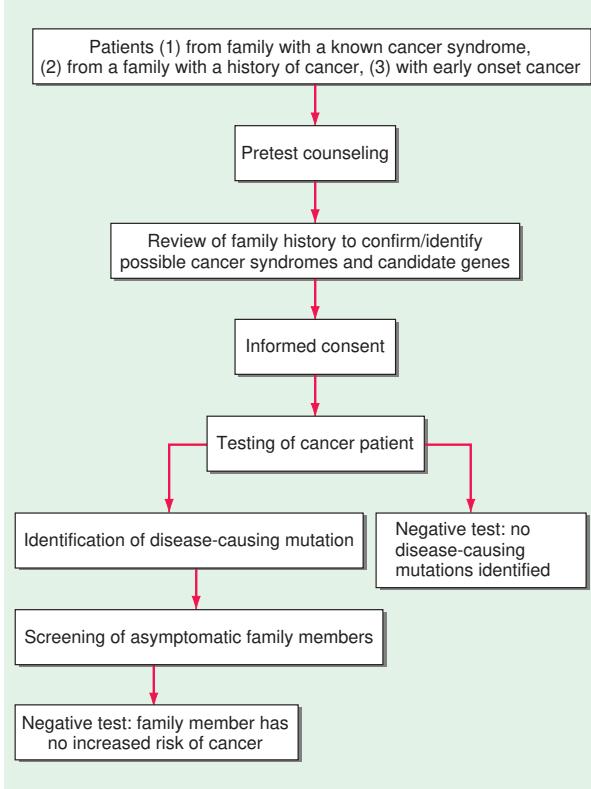


FIGURE 71-6 Algorithm for genetic testing in a family with cancer predisposition. The key step is the identification of a disease mutation in a cancer patient, which is an indication for the testing of asymptomatic family members. Asymptomatic family members who test positive may require increased screening or surgery, whereas those who test negative are at no greater risk for cancer than the general population. It should be emphasized that no molecular assay used for this sort of testing is 100% sensitive; negative results must be interpreted with this caveat in mind.

Jewish population that genetic testing based on ethnicity alone may be warranted.

It is important that genetic test results be communicated to families by trained genetic counselors. To ensure that the families clearly understand its advantages and disadvantages and the impact it may have on disease management and psyche, genetic testing should never be done before counseling. Significant expertise is needed to communicate the results of genetic testing to families.

VIRUSES IN HUMAN CANCER

Several human malignancies are associated with viruses. Examples include Burkitt's lymphoma (Epstein-Barr virus; **Chap. 194**), hepatocellular carcinoma (hepatitis viruses), cervical cancer (human papillomavirus [HPV]; **Chap. 198**), and T-cell leukemia (retroviruses; **Chap. 201**). There are several types of HPV, including the high-risk types 16 and 18 that are strongly associated with the development of cervical, vulvar, vaginal, penile, anal, and oropharyngeal cancer. The mechanisms of action of all these viruses involve inactivation of tumor-suppressor genes. For example, HPV proteins E6 and E7 bind to and inactivate cellular tumor suppressors p53 and pRB, respectively. This is the reason that HPV is such a potent initiator of cancer: infection with a virus is tantamount to having two of the three mutant driver genes required for cancer, that is, one viral oncogene inactivates p53 and the other inactivates Rb. Once these two inactivated gene products initiate tumorigenesis, only one additional mutant gene is required for these tumors to progress to malignancy.

CANCER GENOMES

The advent of relatively inexpensive technologies for rapid and high-throughput DNA sequencing has facilitated the comprehensive analysis of numerous genomes from many types of tumors. This unprecedented view into the genetic nature of cancer has provided remarkable insights. Most cancers do not arise in the context of a mutator phenotype, and accordingly, the number of mutations in even the most advanced cancers is relatively modest. Common solid tumors harbor 30–70 subtle mutations that are nonsynonymous (i.e., result in an amino acid change in the encoded protein). Liquid tumors such as leukemias, as well as pediatric tumors, typically have fewer than 20 mutations. The vast majority of the mutations detected in tumors are not functionally significant; they simply arose by chance in a single cell that gave rise to an expanding clone. Such mutations, which provide no selective advantage to the cell in which they occur, are known as *passenger* mutations. As noted above, only a small number of the mutations confer a selective growth advantage and thereby promote tumorigenesis. These functional mutations are known as *driver* mutations, and the genes in which they occur are called driver genes.

The frequency and distribution of driver mutations within a single tumor type can be represented as a topographical landscape. The picture that emerges from these studies reveals that most genes that are mutated in tumors are actually mutated at relatively low frequencies, as would be expected of passenger genes, whereas a small number of genes (the driver genes) are mutated in a large proportion of tumors. It appears that there are only a total of ~120 bona fide driver genes contributing to the development of solid tumors of all kinds, though other driver genes that play a role in a small fraction of cancers are still being discovered. The majority of the mutations in driver genes provide a direct selective growth advantage by altering the signaling pathways that mediate cell survival or the determination of cell fate. The remaining driver gene mutations indirectly provide a selective growth advantage by accelerating the mutation rate of proto-oncogenes and tumor-suppressor genes. That the same driver genes play a role in multiple cancer types was unexpected before their discovery and has important implications for the development of new “tumor-agnostic” therapeutic and diagnostic approaches. Moreover, the functions of all these driver genes can be organized into a small number of signaling pathways, as shown in **Table 71-4**.

As a consequence of the mutations they harbor, cancer cells invariably express mutant proteins that are only rarely found in normal cells. Some of these mutant proteins are processed and displayed on the cell surface in the context of major histocompatibility complexes, a process that would normally facilitate their recognition by the adaptive immune system. Thus, all cancers have the theoretical potential to be recognized as foreign, or “nonself,” via the display of these tumor-specific antigens, known as mutation-associated neoantigens (MANAs). In fact, established tumors invariably prevent the activation of local

TABLE 71-4 Signaling Pathways Altered in Cancer

PROCESS	PATHWAY	REPRESENTATIVE DRIVER GENES
Cell survival	Cell cycle regulation/apoptosis	<i>RB1, BCL2</i>
	RAS	<i>KRAS, BRAF</i>
	PIK3CA	<i>PTEN, PIK3CA</i>
	JAK/STAT	<i>JAK2, FLT3</i>
	MAPK	<i>MAP3K, ERK</i>
	TGF-β	<i>BMPR1A, SMAD4</i>
Cell fate	Notch	<i>NOTCH1, FBXW7</i>
	Hedgehog	<i>PTCH1, SMO</i>
	WNT/APC	<i>APC, CTNNB1</i>
	Chromatin modification	<i>DNMT1, IDH1</i>
	Transcriptional regulation	<i>AR, KLF4</i>
Genome maintenance	DNA damage signaling and repair	<i>ATM, BRCA1</i>

T cells by inducing an intercellular suppressive mechanism known as an *immune checkpoint*. Therapeutic approaches to exploit this potential vulnerability by blocking immune checkpoints have elicited striking responses in patients with several types of cancer.

It was hypothesized that the potential immunogenicity of a tumor would be related to the total number of distinctive neoantigens it can express, which in turn is directly determined by the total number of mutations in the cancer genome. This does seem to be the case. Colorectal cancers that develop as a result of mismatch repair deficiency and smoking-related lung cancers, both of which characteristically harbor large numbers of mutations, exhibit more robust responses to therapeutic immune checkpoint blockade than most other tumor types. Notably, driver mutations as well as passenger mutations that result in the expression of mutant proteins can both contribute to the display of immunogenic neoantigens. Thus, the total number of coding mutations, a metric known as *mutational load*, is one of the determinants of potential immunogenicity.

TUMOR HETEROGENEITY

The mutant cells that compose a single tumor are not genetically identical. Rather, cells obtained from different sites on a tumor will harbor common mutations as well as mutations that are unique to each sample. Genetic heterogeneity results from the ongoing acquisition of mutations during tumor growth. Each time a genome is replicated, there is a small but quantifiable probability that a mutation will spontaneously arise as a result of a replication error and be passed on to the cellular progeny. This is true in normal cells or in tumor cells. Any randomly chosen cell from the skin of one individual will harbor hundreds of genetic alterations that distinguish it from a different randomly chosen skin cell, and the same is true for all organs of self-renewing tissues. Tumors are actually *less* genetically heterogeneous than normal tissues; any two randomly chosen cells from a tumor of an individual will have fewer differences than any two randomly chosen cells from that individual's normal tissues. The reason for this decrease in heterogeneity is

clonal expansion, the fundamental feature of tumorigenesis. Every time a clonal expansion occurs, a genetic bottleneck wipes out heterogeneity among the cells that did not expand; these unexpanded cells either die or form only a minute proportion of the total cells in the expanding tumor.

The mutations that vary between cells of a given tumor are invariably passenger mutations that arose since the last evolutionary bottleneck, that is, those mutations that arose during the expansion of the founder cell that gave rise to the final clonal expansion. In contrast, the passenger mutations that were present in the founder cell will be uniformly present in every cell in the tumor. In that respect, these passenger mutations are not heterogeneously distributed and are in fact uniformly present in virtually all cancer cells. These "clonal" mutations, i.e., present in all cells of the cancers, are the main source of MANAs that can be exploited through immune checkpoint inhibitors. The total number of mutations and their distribution within tumor cells represent a complex interplay between the age of the patient (the older the patient, the more passenger mutations will have accumulated in the founding cell of the first clonal expansion) and the evolutionary history of the cancer (its age and number of clonal expansions it experienced).

Tumor heterogeneity has been recognized for decades at the cytogenetic, biochemical, and histopathologic levels. However, it is only recently, with the advent of a deep understanding of cancer genetics, that genetic heterogeneity can be interpreted in a medically relevant fashion. The first important point to recognize about tumor heterogeneity is that it is only the variation in driver gene alterations that is important; the cellular distribution of passenger gene mutations is irrelevant except for immune-related phenomena. In this discussion of heterogeneity, we can expand the definition of "driver genes" to include those that provide a selective growth advantage in the face of therapy in addition to those that provide a selective growth advantage during tumor evolution, prior to treatment.

Type I heterogeneity refers to that among tumors of the same type from different patients (Fig. 71-7). Though adenocarcinomas of the

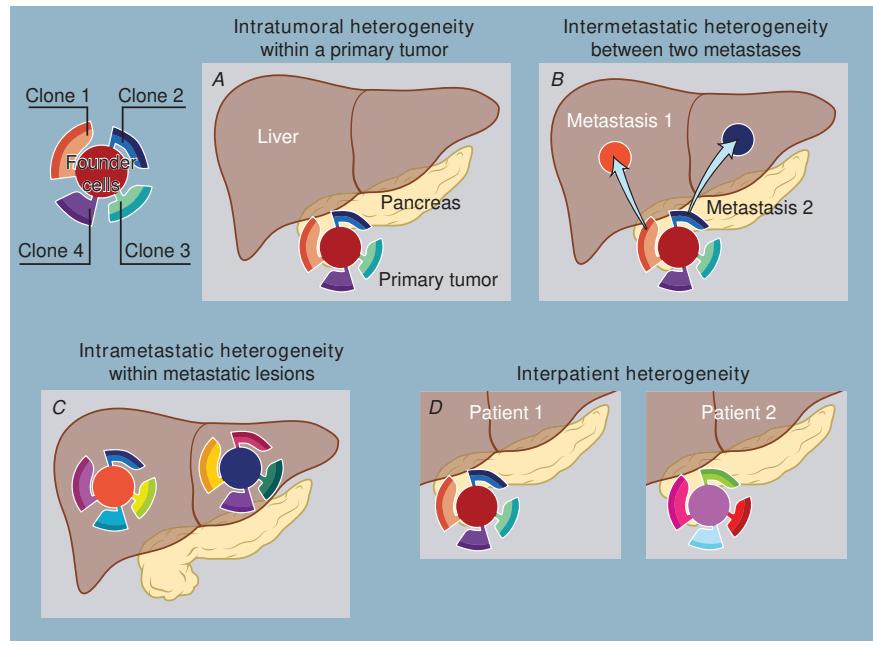


FIGURE 71-7 Four types of tumor heterogeneity. Tumor heterogeneity is the inevitable result of cell proliferation, as new mutations are introduced during clonal expansion. In a typical tumor (*upper left*), founder cells that harbor a large fraction of the total mutations give rise to subclones, which continue to evolve independently. The tumors of the founding populations are shown in the middle of each circle; the distinct subclones are shown around the periphery. *A*, Heterogeneity among the cells of a primary tumor is known as intratumoral heterogeneity. *B*, Heterogeneity among the founding cells of distinct metastatic lesions (marked as 1 and 2) that arise in the same patient is known as intermetastatic heterogeneity. *C*, Heterogeneity among the cells of each metastatic tumor is known as intrametastatic heterogeneity. *D*, Interpatient heterogeneity. The mutations in the tumors of two patients are almost completely distinct. (*From B Vogelstein et al: Cancer genome landscapes. Science 339:1546, 2013. Reprinted with permission from AAAS.*)

lung generally harbor mutations in three or more driver genes, the genes differ among the patients, and the precise mutations within the same gene can vary considerably. Type I heterogeneity is the basis for precision medicine, where the goal is to treat patients with drugs that target the proteins encoded by genetic alterations within their specific tumors. Type II heterogeneity refers to the genetic heterogeneity among different cells from the same primary tumor. Tumors continue to evolve as they grow, and different cells of the same cancer, in its original site (e.g., the pancreas), may acquire other driver gene mutations that are not shared among the other cells of the tumor. Such a mutation can result in a small clonal expansion that may or may not be important biologically. In cases in which the primary tumor can be surgically excised, such mutations are unimportant unless they give rise to type III heterogeneity (described below). The reason they are unimportant is because all primary tumor cells, whether homogeneous or not, are removed by the surgical procedure. In primary tumors that cannot be completely excised (such as most advanced brain tumors and many pancreatic ductal adenocarcinomas), heterogeneity is biomedically important because it can give rise to drug resistance, analogously to that described for type IV heterogeneity (see below). Type III heterogeneity refers to the genetic differences among the founder cells of the metastatic lesions from the same patient. For example, a patient with melanoma may have 100 different metastases distributed throughout various organs. Only if a mutant *BRAF* is present in every founder cell of every metastasis, then the patient has a chance at a complete response to a *BRAF* inhibitor. There have been several recent detailed studies of the metastases from various tumor types. Fortunately, these studies suggest there is very little, if any, type III heterogeneity among driver genes, a necessary prerequisite for the successful implementation of current and future targeted therapies. Finally, type IV heterogeneity refers to that among cells of individual metastatic lesions. As the founder cell of each metastasis expands to become detectable, it acquires mutations, a small number of which can act as “drivers” when the patient is exposed to therapeutics. This type of heterogeneity is of major clinical importance, as it has been shown to be responsible for the development of resistance in virtually all targeted therapies. The development of such resistance is a fait accompli based simply on known mutation rates and genetic resistance mechanisms. The only way to circumvent acquired resistance is to treat metastatic tumors earlier (i.e., in adjuvant setting, before much tumor expansion has occurred) or to treat with combinations of drugs for which cross-resistance is genetically impossible.

PERSONALIZED CANCER DETECTION AND TREATMENT

High-throughput DNA sequencing has led to an unprecedented understanding of cancer at the molecular level. A comprehensive mutation profile provides a molecular history of a given tumor and insights into how it arose. Because tumor cells and tumor DNA are shed into the blood and other bodily fluids, common driver mutations can be used as highly specific biomarkers for early detection. For diagnosed tumors, tumor-specific mutations can be used to estimate tumor burden, assess treatment responses, and detect recurrence.

In some cases, information regarding specific genes and pathways that are altered provides patients and physicians with options for personalized therapy. This general approach is sometimes referred to as *precision medicine*. Because tumor behavior is highly variable, even within a tumor type, personalized information-based medicine can supplement and perhaps eventually supplant histology-based tumor assessment, especially in the case of tumors that are resistant to conventional therapeutic approaches. Conversely, molecular nosology has revealed similarities in tumors of diverse histotype. The success

of the precision medicine approach in any given patient depends on the presence of tumor-associated genetic alterations that are actionable (i.e., can be targeted with a specific drug). Examples of currently actionable changes include mutations in *BRAF* (targeted by the drug vemurafenib), *RET* (targeted by sunitinib and sorafenib), *ALK* rearrangements (targeted by crizotinib), and mismatch repair genes (targetable by immune checkpoint inhibitors). At present, the proportion of tumors that can be treated with such precision medicine approaches is relatively small, but future therapeutic development will hopefully change this situation. The development of new targeted agents is at present hindered by the fact that most such agents can only target activated oncogenes, while the great majority of genetic alterations in common solid tumors are those that inactivate tumor-suppressor genes. Because all drugs, whether for use in oncology or any other purpose, can only inhibit protein actions, drugs cannot be used to directly target the proteins encoded by inactivated tumor-suppressor genes; these proteins are already inactive. More information about the pathways through which tumor-suppressor genes act may provide a way around this obstacle. For example, when a tumor-suppressor gene is inactivated, some downstream component of the pathway is likely to be activated, thereby presenting a realistic target. An example of this is provided by PARP-1 inhibitors, which have been successfully used to treat patients whose tumors have inactivating mutations of genes involved in DNA repair processes, such as *BRCA1*. Patterns of global gene expression can be used to help unravel such pathways and are already being used to predict drug sensitivities and provide prognostic information in addition to that provided by DNA sequence analysis. Evaluation of proteomic and metabolomics patterns may also prove useful for this purpose.

THE FUTURE

A revolution in cancer genetics has occurred in the past 30 years. Most types of cancer are now understood at the DNA sequence level, and this accomplishment has led to an increasingly refined understanding of tumorigenesis. Cancer gene mutations have proven to be reliable biomarkers for cancer detection and monitoring as well as for informing therapeutics through precision medicine approaches. Gene-based tests are already standard of care for patients with certain tumor types, such as melanoma and colorectal and lung cancers, and the utility of these tests will undoubtedly be expanded in the coming years as new therapies and ways of predicting responses to therapies are developed. While effective treatment of advanced cancers remains difficult, the early promise shown by immune-based therapies notwithstanding, it is expected that breakthroughs in these areas will continue to emerge and be applicable to an ever-increasing number of cancers. Moreover, with the hoped-for advances in diagnostics, particularly in the earlier detection of cancers, the new and old therapies for cancer can be expected to have a much greater impact on reducing cancer deaths.

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FURTHER READING

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CANCER CELL BIOLOGY

Cancers are characterized by unregulated cell division, avoidance of cell death, tissue invasion, and the ability to spread to other areas of the body (metastasize). A neoplasm is *benign* when it grows in an unregulated fashion without tissue invasion or metastasizing. The presence of unregulated growth, tissue invasion, and the ability to metastasize is characteristic of *malignant* neoplasms. Cancers are named based on their origin: those derived from epithelial tissue are called *carcinomas*, those derived from mesenchymal tissues are *sarcomas*, and those derived from hematopoietic tissue are *leukemias*, *lymphomas*, and *plasma cell dyscrasias* (including *multiple myeloma*).

Cancers nearly always arise as a consequence of genetic alterations, the vast majority of which begin in a single cell and therefore are monoclonal in origin. However, because a wide variety of genetic and epigenetic changes can occur in different cells within malignant tumors over time, most cancers are characterized by marked heterogeneity in the populations of cells. In addition, extrinsic factors in the cancer environment (e.g., the stroma, infiltrating cells, various cell-to-cell interactions, spatial orientation, secreted factors, and availability of oxygen and nutrients) vary in different areas within the tumor or different metastases, compounding this heterogeneity. This heterogeneity significantly complicates the treatment of most cancers because it is likely that there are subsets of cells that will be resistant to therapy for a variety of reasons and will therefore survive and proliferate even if the majority of cells are killed.

A few cancers appear to, at least initially, be primarily driven by an alteration in a dominant gene that produces uncontrolled cell proliferation. Examples include chronic myeloid leukemia (*abl*), about half of melanomas (*braf*), Burkitt's lymphoma (*c-myc*), and subsets of lung adenocarcinomas (*egfr*, *alk*, *ros1*, *met*, *ret*, *braf*, and *ntrk*). Genes that can promote cell growth when altered are often called *oncogenes*. They were first identified as critical elements of viruses that cause animal tumors; it was subsequently found that the viral genes had normal counterparts with important functions in the cell and had been captured and mutated by viruses as they passed from host to host.

However, most human cancers are characterized by a multiple-step process involving many genetic abnormalities, each of which contributes to the loss of control of cell proliferation and differentiation and the acquisition of capabilities, such as tissue invasion, the ability to metastasize, and angiogenesis (development of new blood vessels required for tumor growth). These properties are not found in the normal adult cell from which the tumor is derived. Indeed, normal cells have a large number of safeguards against DNA damage (including multiple DNA repair and extensive DNA damage response mechanisms), uncontrolled proliferation, and invasion. Many cancers go through recognizable steps of progressively more abnormal phenotypes: hyperplasia, to adenoma, to dysplasia, to carcinoma *in situ*, to invasive cancer with the ability to metastasize (Table 72-1). For most cancers, these changes occur over a prolonged period of time, usually many years.

In most organs, only primitive undifferentiated cells are capable of proliferating and cells lose the capacity to proliferate as they differentiate and acquire functional capabilities. The expansion of the primitive cells (stem cells) is linked to some functional need in the host through receptors that receive signals from the local environment or through hormonal and other influences delivered by the vascular supply. In the absence of such signals, the cells are at rest. The signals that keep the primitive cells at rest remain incompletely understood. These signals must be environmental, based on the observations that a regenerating liver stops growing when it has replaced the portion that has been surgically removed after partial hepatectomy and regenerating bone marrow stops growing when the peripheral blood counts return to

TABLE 72-1 Phenotypic Characteristics of Malignant Cells

Deregulated cell proliferation: Loss of function of negative growth regulators (tumor suppressor genes, i.e., *Rb*, *p53*), and increased action of positive growth regulators (oncogenes, i.e., *Ras*, *Myc*). Leads to aberrant cell cycle control and includes loss of normal checkpoint responses.

Failure to differentiate: Arrest at a stage before terminal differentiation. May retain stem cell properties. (Frequently observed in leukemias due to transcriptional repression of developmental programs by the gene products of chromosomal translocations.)

Loss of normal apoptosis pathways: Inactivation of *p53*, increases in *Bcl-2* (antiapoptotic) family members. This defect enhances the survival of cells with oncogenic mutations and genetic instability and allows clonal expansion and diversification within the tumor without activation of physiologic cell death pathways.

Genetic instability: Defects in DNA repair pathways leading to either single nucleotide or oligonucleotide mutations (as in microsatellite instability, MIN) or, more commonly, chromosomal instability (CIN) leading to aneuploidy (abnormal number of chromosomes in a cell). Caused by loss of function of a number of proteins including *p53*, *BRCA1/2*, mismatch repair genes, DNA repair enzymes, and the spindle checkpoint. Leads to accumulation of a variety of mutations in different cells within the tumor and heterogeneity.

Loss of replicative senescence: Normal cells stop dividing *in vitro* after 25–50 population doublings. Arrest is mediated by the *Rb*, *p16^{INK4a}*, and *p53* pathways. While most cells remain arrested, genetic and epigenetic changes in a subset of cells allow further replication, leading to telomere loss, with crisis leading to death of many cells. Cells that survive often harbor gross chromosomal abnormalities and the ability to continue to proliferate. These cells express telomerase, which maintains telomeres and is important for ongoing growth of these cells. Relevance to human *in vivo* cancer remains uncertain. Many human cancers express telomerase.

Nonresponsiveness to external growth-inhibiting signals: Cancer cells have lost responsiveness to signals normally present to stop proliferating when they have overgrown the niche normally occupied by the organ from which they are derived. Our understanding about this mechanism of growth regulation remains limited.

Increased angiogenesis: Due to increased gene expression of proangiogenic factors (VEGF, FGF, IL-8, angiopoietin) by tumor or stromal cells, or loss of negative regulators (endostatin, tumstatin, thrombospondin).

Invasion: Cell mobility and ability to move through extracellular matrix and into other tissues or organs. Loss of cell-cell contacts (gap junctions, cadherins) and increased production of matrix metalloproteinases (MMPs). Can take the form of epithelial-to-mesenchymal transition (EMT), with anchored epithelial cells becoming more like motile fibroblasts.

Metastasis: Spread of tumor cells to lymph nodes or distant tissue sites. Limited by the ability of tumor cells to migrate out of initial site and to survive in a foreign environment, including evading the immune system (see below).

Evasion of the immune system: Downregulation of MHC class I and II molecules; induction of T-cell tolerance; inhibition of normal dendritic cell and/or T-cell function; antigenic loss variants and clonal heterogeneity; increase in regulatory T cells.

Shift in cell metabolism: Complex changes including alterations due to tumor stress such as hypoxia and energy generation shifts from oxidative phosphorylation to aerobic glycolysis generate building blocks for malignant cell production and proliferation.

Abbreviations: FGF, fibroblast growth factor; IL, interleukin; MHC, major histocompatibility complex; VEGF, vascular endothelial growth factor.

normal. Cancer cells clearly have lost responsiveness to such controls and do not recognize when they have overgrown the niche normally occupied by the organ from which they are derived. A better understanding of these mechanisms of growth regulation in the context of organ homeostasis is evolving.

CANCER AS AN ORGAN THAT IGNORES ITS NICHE

The fundamental cellular defects that create a malignant neoplasm act at the cellular level, and some of these are cell autonomous. However, that is not the entire story. Cancers consist of both malignant cells as well as other cells, blood vessels, extracellular matrix, and signaling and other molecules in the cancer microenvironment. They behave as organs that have lost their specialized function and stopped responding

to signals that would limit their growth in tightly regulated normal tissue homeostasis. Most human cancers usually become clinically detectable when a primary mass is approximately 1 cm in diameter—such a mass consists of about 10^9 cells. Often, patients present with tumors that are approximately 10^{10} cells. Although it varies by type of cancer and where the primary tumor and metastases are located, a lethal tumor burden is usually about 10^{12} – 10^{13} cells. If all malignant cells were dividing without any cell death at the time of diagnosis, most patients would reach a lethal tumor burden in a very short time. However, human tumors grow by Gompertzian kinetics—this means that not every daughter cell produced by a cell division is actively dividing. In addition, the overall growth rate of a tumor depends on differences between growth rates of different cells within the tumor and rate of cell loss. The growth fraction of a tumor declines with time, largely due to factors in the microenvironment. The growth fraction of the first malignant cell is 100%, and by the time a patient presents for medical care, the growth fraction is estimated to be <10%, although the fraction varies between different types of cancers and even different cancers of the same type in different individuals. This fraction is often similar to the growth fraction of normal bone marrow and normal intestinal epithelium, the most highly proliferative normal tissues in the human body, a fact that may explain the dose-limiting toxicities to these tissues of agents that target dividing cells.

The implication of these data is that the tumor is slowing its own growth over time. How does it do this? The tumor cells have multiple genetic lesions that tend to promote proliferation, yet by the time the tumor is clinically detectable, its capacity for proliferation has declined. Better understanding of how a tumor slows its own growth would provide important clues for better cancer treatment. A number of factors, including those in the tumor microenvironment, are known to contribute to the decreased proliferation of tumor cells over time *in vivo*. Some cells are hypoxicemic and have inadequate supply of nutrients and energy. Some have sustained too much genetic damage to complete the cell cycle but have lost the capacity to undergo apoptosis and therefore survive but do not proliferate. However, an important subset is not actively dividing but retains the capacity to divide and can start dividing again under certain conditions such as when the tumor mass is reduced by treatments leading to improved conditions in the tumor microenvironment favorable for cell proliferation. Just as the bone marrow increases its rate of proliferation in response to bone marrow-damaging agents, the tumor also seems to sense when tumor cell numbers have been reduced and can respond by increasing growth rate. However, the critical difference is that the marrow stops growing when it has reached its production goals, whereas tumors do not.

The ultimate structure and organization of an organ are based on a number of factors including growth, migration, elimination, and death of various cells; communication between cells to establish the correct architecture; competition between cells; and the composition of the extracellular matrix that is produced. In addition to normal cells stopping proliferation in an organ when that is appropriate, normal tissues have various mechanisms for eliminating cells both in the process of development as well as ongoing homeostasis of an organ. These include mechanical processes based on a number of factors including cell size, shape, and topology between cells that can determine cell fate as well as an active process of cell extrusion, which plays a major role in the elimination of both cells that are no longer needed by the organ and cells that are damaged and potentially dangerous (such as those with mutations that might be precursors for malignancy). The process of cell extrusion may depend on cell cycle arrest in the S phase; aberrations in this process may contribute to the metastatic process. A variety of processes, including major alterations in cell cycle control, apoptosis and other mechanisms of cell death, and uncontrolled cell signaling, all contribute to defects in appropriate cell extrusion contributing to the development of cancer.

Additional tumor cell vulnerabilities are likely to be detected when we learn more about how normal cells respond to “stop” signals from their environment, and why and how tumor cells and tissues fail to heed such signals.

■ CELL CYCLE CHECKPOINTS

The cell division cycle consists of four phases—G₁ (growth and preparation for DNA synthesis), S (DNA synthesis), G₂ (preparation to divide), and M (mitosis, cell division). Cells can also exit the cell cycle and be quiescent (G₀). Progression of a cell through the cell cycle is tightly regulated at a number of checkpoints (especially at the G₁/S boundary, the G₂/M boundary, and during M [spindle checkpoint]) by an array of genes that are targeted by specific genetic alterations in cancer. Critical proteins in these control processes that are frequently mutated or otherwise inactivated in cancers are called tumor-suppressor genes. Examples include p53 and Rb (discussed below). In the first phase, G₁, preparations are made to replicate the genetic material. The cell stops before entering the DNA synthesis phase, or S phase, to take inventory. Are we ready to replicate our DNA? Is the DNA repair machinery in place to fix any mutations that are detected? Are the DNA replicating enzymes available? Is there an adequate supply of nucleotides? Is there sufficient energy to proceed? The retinoblastoma protein, Rb, plays a central role in placing a brake on the process until the cell is ready. When the cell determines that it is prepared to move ahead, sequential activation of cyclin-dependent kinases (CDKs) results in the inactivation of the brake, Rb, by phosphorylation. Phosphorylated Rb releases the S phase-regulating transcription factor, E2F/DP1, and genes required for S-phase progression are expressed. If the cell determines that it is unready to move ahead with DNA replication, a number of inhibitors are capable of blocking the action of the CDKs, including p21Cip2/Waf1, p16Ink4a, and p27Kip1. Nearly every cancer has one or more defects in the G₁ checkpoint that permit progression to S phase despite abnormalities in DNA repair machinery or other deficiencies that would affect normal DNA synthesis.

At the end of the G₂ phase and prior to the M phase, after the cell has exactly duplicated its DNA content, a second inventory is taken at the G₂ checkpoint. Have all of the chromosomes been fully duplicated? Were all segments of DNA copied only once? Has all damaged DNA been repaired? Do we have the right number of chromosomes and the right amount of DNA? If so, the cell proceeds to prepare for division by synthesizing mitotic spindle and other proteins needed to produce two daughter cells. If DNA damage is detected, the p53 pathway is normally activated. Called the guardian of the genome, p53 is a transcription factor that is normally present in the cell in very low levels. This level is generally regulated through its rapid turnover. Normally, p53 is bound to mdm2, a ubiquitin ligase that both inhibits p53 transcriptional activation and also targets p53 for degradation in the proteasome. When DNA damage is sensed, the ATM (ataxia-telangiectasia mutated) pathway is activated; ATM phosphorylates mdm2, releasing it from its inhibitory bond to p53. p53 then stops cell cycle progression, directs the synthesis of repair enzymes, or if the damage is too great, initiates apoptosis (programmed cell death) of the cell to prevent the propagation of a damaged cell (Fig. 72-1).

A second method of activating p53 involves the induction of p14ARF by hyperproliferative signals from oncogenes. p14ARF competes with p53 for binding to mdm2, allowing p53 to escape the effects of mdm2 and accumulate in the cell. p53 then stops cell cycle progression by activating CDK inhibitors such as p21 and/or initiating the apoptosis pathway. Not surprisingly given its critical role in controlling cell cycle progression, mutations in the gene for p53 on chromosome 17p are among the most frequent mutations in human cancers, although percentages vary between different cancers. Most commonly these mutations are acquired in the malignant tissue in one allele and the second allele is inactivated (such as by deletion or epigenetic silencing), leaving the cell unprotected from DNA-damaging agents or activated oncogenes. Some environmental exposures produce signature mutations in p53; for example, aflatoxin exposure leads to mutation of arginine to serine at codon 249 and leads to hepatocellular carcinoma. In rare instances, p53 mutations are in the germline (Li-Fraumeni syndrome) and produce a familial cancer syndrome. Another mechanism for inactivation of p53 in malignant cells is due to alterations in regulators such as overexpression of the inhibitory mdm2 protein. Whether inactivated by mutation or inhibited by regulatory factors, absence of normal p53 function leads to chromosomal instability and accumulation of DNA

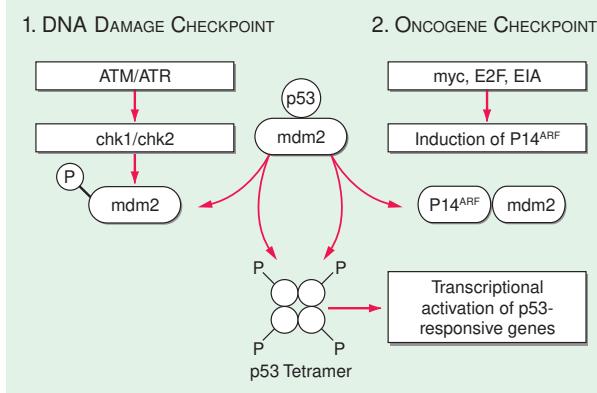


FIGURE 72-1 Induction of p53 by the DNA damage and oncogene checkpoints. In response to noxious stimuli, p53 and mdm2 are phosphorylated by the ataxiatelangiectasia mutated (ATM) and related ATR serine/threonine kinases, as well as the immediate downstream checkpoint kinases, Chk1 and Chk2. This causes dissociation of p53 from mdm2, leading to increased p53 protein levels and transcription of genes leading to cell cycle arrest ($p21^{Cip1/Waf1}$) or apoptosis (e.g., the proapoptotic Bcl-2 family members Noxa and Puma). Inducers of p53 include hypoxemia, DNA damage (caused by ultraviolet radiation, gamma irradiation, or chemotherapy), ribonucleotide depletion, and telomere shortening. A second mechanism of p53 induction is activated by oncogenes such as *Myc*, which promote aberrant G/S transition. This pathway is regulated by a second product of the Ink4a locus, p14^{ARF} (p19 in mice), which is encoded by an alternative reading frame (ARF) of the same stretch of DNA that codes for p16^{Ink4a}. Levels of ARF are upregulated by *Myc* and E2F, and ARF binds to mdm2 and rescues p53 from its inhibitory effect. This *oncogene checkpoint* leads to the death or senescence (an irreversible arrest in G₁ of the cell cycle) of renegade cells that attempt to enter S phase without appropriate physiologic signals. Senescent cells have been identified in patients whose premalignant lesions harbor activated oncogenes, for instance, dysplastic nevi that encode an activated form of *BRAF* (see below), demonstrating that induction of senescence is a protective mechanism that operates in humans to prevent the outgrowth of neoplastic cells.

damage including acquisition of properties that give the abnormal cell a proliferative and survival advantage. Like Rb dysfunction, most cancers have mechanisms that disable the p53 pathway. Indeed, the importance of p53 and Rb in the development of cancer is underscored by the neoplastic transformation mechanism of human papillomavirus. This virus has two main oncogenes, E6 and E7. E6 acts to increase the rapid turnover of p53, and E7 acts to inhibit Rb function; inhibition of these two targets is required for transformation of epithelial cells.

Another cell cycle checkpoint exists when the cell is undergoing division (M phase); this is the spindle checkpoint, which acts to ensure that there is proper attachment of chromosomes to the mitotic spindle before progression through the cell cycle can occur. If the spindle apparatus does not properly align the chromosomes for division, if the chromosome number is abnormal (i.e., greater or less than $4n$), or if the centromeres are not properly paired with their duplicated partners, then the cell initiates a cell death pathway to prevent the production of aneuploid progeny (having an altered number of chromosomes). Abnormalities in the spindle checkpoint facilitate the development of aneuploidy, which is frequently found in cancers. In some tumors, aneuploidy is a predominant genetic feature.

In other tumors, a defect in the cells' ability to repair errors in the DNA, such as due to mutations in genes coding for the proteins critical for mismatched DNA repair, is the primary genetic lesion. Cancer cells can have defects in any of several DNA repair pathways in addition to mismatch repair, including deficient interstrand cross-link, double-strand breaks (homologous recombination or nonhomologous end joining repair), single-strand breaks, base excision, nucleotide excision, and translesional synthesis.

In general, tumors have either defects in chromosome number or defective DNA repair pathways but not both. Defects that lead to cancer include abnormal cell cycle checkpoints, inadequate DNA repair, and failure to preserve genome integrity leading to DNA damage. These

defects and the stress of the resultant increased DNA damage make cancer cells more vulnerable to additional DNA damage, which can be exploited by chemotherapy, radiation therapy, targeted therapy, and immunotherapy—the major systemic therapeutic approaches effective against cancer.

Alternatively, research is ongoing in an attempt to therapeutically restore the defects in cell cycle regulation and DNA repair that characterize cancer, although this remains a challenging problem because it is much more difficult to restore normal biologic function than to inhibit abnormal function of proteins driving cell proliferation, such as occurs with oncogenes. Newer approaches to gene editing (e.g., clustered regularly interspaced short palindromic repeats [CRISPR]) and subsequent modifications to this approach should make this more feasible.

■ CELLULAR SENESCENCE

The irreversible cessation of growth of normal cells while the cells remain viable has been termed cellular senescence. This was initially identified by the fact that when normal cells are placed in culture *in vitro*, most are not capable of sustained growth. They quickly reach a point where they either undergo cell death due to excessive DNA damage or other factors or they become senescent. Fibroblasts are an exception to this rule. When they are cultured, fibroblasts may divide 30–50 times and then they undergo what has been termed a “crisis” during which the majority of cells stop dividing (usually due to an increase in p21 expression, a CDK inhibitor). This form of senescence is termed replicative senescence. Many other cells die, and a small fraction emerge that have acquired genetic and epigenetic changes that permit their uncontrolled growth.

Among the cellular changes during *in vitro* propagation is telomere shortening. DNA polymerase is unable to replicate the tips of chromosomes, resulting in the loss of DNA at the specialized ends of chromosomes (called *telomeres*) with each replication cycle. At birth, human telomeres are 15- to 20-kb pairs long and are composed of tandem repeats of a six-nucleotide sequence (TTAGGG) that associate with specialized telomere-binding proteins to form a T-loop structure that protects the ends of chromosomes from being mistakenly recognized as damaged. The loss of telomeric repeats with each cell division cycle causes gradual telomere shortening, leading to growth arrest when one or more critically short telomeres trigger a p53-regulated DNA-damage checkpoint response. Cell death usually ensues when the unprotected ends of chromosomes lead to chromosome fusions or other catastrophic DNA rearrangements. Cells with certain abnormalities, such as those with nonfunctional pRb and p53, can bypass this growth arrest. *The ability to bypass telomere-based growth limitations is thought to be a critical step in the evolution of most malignancies.* This occurs by reactivation of telomerase expression in cancer cells. Telomerase is an enzyme that adds TTAGGG repeats onto the 3' ends of chromosomes. It contains a catalytic subunit with reverse transcriptase activity (hTERT) and an RNA component that provides the template for telomere extension. Most normal somatic cells do not express sufficient telomerase to prevent telomere attrition with each cell division. Exceptions include stem cells (such as those found in hematopoietic tissues, gut and skin epithelium, and germ cells) that require extensive cell division to maintain tissue homeostasis. More than 90% of human cancers express high levels of telomerase that prevent telomere shortening to critical levels and allow indefinite cell proliferation. *In vitro* experiments indicate that inhibition of telomerase activity leads to tumor cell apoptosis. Major efforts are underway to develop methods to inhibit telomerase activity in cancer cells. For example, the protein component of telomerase (hTERT) may act as one of the most widely expressed tumor-associated antigens and can be targeted by vaccine approaches. However, a caveat to targeting telomerase for anticancer treatment is the potential for inhibiting its activity in certain normal cells (such as stem cells) required for maintaining the normal physiologic state.

Although most of the functions of telomerase relate to cell division, it also has several other effects including interfering with the differentiated functions of at least certain stem cells. However, the impact on differentiated function of normal nonstem cells is less clear. The picture is further complicated by the fact that rare genetic defects in the

telomerase enzyme seem to cause dyskeratosis congenita (characterized by abnormalities in various rapidly dividing cells in the body including skin, nails, oral mucosa, hair, and bone marrow with increased risk for leukemia and certain other cancers). This can be associated with a number of other abnormalities including pulmonary fibrosis, bone marrow failure (aplastic anemia), or liver fibrosis. However, paradoxically, defects in nutrient absorption in the gastrointestinal tract, a site that should be highly sensitive to defective cell proliferation, are not seen. Much remains to be learned about how telomere shortening and telomere maintenance are related to human illness in general and cancer in particular.

A variety of other stresses on cells (both environmental and intrinsic including radiation, chemotherapy, reactive oxygen species, and oncogenic mutations) can also lead to senescence, primarily those that induce DNA damage similar to that seen in cells with shortened telomeres. This is termed *replicative senescence*.

SIGNAL TRANSDUCTION PATHWAYS IN CANCER CELLS

Signals that affect cell behavior come from adjacent cells, the stroma in which the cells are located, hormonal signals that originate remotely, and the cells themselves (autocrine signaling). These signals generally exert their influence on the receiving cell through activation of signal transduction pathways that have as their end result the induction of activated transcription factors that mediate a change in cell behavior or function or the acquisition of effector machinery to accomplish a new task. Although signal transduction pathways can lead to a wide variety of outcomes, many such pathways rely on cascades of signals that sequentially activate different proteins or glycoproteins and lipids or glycolipids, and the activation steps often involve the addition or removal of one or more phosphate groups on a downstream target. Other chemical changes can result from signal transduction pathways, but reversible phosphorylation and dephosphorylation play a major role. Proteins that add phosphate groups to other molecules (proteins, lipids, or nucleic acids) are called kinases. Two major classes of kinases involved in signal transduction pathways important for cancer cells are tyrosine kinases that phosphorylate tyrosine and serine/threonine kinases that phosphorylate serine/threonine either directly or indirectly. However, some kinases can phosphorylate both, such as the MEK kinases that can phosphorylate both threonine and tyrosine. Phosphatases (protein tyrosine phosphatases and protein serine/threonine phosphatases) remove the phosphate groups to reverse the kinase activity.

Various kinases play critical roles in signal transduction pathways important for malignant cells. These include a number of receptor tyrosine kinases (RTKs) as well as various protein kinases (both tyrosine and serine/threonine kinases) downstream of receptors that transmit the signals within the cell (Fig. 72-2). Two important signaling pathways are the RAS-RAF-MEK-ERK pathway and the phosphatidylinositol-3-kinase (PI3K) pathway (Fig. 72-2). Although pathways are depicted as distinct, there are complex interactions between pathways within cells.

Normally, kinase activity is short-lived and reversed by protein phosphatases. However, in many human cancers, RTKs or components of their downstream pathways are activated by mutation, gene amplification, or chromosomal translocations to have enhanced and/or prolonged activity. Because these pathways are important in regulating proliferation, survival, migration, and angiogenesis, they have been identified as important targets for cancer therapeutics.

Inhibition of kinase activity is effective in the treatment of a number of neoplasms. Lung cancers with mutations in the epidermal growth factor receptor are highly responsive to osimertinib as well as other inhibitors (Table 72-2). Inhibitors have been developed to treat lung cancers with other tyrosine kinase-activating mutations (including anaplastic lymphoma kinase [ALK], ROS1, NTRK, MET, and RET). BRAF (a serine/threonine kinase) inhibitors are highly effective in melanomas and thyroid cancers and are also used in combination with other agents for lung and colon cancers in which BRAF is mutated. Targeting the MEK protein (which phosphorylates both threonine and

tyrosine residues) downstream of BRAF also has activity against BRAF mutant melanomas, and combined inhibition of BRAF and MEK is more effective than either alone with activity that extends to BRAF mutant lung cancer. Janus kinase (JAK) inhibitors are active in myeloproliferative syndromes in which JAK2 activation is a pathogenetic event. Imatinib (which targets a number of tyrosine kinases) is an effective agent in tumors that have translocations of the c-Abl and BCR gene (such as chronic myeloid leukemia), mutant c-Kit (gastrointestinal stromal cell tumors), or mutant platelet-derived growth factor receptor (PDGF α ; gastrointestinal stromal tumors). Second-generation inhibitors of BCR-Abl, dasatinib and nilotinib, are even more effective, and the third-generation agent bosutinib has activity in some patients who have progressed on other inhibitors, while the third-generation agent ponatinib has activity against the T315I mutation, which is resistant to the other agents. Although almost all tyrosine kinase inhibitors are not entirely selective for one protein, certain inhibitors have significant activity against a broad number of proteins. These include sorafenib, regorafenib, cabozantinib, sunitinib, and lenvatinib. These have shown antitumor activity in various malignancies, including renal cell cancer (RCC) (sorafenib, sunitinib, cabozantinib, lenvatinib), hepatocellular carcinoma (sorafenib, regorafenib, lenvatinib), gastrointestinal stromal tumor (GIST) (sunitinib, regorafenib), thyroid cancer (sorafenib, cabozantinib, lenvatinib), colorectal cancer (regorafenib), and pancreatic neuroendocrine tumors (sunitinib).

Inhibitors of the PI3K pathway also have been approved for cancer therapy. The PI3K family includes three classes and several isoforms within each class. Inhibitors against different isoforms have proved effective against different types of malignancies, with inhibitors of the delta isoform (either specifically or also with inhibition of other isoforms; e.g., idelalisib) having activity against lymphoid malignancies, whereas the specific inhibitor of a mutation in the alpha isoform (alpelisib) has activity against breast cancers with this mutation. Inhibitors of mammalian target of rapamycin (mTOR; which is downstream of PI3K; e.g., everolimus, temsirolimus) are active in RCC, pancreatic neuroendocrine tumors, and breast cancer. Additional inhibitors of the PI3K pathway and other phospholipid signaling pathways such as the phospholipase C-gamma pathway, which are involved in a large number of cellular processes important in cancer development and progression, are being evaluated.

The list of active agents and treatment indications is growing rapidly (Table 72-2). These agents have ushered in a new era of personalized therapy. It is becoming more common for tumor biopsies to be assessed for specific molecular changes that predict response and to have clinical decision-making guided by those results. This is now an important component of standard therapy for metastatic lung, gastroesophageal, melanoma, breast, and colorectal cancers as well as in adjuvant therapy for breast cancer.

An alternative approach to testing samples directly from tumors is to test blood for the presence of mutations or amplification in circulating tumor DNA, which has the significant advantage of being noninvasive. As cancers grow, some of the cells die and break down with release of cellular contents, including DNA, into the circulation. Sensitive methods have been developed to detect this DNA and to identify mutations and other DNA changes in the malignant cells. This has the potential advantage over tumor biopsies of sampling all of the tumor and not being limited to one site that may not be representative of the overall tumor heterogeneity. In addition to identifying potential changes that can be targeted for therapy, there is also the potential for monitoring a patient's response to therapy, identifying resistance mechanisms to therapy earlier, detecting disease recurrence before it can be detected by tumor markers or scans, monitoring bodily fluids in addition to blood, and possibly providing a means of earlier initial detection of cancer if sufficiently sensitive and specific detection methods can be developed.

However, none of these targeted therapies has yet been curative by themselves for any malignancy, although prolonged periods of disease control lasting many years frequently occur in chronic myeloid leukemia (CML), including a >80% survival rate at 10 years. The reasons for the failure to cure are not completely defined, although resistance

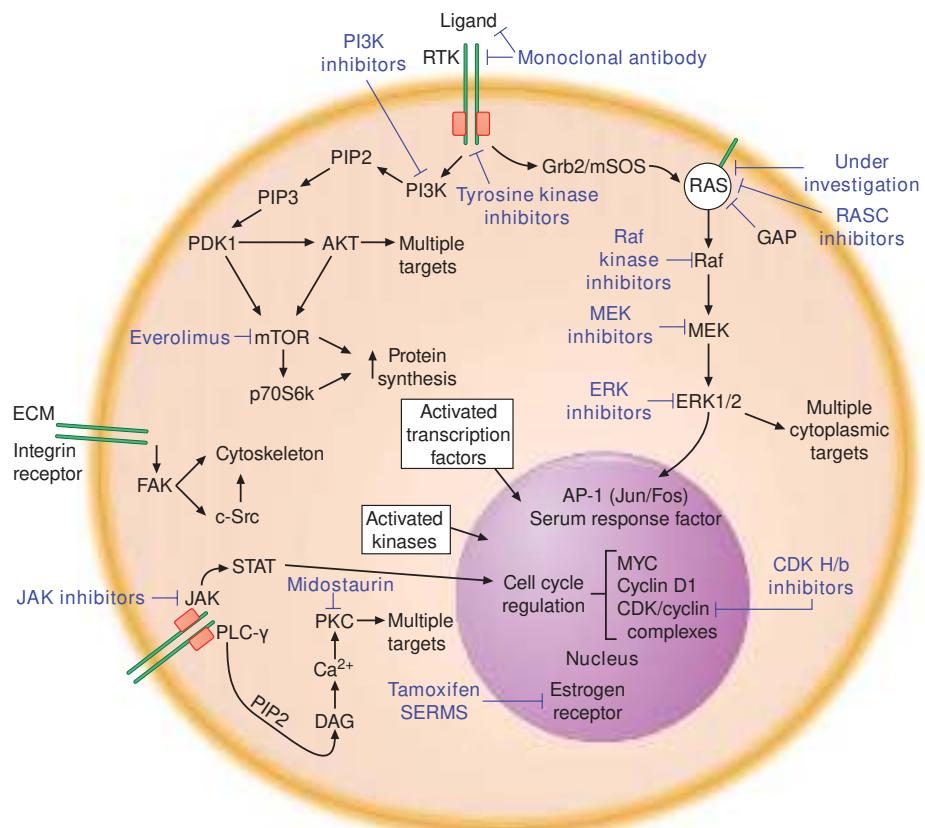


FIGURE 72-2 Therapeutic targeting of signal transduction pathways in cancer cells. Three major signal transduction pathways are activated by receptor tyrosine kinases (RTKs). 1. The protooncogene Ras is activated by the Grb2/mSOS guanine nucleotide exchange factor, which induces an association with Raf and activation of downstream kinases (MEK and ERK1/2). 2. Activated PI3K phosphorylates the membrane lipid PIP₂ to generate PIP₃, which acts as a membrane-docking site for a number of cellular proteins including the serine/threonine kinases PDK1 and Akt. PDK1 has numerous cellular targets, including Akt and mTOR. Akt phosphorylates target proteins that promote resistance to apoptosis and enhance cell cycle progression, while mTOR and its target p70S6K upregulate protein synthesis to potentiate cell growth. 3. Activation of PLC-γ leads the formation of diacylglycerol (DAG) and increased intracellular calcium, with activation of multiple isoforms of PKC and other enzymes regulated by the calcium/calmodulin system. Other important signaling pathways involve non-RTKs that are activated by cytokine or integrin receptors. Janus kinases (JAK) phosphorylate STAT (signal transducer and activator of transcription) transcription factors, which translocate to the nucleus and activate target genes. Integrin receptors mediate cellular interactions with the extracellular matrix (ECM), inducing activation of FAK (focal adhesion kinase) and c-Src, which activate multiple downstream pathways, including modulation of the cell cytoskeleton. Many activated kinases and transcription factors migrate into the nucleus, where they regulate gene transcription, thus completing the path from extracellular signals, such as growth factors, to a change in cell phenotype, such as induction of differentiation or cell proliferation. The nuclear targets of these processes include transcription factors (e.g., Myc, AP-1, and serum response factor) and the cell cycle machinery (cyclin-dependent kinases [CDKs] and cyclins). Inhibitors of many of these pathways have been developed for the treatment of human cancers. Examples of inhibitors that are either approved or are currently being evaluated in clinical trials are shown in purple type.

to the treatment ultimately develops in most patients. In some tumors, resistance to kinase inhibitors is related to proliferation of cells with a mutation in the target kinase that inhibits drug binding. Many of these kinase inhibitors act as competitive inhibitors of the ATP-binding pocket. ATP is the phosphate donor in these phosphorylation reactions. For example, mutation in the critical BCR-ABL kinase in the ATP-binding pocket (such as the threonine to isoleucine change at codon 315 [T315I]) can prevent imatinib binding. Other resistance mechanisms include alterations in other signal transduction pathways to bypass the inhibited pathway. As resistance mechanisms continue to be better defined, rational strategies to overcome resistance are emerging. In addition, many kinase inhibitors are less specific for an oncogenic target than was hoped, and toxicities related to off-target inhibition of kinases limit the use of the agent at a dose that would optimally inhibit the cancer-relevant kinase.

Antibodies against protein targets more highly expressed on malignant than normal cells can also be used to deliver highly toxic compounds relatively specifically to cancer cells. Examples of protein targets for currently approved antibody-drug conjugates include CD30 for Hodgkin's and anaplastic lymphomas; HER2 on breast cancer; CD33 on

acute myeloid leukemias; CD22 on B-cell acute lymphocytic and hairy cell leukemias; and CD79b on diffuse large B-cell lymphomas.

Another strategy to enhance the antitumor effects of targeted agents is to use them in rational combinations with each other as well as with chemotherapy or immunotherapy agents that kill cells in ways distinct from agents targeting specific mutant or overexpressed proteins. Combinations of trastuzumab (a monoclonal antibody that targets the HER2 receptor [member of the EGFR family]) with chemotherapy have significant activity against breast and stomach cancers that have high levels of expression of the HER2 protein. The activity of trastuzumab and chemotherapy can be enhanced further by combinations with another targeted monoclonal antibody (pertuzumab), which prevents dimerization of the HER2 receptor with other HER family members including HER3.

Although targeted therapies have not yet resulted in cures when used alone, their use in the adjuvant setting and when combined with other effective treatments has substantially increased the fraction of patients cured. For example, the addition of rituximab, an anti-CD20 antibody, to combination chemotherapy in patients with diffuse large B-cell lymphoma improves cure rates by ~15%. The addition

TABLE 72-2 Some FDA-Approved Molecularly Targeted Agents for the Treatment of Cancer

DRUG	MOLECULAR TARGET	DISEASE	MECHANISM OF ACTION
All- <i>trans</i> retinoic acid	PML-RAR α oncogene	Acute promyelocytic leukemia M3 AML, t(15;17)	Inhibits transcriptional repression by PML-RAR α
Imatinib	Bcr-Abl, c-Abl, c-Kit, PDGFR- α/β	Chronic myeloid leukemia, GIST	Blocks ATP binding to tyrosine kinase active site
Ripretinib	c-Kit, PDGFR- α	GIST	Inhibits tyrosine kinase activity
Dasatinib, nilotinib, ponatinib, bosutinib	Bcr-Abl (primarily)	Chronic myeloid leukemia	Blocks ATP binding to tyrosine kinase active site
Sunitinib	c-Kit, VEGFR-2, PDGFR- β , Flt-3	GIST, RCC, PNET	Inhibits activated c-Kit and PDGFR in GIST; inhibits VEGFR in RCC and probably in PNET
Sorafenib	RAF, VEGFR-2, PDGFR- α/β , Flt-3, c-Kit	RCC, hepatocellular carcinoma (HCC), differentiated thyroid cancer, desmoid	Targets VEGFR pathways in RCC and HCC. Possible activity against BRAF in thyroid cancer
Regorafenib	VEGFR1–3, TIE-2, FGFR1, KIT, RET, PDGFR	Colorectal cancer, GIST, HCC	Competitive inhibitor ATP binding site of tyrosine kinase domain multiple kinases including VEGFR
Larotrectinib, entrectinib	NTRK	Cancers with NTRK mutation	Competitive inhibitor of ATP binding site of the tyrosine kinase domain of NTRK
Axitinib	VEGFR1–3	RCC	Competitive inhibitor ATP binding site of tyrosine kinase domain VEGF receptors
Erlotinib	EGFR	NSCLC, pancreatic cancer	Competitive inhibitor of the ATP-binding site of the EGFR
Afatinib	EGFR (and other HER family)	NSCLC	Irreversible inhibitor of ATP-binding site of HER family members
Osimertinib	EGFR (T790M)	NSCLC	Inhibits EGFR mutations including T790M mutant NSCLC
Dacomitinib	EGFR	NSCLC (exon19 deletion/exon 21 L858R)	Inhibits EGFR mutant lung cancer
Erdafitinib, pemigatinib	FGFR2, FGFR3	Urothelial (erdafitinib), cholangiocarcinoma (pemigatinib)	Inhibits tyrosine kinase of FGFR
Lapatinib, tucatinib	HER2/neu	Breast cancer	Competitive inhibitor of the ATP-binding site of HER2
Crizotinib, ceritinib, alectinib, brigatinib, lorlatinib	ALK	NSCLC	Inhibitor of ALK tyrosine kinase
Crizotinib, entrectinib	ROS1	NSCLC	Inhibitor of ROS1 tyrosine kinase
Palbociclib, ribociclib, abemaciclib	CDK4/6	Breast	Inhibitor of CDK4/6
Bortezomib, carfilzomib, ixazomib	Proteasome	Multiple myeloma	Inhibits proteolytic degradation of multiple cellular proteins
Vemurafenib, dabrafenib	BRAF	Melanoma	Inhibitor of serine-threonine kinase domain of V600E mutant of BRAF
Encorafenib	MEK	CRC	Inhibits BRAFV600E mutation; used in combination with cetuximab
Trametinib, Cobimetinib	MEK	Melanoma	Inhibitor of serine-threonine kinase domain of MEK
Cabozantinib	RET, MET, VEGFR	MTC, RCC	Competitive inhibitor of ATP-binding site of tyrosine kinase domain of multiple kinases, including VEGFR2 and RET
Capmatinib	MET	NSCLC with MET exon14 deletions	
Vandetanib	RET, VEGFR, EGFR	MTC	Competitive inhibitor of ATP-binding site of tyrosine kinase domain of multiple kinases, including RET
Selpercatinib	RET	NSCLC, MTC, RET fusion thyroid cancer	Inhibitor of RET, VEGFR1, VEGFR2 tyrosine kinases
Tensirolimus	mTOR	RCC	Competitive inhibitor of mTOR serine-threonine kinase
Everolimus	mTOR	RCC, PNET	Binds to immunophilin FK binding protein-12, which forms a complex that inhibits mTOR kinase
Vorinostat, romidepsin, belinostat	HDAC	CTCL/PTL	HDAC inhibitor, epigenetic modulation
Panobinostat	HDAC	MM	HDAC inhibitor, epigenetic modulation
Ruxolitinib	JAK-1, 2	Myelofibrosis	Competitive inhibitor of tyrosine kinase
Vismodegib	Hedgehog pathway	Basal cell cancer (skin)	Inhibits smoothened in hedgehog pathway
Lenvatinib	Multikinase inhibitor (VEGFR, FGFR, PGFR- α , others)	RCC, thyroid cancer, HCC	Competitive inhibitor of ATP-binding site of tyrosine kinase domain of multiple kinases
Olaparib, rucaparib, niraparib, talazoparib	PARP	BRCA mutant ovarian, breast, prostate, pancreas cancers; not all agents approved for all cancers	Inhibits PARP and DNA repair
Venetoclax	BCL-2	CLL (with 17p deletion)	Inhibits BCL-2 and enhances apoptosis
Ibrutinib, acalabrutinib	Bruton tyrosine kinase (BTK)	CLL, MCL, MZL, SLL, WM	Inhibitor of BTK

(Continued)

TABLE 72-2 Some FDA-Approved Molecularly Targeted Agents for the Treatment of Cancer (Continued)

DRUG	MOLECULAR TARGET	DISEASE	MECHANISM OF ACTION
Ivosidenib	IDH1	AML	IDH1 inhibitor
Gilteritinib	FLT3	AML	FLT3 inhibitor
Idelalisib	PI3K-delta	CLL, SLL, FL	Inhibits PI3k-delta, preventing proliferation and inducing apoptosis
Alpelisib	PIK3CA	Breast cancer with a PIK3CA mutation	Inhibits PIK3CA
Monoclonal Antibodies Alone			
Trastuzumab	HER2/neu (ERBB2)	Breast cancer, gastric cancer	Binds HER2 on tumor cell surface and induces receptor internalization
Pertuzumab	HER2/neu (ERBB2)	Breast cancer	Binds HER2 on tumor cell surface at distinct site from trastuzumab and prevents binding to other receptors
Cetuximab	EGFR	Colon cancer, squamous cell carcinoma of the head and neck	Binds extracellular domain of EGFR and blocks binding of EGF and TGF- α ; induces receptor internalization. Potentiates the efficacy of chemotherapy and radiotherapy
Panitumumab	EGFR	Colon cancer	Similar to cetuximab but fully humanized rather than chimeric
Necitumumab	EGFR	Squamous NSCLC	Binds EGFR
Rituximab	CD20	B-cell lymphomas and leukemias that express CD20	Multiple potential mechanisms, including direct induction of tumor cell apoptosis and immune mechanisms
Alemtuzumab	CD52	Chronic lymphocytic leukemia and CD52-expressing lymphoid tumors	Immune mechanisms
Bevacizumab	VEGF	Colorectal, lung cancers, RCC, glioblastoma	Inhibits angiogenesis by high-affinity binding to VEGF
Ziv-aflibercept	VEGFA, VEGFB, PLGF	Colorectal cancers	Inhibits angiogenesis by high-affinity binding to VEGFA, VEGFB, and PLGF
Ramucirumab	VEGFR	Gastric, colorectal, lung cancers	Inhibits angiogenesis by binding to VEGFR
Ipilimumab	CTLA-4	Melanoma, HCC, MSI-high colorectal cancer	Blocks CTLA-4, preventing interaction with CD80/86 and T-cell inhibition
Nivolumab, pembrolizumab	PD-1	Melanoma, head and neck cancer, NSCLC, SCLC, Hodgkin's disease, urothelial cancer, RCC, HCC, gastric cancer, MSI-high cancers, endometrial cancer	Blocks PD-1, preventing interaction with PD-L1 and T-cell inhibition
Atezolizumab, durvalumab, avelumab	PD-L1	NSCLC, urothelial cancer, SCLC (durvalumab), HCC (atezolizumab), Merkel cell cancer (avelumab)	Blocks PD-L1, preventing interaction with PD-1 and T-cell inhibition
Denosumab	Rank ligand	Breast, prostate	Inhibits Rank ligand, primary signal for bone removal
Dinutuximab	Glycolipid GD2	Neuroblastoma (pediatric)	Immune-mediated attack on GD2-expressing cells
Daratumumab	CD38	MM	Binds to CD38 on MM cells causing apoptosis by antibody-dependent or complement-mediated cytotoxicity
Botuzumab	SLAM F7	MM	Activating NK cells to kill MM cells
Olaratumab	PDGFR α	Soft tissue sarcomas	Blocks PDGFR α activity
Blinatumomab	CD19 and CD3	Ph-relapsed precursor B-cell ALL	Binds CD19 on ALL cells and CD3 on T cells; immune attack on CD19-expressing cells
Antibody-Chemotherapy Conjugates			
Brentuximab vedotin	CD30	Hodgkin's disease, anaplastic lymphoma	Delivery of chemotherapeutic agent (MMAE) to CD30-expressing tumor cells
Ado-trastuzumab emtansine	HER2	Breast cancer	Delivery of chemotherapeutic agent emtansine to HER2-expressing breast cancer cells
Fam-trastuzumab	HER2	Breast cancer, gastric cancer	Delivery of chemotherapeutic agent deruxtecan to HER2-expressing breast cancer cells
CAR-T Cells			
Tisagenlecleucel, axicabtagene ciloleucel	CD19	ALL (tisagenlecleucel), DLBCL/high-grade BCL (axicabtagene ciloleucel)	Targeted T cells to protein on surface of malignant cells

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BCL, B-cell lymphoma; CAR-T, chimeric antigen receptor T cells; CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; CTCL, cutaneous T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; EGFR, epidermal growth factor receptor; FDA, U.S. Food and Drug Administration; FGFR, fibroblast growth factor receptor; FL, follicular lymphoma; Flt-3, fms-like tyrosine kinase-3; GIST, gastrointestinal stromal tumor; HDAC, histone deacetylases; MCL, mantle cell lymphoma; MM, multiple myeloma; MSI, microsatellite instability; MTC, medullary thyroid cancer; mTOR, mammalian target of Rapamycin; MZL, mantle zone lymphoma; NK, natural killer; NSCLC, non-small-cell lung cancer; PARP, poly-ADP ribose polymerase; PDGFR, platelet-derived growth factor receptor; PLGF, placenta growth factor; PML-RAR α , promyelocytic leukemia-retinoic acid receptor-alpha; PNET, pancreatic neuroendocrine tumors; PTL, peripheral T-cell lymphoma; RCC, renal cell cancer; t(15;17), translocation between chromosomes 15 and 17; SCLC, small-cell lung cancer; SLL, small lymphocytic lymphoma; TGF- α , transforming growth factor-alpha; VEGFR, vascular endothelial growth factor receptor; WM, Waldenström's macroglobulinemia.

of trastuzumab, antibody to HER2, to combination chemotherapy in the adjuvant treatment of HER2-positive breast cancer significantly improves overall survival.

A major effort continues to develop targeted therapies for mutations in the *ras* family of genes, which play a critical role in transmitting signals through a number of downstream signaling pathways including the MAP (mitogen-activated protein) kinase and PI3K pathways. Mutations in *ras* are the most common mutations in oncogenes in cancers (especially *kras*) but have proved to be very difficult targets for a number of reasons related to the structure of RAS proteins as well as mechanisms of activation and inactivation (active when bound to guanosine triphosphate [GTP] and inactive when bound to guanosine diphosphate [GDP]). RAS proteins are not kinases but bind directly to the BRAF serine/threonine kinase with preferential binding when RAS is in the active GTP bound state. Preliminary evidence indicates antitumor activity of agents that target one of the mutant forms of KRAS (12C) that is found in a subset of cancers. Indirect inhibition of RAS function by inhibiting farnesyl transferase, which is important for RAS binding to the membrane and is required for activation, has shown some promise against HRAS mutant head and neck cancers. Targeted therapies against a subset of proteins downstream of RAS in the MAP kinase signaling pathway (including BRAF and MAP kinase) have proven to have significant antitumor activity against V600E *BRAF* mutant melanoma, with improved efficacy when they are used in combination. However, similar activity is not seen against *ras* mutant tumors. Additional targeted therapies against other proteins downstream of RAS (including ERK, or combinations of MAP kinase inhibitors and immunotherapy) are being studied, both individually and in combination. However, at this time, there is no clinically approved approach to inhibiting RAS mutant tumors.

One of the strategies for new drug development is to take advantage of so-called oncogene addiction. This situation (Fig. 72-3) is created when a tumor cell develops an activating mutation in an oncogene that becomes a dominant pathway for survival and growth with reduced contributions from other pathways, even when there may be abnormalities in those pathways. This dependency on a single pathway creates a cell that is vulnerable to inhibitors of that oncogene pathway. For example, cells harboring mutations in *BRAF* are very sensitive to MEK inhibitors that inhibit downstream signaling in the *BRAF* pathway.

Proteins critical for transcription of other proteins essential for malignant cell survival or proliferation provide another potential target for treating cancers. The transcription factor nuclear factor (NF)- κ B is a heterodimer composed of p65 and p50 subunits that associate with an inhibitor, I κ B, in the cell cytoplasm. In response to growth factor or cytokine signaling, a multi-subunit kinase called IKK (I κ B-kinase) phosphorylates I κ B and directs its degradation by the ubiquitin/proteasome system. NF- κ B, free of its inhibitor, translocates to the nucleus and activates target genes, many of which promote the survival of tumor cells. One of the mechanisms by which novel drugs called *proteasome inhibitors* are thought to produce an anticancer effect is by blocking the proteolysis of I κ B, thereby preventing NF- κ B activation.

For reasons that have not been fully elucidated, this has a differential toxicity effect on tumor, as compared to normal, cells. Although this mechanism appears to be an important aspect of the antitumor effects of proteasome inhibitors, there are other effects involving the inhibition of the degradation of multiple cellular proteins important in malignant cell survival or proliferation. Proteasome inhibitors (bortezomib, carfilzomib, ixazomib) have activity in patients with multiple myeloma, including partial and complete remissions. Inhibitors of IKK are also in development, with the hope of more selectively blocking the degradation of I κ B, thus “locking” NF- κ B in an inhibitory complex and rendering the cancer cell more susceptible to apoptosis-inducing agents. Many other transcription factors are activated by phosphorylation, which can be prevented by tyrosine or serine/threonine kinase inhibitors, a number of which are currently in clinical trials.

Estrogen receptors (ERs) and androgen receptors (ARs), members of the steroid hormone family of nuclear receptors, are targets of inhibition by drugs used to treat breast and prostate cancers, respectively. Selective estrogen receptor modulators (SERMs) have been developed as a treatment approach for ER-positive breast cancer. Tamoxifen, a partial agonist and antagonist of ER function, is frequently used in breast cancer and can mediate tumor regression in metastatic breast cancer and can prevent disease recurrence in the adjuvant setting. Tamoxifen binds to the ER and modulates its transcriptional activity, inhibiting activity in the breast but promoting activity in bone but unfortunately also in uterine epithelium, leading to a small increased risk of uterine cancer. Attempts have been made to develop SERMs that would have antiestrogenic effects in both breast and uterus while maintaining protective effects on bone. However, none of these to date has been an improvement over tamoxifen. Aromatase inhibitors, which

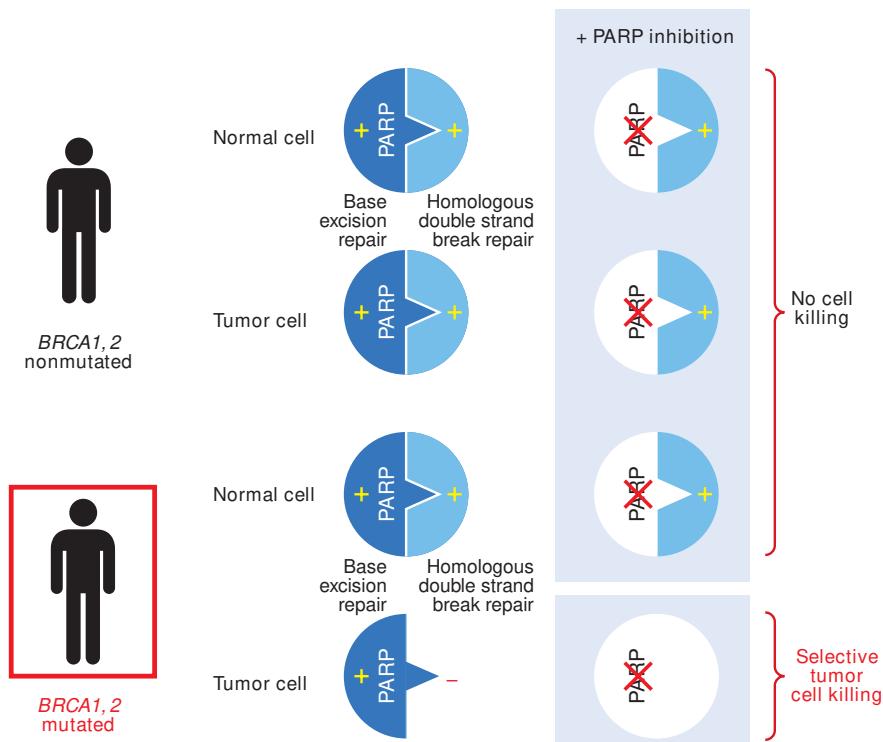


FIGURE 72-3 Synthetic lethality. Genes are said to have a synthetic lethal relationship when mutation of either gene alone is tolerated by the cell, but mutation of both genes leads to lethality, as originally noted by Bridges and later named by Dobzhansky. Thus, mutant *gene a* and *gene b* have a synthetic lethal relationship, implying that the loss of one gene makes the cell dependent on the function of the other gene. In cancer cells, loss of function of a DNA repair gene like *BRCA1*, which repairs double-strand breaks, makes the cell dependent on base excision repair mediated in part by *PARP*. If the *PARP* gene product is inhibited, the cell attempts to repair the break using the error-prone nonhomologous end-joining method, which results in tumor cell death. High-throughput screens can now be performed using isogenic cell line pairs in which one cell line has a defined defect in a DNA repair pathway. Compounds can be identified that selectively kill the mutant cell line; targets of these compounds have a synthetic lethal relationship to the repair pathway and are potentially important targets for future therapeutics.

block the conversion of androgens to estrogens in breast and subcutaneous fat tissues, have demonstrated improved clinical efficacy compared with tamoxifen in postmenopausal women and are often used as first-line therapy in postmenopausal patients with ER-positive disease. They are occasionally used in premenopausal patients with ER-positive disease in combination with ovarian suppression approaches such as luteinizing hormone-releasing hormone (LHRH) agonists. A number of approaches have been developed for blocking androgen stimulation of prostate cancer, including decreasing production by the testicles (e.g., orchectomy, LHRH agonists or antagonists), directly blocking actions of androgen (a number of agents have been developed to do this), or blocking production by inhibiting the enzyme CYP17, which is central in production of androgens from cholesterol (Chap. 79).

CANCER SPECIFIC GENETIC CHANGES AND SYNTHETIC LETHALITY

The concepts of oncogene addiction and synthetic lethality have spurred new drug development targeting oncogene- and tumor-suppressor pathways. As discussed earlier in this chapter and outlined in Fig. 72-3, cancer cells can become dependent upon signaling pathways containing activated oncogenes; this can effect proliferation (i.e., mutated KRAS, BRAF, overexpressed MYC, or activated tyrosine kinases). Additional genetic changes in malignant cells or unique features of tumors including defects in DNA repair (e.g., loss of *BRCA1* or *BRCA2* gene function), modifications in cell cycle control (e.g., changes in protein levels or mutations in cyclins and cyclin-dependent kinases), enhanced survival mechanisms (overexpression of Bcl-2 or NF-κB), altered cell metabolism (such as occurs when mutant KRAS enhances glucose uptake and aerobic glycolysis), tumor-stromal interactions, and angiogenesis (e.g., production of vascular endothelial growth factor [VEGF] in response to HIF-2α in RCC) can also be successfully exploited to relatively specifically target cancers. However, resistance to inhibition of specific oncogenic pathways almost always eventually develops. In addition, targeting defects in tumor-suppressor genes has been much more difficult, both because the target of mutation is often deleted and because it is much more difficult to restore normal function than to inhibit abnormal function of a protein.

Synthetic lethality occurs when loss of function in either of two or more genes individually has limited effects on cell survival but loss of function in both (or more) genes leads to cell death. In the case of oncogene addicted pathways, identifying genes that have a synthetic lethal relationship with the activated pathway may allow enhanced cell killing and decreased resistance by targeting those genes or their proteins. In the case of mutant tumor-suppressor genes, identifying genes that have a synthetic lethal relationship to those mutated pathways may allow targeting by inhibiting proteins required uniquely by those cells for survival or proliferation (Fig. 72-3). This is a much more tractable approach than attempting to repair normal function of the mutant suppressor gene itself. Examples of synthetic lethality with clinical impact have been identified. For instance, cells with mutations in the *BRCA1* or *BRCA2* tumor-suppressor genes (e.g., a subset of breast and ovarian cancers) are unable to repair DNA damage by homologous recombination. Poly-ADP ribose polymerase (PARP) is a family of proteins important for single-strand break (SSB) DNA repair. PARP inhibition results in selective killing of cancer cells that have lost *BRCA1* or *BRCA2* function. A number of PARP inhibitors have been approved for treatment of ovarian, breast, prostate, and pancreatic cancers that have mutations in *BRCA* genes, as well as for maintenance therapy of ovarian cancer and are likely to have activity in other tumors with defective DNA repair mechanisms. The concept of synthetic lethality provides a framework for genetic screens to identify other synthetic lethal combinations involving known tumor-suppressor genes and development of novel therapeutic agents to target dependent pathways. Other unique aspects of malignant tumors, including those outlined elsewhere in the chapter, may also be vulnerable to synthetic lethal interactions.

EPIGENETIC INFLUENCES ON CANCER GENE TRANSCRIPTION

Chromatin structure regulates the hierarchical order of sequential gene transcription that governs differentiation and tissue homeostasis.

Disruption of chromatin remodeling (the process of modifying chromatin structure to control exposure of specific genes to transcriptional proteins, thereby controlling the expression of those genes) leads to aberrant gene expression that can significantly alter the biology of cells including inducing proliferation or migration of cells. *Epigenetic* changes are those that alter the pattern of gene expression that persist across at least one cell division, but are not caused by changes in the DNA code. These include alterations of chromatin structure mediated by methylation of cytosine residues of DNA (primarily in context of CpG dinucleotides in somatic cells), modification of histones by altering acetylation or methylation, or changes in higher-order chromosome structure (Fig. 72-4). Appropriate control of DNA methylation is essential for normal cell function and development, and both altered methylation and hypomethylation of histones occur in cancers. Hypermethylation of DNA promoter regions is a common mechanism by which tumor-suppressor loci are epigenetically silenced in cancer cells. Thus, one allele of a tumor-suppressor gene may be inactivated by mutation or deletion, while expression of the other allele is epigenetically silenced, usually by methylation, leading to loss of gene function. Aberrant hypomethylation is also frequently found in a number of cancers consistent with the dysregulated pattern of gene transcription that is a hallmark of cancer cells, with some genes being inappropriately turned off while others are inappropriately turned on.

Acetylation of the amino terminus of the core histones H3 and H4 induces an open chromatin conformation that promotes transcription initiation. Histone acetylases are components of coactivator complexes recruited to promoter/enhancer regions by sequence-specific transcription factors during the activation of genes (Fig. 72-4). Histone deacetylases (HDACs; multiple HDACs are encoded in the human genome) are recruited to genes by transcriptional repressors and prevent the initiation of gene transcription. Methylated cytosine residues in promoter regions become associated with methyl cytosine-binding proteins that recruit protein complexes with HDAC activity. The balance between permissive and inhibitory chromatin structure is therefore largely determined by the activity of transcription factors in modulating the "histone code" and the methylation status of the genetic regulatory elements of genes.

The pattern of gene transcription is aberrant in all human cancers, and in many cases, epigenetic events are responsible. Epigenetic events play a critical role in carcinogenesis (e.g., long-lasting changes in methylation induced by smoking) and are found in premalignant lesions. Unlike genetic events that alter DNA primary structure (e.g., deletions), epigenetic changes are potentially reversible and appear amenable to therapeutic intervention. In certain human cancers, including a subset of pancreatic cancers and multiple myeloma, the p16^{Ink4a} promoter is inactivated by methylation, thus permitting the unchecked activity of CDK4/cyclin D and rendering pRb nonfunctional. In sporadic forms of renal, breast, and colon cancer, the von Hippel-Lindau (*VHL*), breast cancer 1 (*BRCA1*), and serine/threonine kinase 11 (*STK11*) genes, respectively, can be epigenetically silenced. Other targeted genes include the p15^{INK4b} CDK inhibitor, glutathione-S-transferase (which detoxifies reactive oxygen species [ROS]), and the E-cadherin molecule (important for junction formation between epithelial cells). Epigenetic silencing can affect genes involved in DNA repair, thus predisposing to further genetic damage. Examples include MLH1 (mutL homologue in sporadic colon cancers that have microsatellite instability) and MSH2 in a subset of hereditary nonpolyposis colon cancer patients who have a mutation in the 3' end of epithelial cell adhesion molecule (EPCAM). These are critical genes involved in repair of mismatched bases that occur during DNA synthesis, and their silencing can lead to mutations in the DNA.

Human leukemias often have chromosomal translocations that code for novel fusion proteins with activities that alter chromatin structure by interacting with HDACs or histone acetyl transferases (HATs). For example, the promyelocytic leukemia-retinoic acid receptor α (PML-RARα) fusion protein, generated by the t(15;17) translocation observed in most cases of acute promyelocytic leukemia (APL), binds to promoters containing retinoic acid response elements and recruits HDACs to these promoters, effectively inhibiting gene expression.

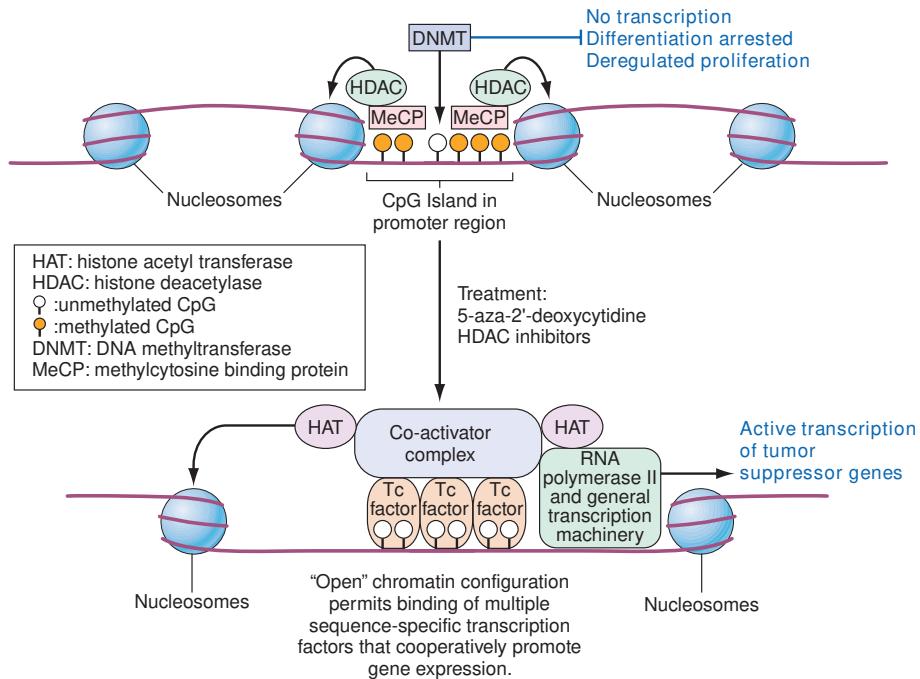


FIGURE 72-4 Epigenetic regulation of gene expression in cancer cells. Tumor-suppressor genes are often epigenetically silenced in cancer cells. In the upper portion, a CpG island within the promoter and enhancer regions of the gene has been methylated, resulting in the recruitment of methyl-cytosine binding proteins (MeCP) and complexes with histone deacetylase (HDAC) activity. Chromatin is in a condensed, nonpermissive conformation that inhibits transcription. Clinical trials are under way utilizing the combination of demethylating agents such as 5-aza-2'-deoxycytidine plus HDAC inhibitors, which together confer an open, permissive chromatin structure (*lower portion*). Transcription factors bind to specific DNA sequences in promoter regions and, through protein-protein interactions, recruit coactivator complexes containing histone acetyl transferase (HAT) activity. This enhances transcription initiation by RNA polymerase II and associated general transcription factors. The expression of the tumor-suppressor gene commences, with phenotypic changes that may include growth arrest, differentiation, or apoptosis.

This arrests differentiation at the promyelocyte stage and promotes tumor cell proliferation and survival. Treatment with pharmacologic doses of all-*trans* retinoic acid (ATRA), the ligand for RAR α , results in the release of HDAC activity and the recruitment of coactivators, which overcome the differentiation block. This induced differentiation of APL cells has improved treatment of these patients but also has led to a novel treatment toxicity when newly differentiated tumor cells infiltrate the lungs. ATRA represents a treatment paradigm for the reversal of epigenetic changes in cancer. Other leukemia-associated fusion proteins, such as Tel-acute myeloid leukemia (AML1), AML1-eight-twenty-one (ETO), and the MLL fusion proteins seen in acute myeloid leukemia (AML) and acute lymphocytic leukemia, also lead to repression through the HDAC complex. Therefore, efforts are ongoing to determine the structural basis for interactions between translocation fusion proteins and chromatin-remodeling proteins and to use this information to rationally design small molecules that will disrupt specific protein-protein associations, although this has proven to be technically difficult. Several drugs that block the enzymatic activity of HDACs (HDAC inhibitors [HDACis]) are approved for cancer treatment, and others are being tested. HDACis have demonstrated sufficient antitumor activity against cutaneous T-cell lymphoma (vorinostat, romidepsin), peripheral T-cell lymphoma (romidepsin, belinostat), and multiple myeloma (panobinostat) to be approved by the U.S. Food and Drug Administration (FDA).

HDACis have also demonstrated antitumor activity in clinical studies against some solid tumors, and additional studies are ongoing. HDACis may target cancer cells via a number of mechanisms including both epigenetic modulation via histone acetylation and effects on other proteins that are acetylated. The pleiotropic effects of some HDACis include enhancement of apoptosis by upregulation of a number of proteins that enhance apoptosis including death receptors (DR4/5, FAS, and their ligands) and downregulation of proteins that

inhibit apoptosis (e.g., X-linked inhibitor of apoptosis [XIAP]); upregulation of proteins that inhibit cell cycle progression (e.g., p21Cip1/Waf1); inhibition of DNA repair and generation of ROS leading to increased DNA damage; and disruption of the chaperone protein HSP90.

Efforts are also under way to modulate other epigenetic processes such as reversing the hypermethylation of CpG islands that characterizes many malignancies. Drugs that induce DNA demethylation, such as 5-aza-2-deoxycytidine, can lead to reexpression of silenced genes in cancer cells with restoration of function, and 5-aza-2-deoxycytidine is approved for use in myelodysplastic syndrome. However, 5-aza-2-deoxycytidine has limited aqueous solubility and is myelosuppressive, limiting its usefulness. Other inhibitors of DNA methyltransferases are in development. In ongoing clinical trials, inhibitors of DNA methylation are being combined with HDACis, with the idea that reversing coexisting epigenetic changes will reverse the deregulated patterns of gene transcription in cancer cells.

Epigenetic gene regulation can also occur via microRNAs or long noncoding RNAs (lncRNA). MicroRNAs (miRNA) are short (average 22 nucleotides in length) RNA molecules that silence gene expression after transcription by binding and inhibiting the translation or promoting the degradation of mRNA transcripts. It is estimated that >1000 miRNAs are encoded in the human genome. Each tissue has a distinctive repertoire of miRNA expression, and this pattern is altered in specific ways in cancers. Specific correlations between miRNA expression and tumor biology and clinical behavior are continuing to emerge. Therapies targeting miRNAs are not currently at hand but represent an ongoing area of treatment development. lncRNAs are longer than 200 nucleotides and comprise the largest group of noncoding RNAs. Some of them have been shown to play important roles in gene regulation. The potential for altering these RNAs for therapeutic benefit is an area of active investigation.

APOPTOSIS AND OTHER MECHANISMS OF CELL DEATH

Tissue homeostasis requires a balance between the death of aged, terminally differentiated cells or severely damaged cells and their renewal by proliferation of committed progenitors. Genetic damage to growth-regulating genes of stem cells could lead to catastrophic results for the host as a whole. Thus, genetic events causing activation of oncogenes or loss of tumor suppressors, which would be predicted to lead to unregulated cell proliferation unless corrected, usually activate signal transduction pathways that block aberrant cell proliferation. These pathways can lead to a form of programmed cell death (*apoptosis*) or irreversible growth arrest (*senescence*). Much as a panoply of intra- and extracellular signals impinge upon the core cell cycle machinery to regulate cell division, so too these signals are transmitted to a core enzymatic machinery that regulates cell death and survival.

Apoptosis is a tightly regulated process induced by two main pathways (Fig. 72-5). The extrinsic pathway of apoptosis is activated by

cross-linking members of the tumor necrosis factor (TNF) receptor superfamily, such as CD95 (Fas) and death receptors DR4 and DR5, by their ligands, Fas ligand or TRAIL (TNF-related apoptosis-inducing ligand), respectively. This induces the association of FADD (Fas-associated death domain) and pro-caspase-8 to death domain motifs of the receptors. Caspase-8 is activated and then cleaves and activates effector caspases-3 and -7, which then target cellular constituents (including caspase-activated DNase, cytoskeletal proteins, and a number of regulatory proteins), inducing the morphologic appearance characteristic of apoptosis, which pathologists term *karyorrhexis*. The intrinsic pathway of apoptosis is initiated by the release of cytochrome c and SMAC (second mitochondrial activator of caspases) from the mitochondrial intermembrane space in response to a variety of noxious stimuli, including DNA damage, loss of adherence to the extracellular matrix (ECM), oncogene-induced proliferation, and growth factor deprivation. Upon release into the cytoplasm, cytochrome c associates with dATP, pro-caspase-9, and the adaptor protein APAF-1, leading to the

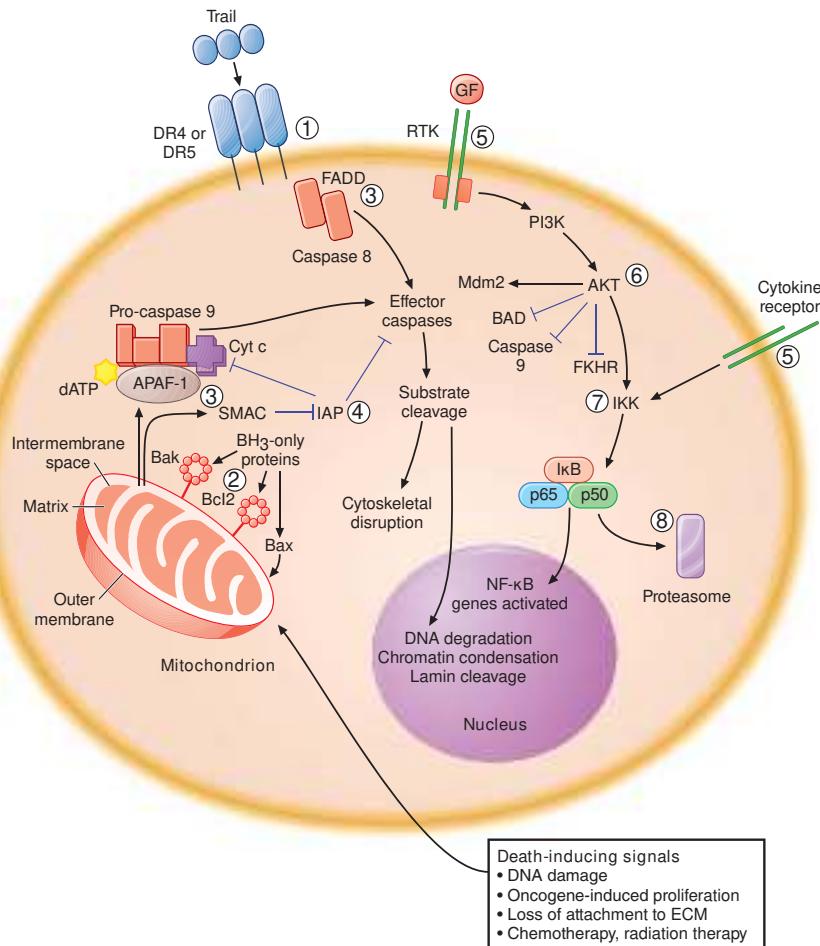


FIGURE 72-5 Therapeutic strategies to overcome aberrant survival pathways in cancer cells. 1. The extrinsic pathway of apoptosis can be selectively induced in cancer cells by TRAIL (the ligand for death receptors 4 and 5) or by agonistic monoclonal antibodies. 2. Inhibition of antiapoptotic Bcl-2 family members with antisense oligonucleotides or inhibitors of the BH₃-binding pocket will promote formation of Bak- or Bax-induced pores in the mitochondrial outer membrane. 3. Epigenetic silencing of APAF-1, caspase-8, and other proteins can be overcome using demethylating agents and inhibitors of histone deacetylases. 4. Inhibitor of apoptosis proteins (IAP) blocks activation of caspases; small-molecule inhibitors of IAP function (mimicking SMAC action) should lower the threshold for apoptosis. 5. Signal transduction pathways originating with activation of receptor tyrosine kinase receptors (RTKs) or cytokine receptors promote survival of cancer cells by a number of mechanisms. Inhibiting receptor function with monoclonal antibodies, such as trastuzumab or cetuximab, or inhibiting kinase activity with small-molecule inhibitors can block the pathway. 6. The Akt kinase phosphorylates many regulators of apoptosis to promote cell survival; inhibitors of Akt may render tumor cells more sensitive to apoptosis-inducing signals; however, the possibility of toxicity to normal cells may limit the therapeutic value of these agents. 7 and 8. Activation of the transcription factor NF-κB (composed of p65 and p50 subunits) occurs when its inhibitor, IκB, is phosphorylated by IκB-kinase (IKK), with subsequent degradation of IκB by the proteasome. Inhibition of IKK activity should selectively block the activation of NF-κB target genes, many of which promote cell survival. Inhibitors of proteasome function are U.S. Food and Drug Administration approved and may work in part by preventing destruction of IκB, thus blocking NF-κB nuclear localization. NF-κB is unlikely to be the only target for proteasome inhibitors.

sequential activation of caspase-9 and effector caspases. SMAC binds to and blocks the function of inhibitor of apoptosis proteins (IAP), negative regulators of caspase activation.

The release of apoptosis-inducing proteins from the mitochondria is regulated by pro- and antiapoptotic members of the Bcl-2 family. Antiapoptotic members (e.g., Bcl-2, Bcl-XL, and Mcl-1) associate with the mitochondrial outer membrane via their carboxyl termini, exposing to the cytoplasm a hydrophobic binding pocket composed of Bcl-2 homology (BH) domains 1, 2, and 3 that is crucial for their activity. Perturbations of normal physiologic processes in specific cellular compartments lead to the activation of BH3-only proapoptotic family members (e.g., Bad, Bim, Bid, Puma, Noxa, and others) that can alter the conformation of the outer-membrane proteins Bax and Bak, which then oligomerize to form pores in the mitochondrial outer membrane resulting in cytochrome c release. If proteins comprised only by BH3 domains are sequestered by Bcl-2, Bcl-XL, or Mcl-1, pores do not form and apoptosis-inducing proteins are not released from the mitochondria. The ratio of levels of antiapoptotic Bcl-2 family members and the levels of proapoptotic BH3-only proteins at the mitochondrial membrane determines the activation state of the intrinsic pathway. The mitochondrion must therefore be recognized not only as an organelle with vital roles in intermediary metabolism and oxidative phosphorylation but also as a central regulatory structure of the apoptotic process.

The evolution of tumor cells to a more malignant phenotype requires the acquisition of genetic changes that subvert apoptosis pathways and promote cancer cell survival and resistance to anticancer therapies. However, cancer cells may be more vulnerable than normal cells to therapeutic interventions that target the apoptosis pathways that cancer cells depend upon. For instance, overexpression of Bcl-2 as a result of the t(14;18) translocation contributes to follicular lymphoma, and it is highly expressed in many lymphoid malignancies including chronic lymphocytic leukemia (CLL). Upregulation of Bcl-2 expression is also observed in other cancers including prostate, breast, and lung cancers and melanoma. Targeting of antiapoptotic Bcl-2 family members has been accomplished by the identification of several low-molecular-weight compounds that bind to the hydrophobic pockets of either Bcl-2 or Bcl-XL and block their ability to associate with death-inducing BH3-only proteins. An oral BH3 mimetic inhibitor of BCL-2, venetoclax, is approved for use in patients with refractory CLL with 17p deletion and is active in acute myeloid leukemia.

Preclinical studies targeting death receptors DR4 and -5 have demonstrated that recombinant, soluble, human TRAIL or humanized monoclonal antibodies with agonist activity against DR4 or -5 can induce apoptosis of tumor cells while sparing normal cells. The mechanisms for this selectivity may include expression of decoy receptors or elevated levels of intracellular inhibitors (such as FLIP, which competes with caspase-8 for FADD) by normal cells but not tumor cells. Synergy has been shown between TRAIL-induced apoptosis and chemotherapeutic agents in some preclinical studies. However, studies have not yet shown significant clinical activity of approaches targeting the TRAIL pathway.

Many of the signal transduction pathways perturbed in cancer promote tumor cell survival (Fig. 72-5). These include activation of the PI3K/Akt pathway, increased levels of the NF- κ B transcription factor, and epigenetic silencing of genes such as APAF-1 (apoptosis protease activating factor-1 involved in activating caspase-9 and essential for apoptosis) and caspase-8. Each of these pathways is a target for therapeutic agents that, in addition to affecting cancer cell proliferation or gene expression, may render cancer cells more susceptible to apoptosis, thus promoting synergy when combined with other chemotherapeutic agents.

Some tumor cells resist drug-induced apoptosis indirectly by eliminating the noxious stimulus-inducing apoptosis through expression of one or more members of the ABC (ATP-binding cassette proteins) family of ATP-dependent efflux pumps that mediate the multidrug-resistance (MDR) phenotype. The prototype member of this family, P-glycoprotein (PGP), spans the plasma membrane 12 times and has two ATP-binding sites. Hydrophobic drugs (e.g., anthracyclines and vinca alkaloids) are recognized by PGP as they enter the cell and are

pumped out. Numerous clinical studies have failed to demonstrate that drug resistance can be overcome using inhibitors of PGP. However, ABC transporters have different substrate specificities, and inhibition of a single family member may not be sufficient to overcome the MDR phenotype. Efforts to reverse PGP-mediated drug resistance continue.

Cells, including cancer cells, can also undergo other mechanisms of cell death including *autophagy* (degradation of proteins and organelles by lysosomal proteases) and *necrosis* (digestion of cellular components and rupturing of the cell membrane). Necrosis usually occurs in response to external forces resulting in release of cellular components, which leads to inflammation and damage to surrounding tissues. Although necrosis was thought to be unprogrammed, evidence now suggests that at least some aspects may also be programmed. The exact role of necrosis in cancer cell death in various settings is still being determined. In addition to its role in cell death, autophagy can also serve as a homeostatic mechanism to promote survival for the cell by recycling cellular components to provide necessary energy. The mechanisms that control the balance between enhancing survival versus leading to cell death are still not fully understood. Autophagy appears to play conflicting roles in the development and survival of cancer. Early in the carcinogenic process, it can act as a tumor suppressor by preventing the cell from accumulating abnormal proteins and organelles. However, in established tumors, it may serve as a mechanism of survival for cancer cells when they are stressed by damage such as from chemotherapy. Preclinical studies have indicated that inhibition of this process can enhance the sensitivity of cancer cells to chemotherapy or radiation therapy, and ongoing trials are evaluating inhibitors of autophagy in combination with chemotherapy and/or radiation therapy. Better understanding of the factors that control the survival-promoting versus death-inducing aspects of autophagy is required in order to know how to best manipulate it for therapeutic benefit.

METASTASIS

The metastatic process accounts for the vast majority of deaths from solid tumors, and therefore, an understanding of this process is critical for improvements in survival from cancer. The biology of metastasis is complex and requires multiple steps. The initial step involves cell migration and invasion through the ECM. The three major features of tissue invasion are cell adhesion to the basement membrane, local proteolysis of the membrane, and movement of the cell through the rent in the membrane and the ECM. Cells that lose contact with the ECM normally undergo programmed cell death (anoikis-apoptosis induced by the loss of contact), and this process has to be suppressed in cells that metastasize. Another process important for many, but not necessarily all, metastasizing epithelial cancer cells is epithelial-mesenchymal transition (EMT). This is a process by which cells lose their epithelial properties and gain mesenchymal properties. This normally occurs during the developmental process in embryos, allowing cells to migrate to their appropriate destinations in the embryo. It also occurs in wound healing, tissue regeneration, and fibrotic reactions, but in all of these processes, cells stop proliferating when the process is complete. Malignant cells that metastasize often undergo EMT as an important step in that process but retain the capacity for unregulated proliferation. However, there is evidence that not all metastasizing cancer cells require EMT, and the exact role of EMT in different metastasizing cancer cells continues to be elucidated. Malignant cells that gain access to the circulation must then repeat those steps at a remote site, find a hospitable niche in a foreign tissue, avoid detection and elimination by host defenses including the immune system, and induce the growth of new blood vessels. Some metastatic cells occur as oligoclonal clusters, which appear to be more potent in establishing metastasis than single cells, perhaps, in part, through differential and cooperative effects in evading host defenses. The rate-limiting step for metastasis is the ability for tumor cells to survive and expand in the novel microenvironment of the metastatic site, and multiple host-tumor interactions determine the ultimate outcome (Fig. 72-6). Few drugs have been developed to attempt to directly target the process of metastasis, in part because the specifics of the critical steps in the process that would be potentially good targets for drugs are still being

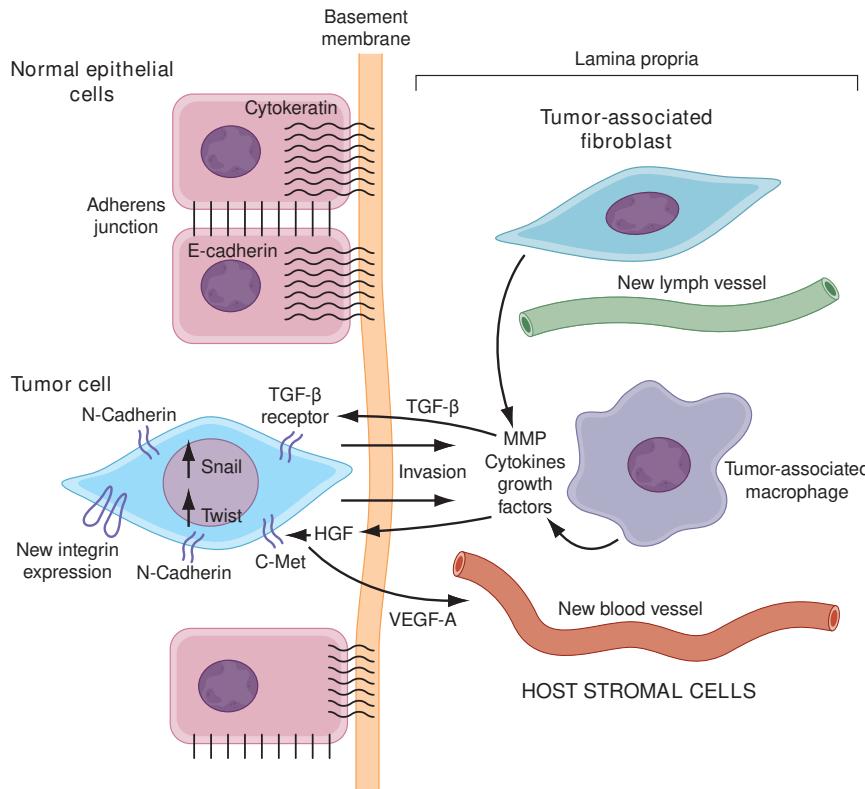


FIGURE 72-6 Oncogene signaling pathways are activated during tumor progression and promote metastatic potential. This figure shows a cancer cell that has undergone epithelial to mesenchymal transition (EMT) under the influence of several environmental signals. Critical components include activated transforming growth factor beta (TGF- β) and the hepatocyte growth factor (HGF)/c-Met pathways, as well as changes in the expression of adhesion molecules that mediate cell-cell and cell-extracellular matrix interactions. Important changes in gene expression are mediated by the Snail and Twist family of transcriptional repressors (whose expression is induced by the oncogenic pathways), leading to reduced expression of E-cadherin, a key component of adherens junctions between epithelial cells. This, in conjunction with upregulation of N-cadherin, a change in the pattern of expression of integrins (which mediate cell-extracellular matrix associations that are important for cell motility), and a switch in intermediate filament expression from cytokeratin to vimentin, results in the phenotypic change from adherent highly organized epithelial cells to motile and invasive cells with a fibroblast or mesenchymal morphology. EMT is thought to be an important step leading to metastasis in some human cancers. Host stromal cells, including tumor-associated fibroblasts and macrophages, play an important role in modulating tumor cell behavior through secretion of growth factors and proangiogenic cytokines, and matrix metalloproteinases that degrade the basement membrane. VEGF-A, -C, and -D are produced by tumor cells and stromal cells in response to hypoxemia or oncogenic signals and induce production of new blood vessels and lymphatic channels through which tumor cells metastasize to lymph nodes or tissues.

identified. However, a number of potential targets are known. HER2 can enhance the metastatic potential of breast cancer cells, and as discussed above, the monoclonal antibody trastuzumab, which targets HER2, improves survival in the adjuvant setting for HER2-positive breast cancer patients. A number of other potential targets that increase metastatic potential of cells in preclinical studies include HIF-1 and -2, transcription factors induced by hypoxia within tumors, growth factors (e.g., cMET and VEGFR), oncogenes (e.g., SRC), adhesion molecules (e.g., focal adhesion kinase [FAK]), ECM proteins (e.g., matrix metalloproteinases 1 and 2), and inflammatory molecules (e.g., COX-2).

The metastatic phenotype is likely restricted to a fraction of tumor cells (Fig. 72-6). A number of genetic and epigenetic changes are required for tumor cells to be able to metastasize, including activation of metastatic-promoting genes and inhibition of genes that suppress the metastatic ability. Given the role of microRNAs in controlling gene expression (see epigenetic section) including those critical to the metastatic process, efforts are under way to modulate these to try to inhibit metastasis. Cells with metastatic capability frequently express chemokine receptors that are likely important in the metastatic process. A number of candidate metastasis-suppressor genes have been identified, including genes coding for proteins that enhance apoptosis, suppress cell division, are involved in the interactions of cells with each other or the ECM, or suppress cell migration. The loss of function of these genes enhances metastasis. Gene expression profiling is being used to

study the metastatic process and other properties of tumor cells that may predict susceptibilities.

An example of the ability of malignant cells to survive and grow in a novel microenvironment is bone metastases. Bone metastases can be extremely painful, cause fractures of weight-bearing bones, can lead to hypercalcemia, and are a major cause of morbidity for cancer patients. Osteoclasts and their monocyte-derived precursors express the surface receptor RANK (receptor activator of NF- κ B), which is required for terminal differentiation and activation of osteoclasts. Osteoblasts and other stromal cells express RANK ligand (RANKL), as both a membrane-bound and soluble cytokine. Osteoprotegerin (OPG), a soluble receptor for RANKL produced by stromal cells, acts as a decoy receptor to inhibit RANK activation. The relative balance of RANKL and OPG determines the activation state of RANK on osteoclasts. Many tumors increase osteoclast activity by secretion of substances such as parathyroid hormone (PTH), PTH-related peptide, interleukin (IL) 1, or Mip1 that perturb the homeostatic balance of bone remodeling by increasing RANK signaling. One example is multiple myeloma, where tumor cell-stromal cell interactions activate osteoclasts and inhibit osteoblasts, leading to the development of multiple lytic bone lesions. Inhibition of RANKL by an antibody (denosumab) can prevent further bone destruction. Bisphosphonates are also effective inhibitors of osteoclast function that are used in the treatment of cancer patients with bone metastases.

CANCER STEM CELLS

Normal tissues have stem cells capable of self-renewal and repairing damaged tissue, whereas the majority of cells in normal tissues do not have this capacity. Similarly, only a small proportion of the cells within a tumor are capable of initiating colonies *in vitro* or forming tumors at high efficiency when injected into immunocompromised NOD/SCID mice. For example, AML and CML have a small population of cells (estimated to be <1%) that have properties of stem cells, such as unlimited self-renewal and the capacity to cause leukemia when serially transplanted in mice. These cells have an undifferentiated phenotype (Thy1+CD34+CD38- and do not express other differentiation markers) and resemble normal stem cells in many ways but are no longer under homeostatic control (Fig. 72-7). Solid tumors may also contain a population of stem cells. It is not yet known how often cancers may originate within a stem cell population. Cancer stem cells, like their normal counterparts, have unlimited proliferative capacity and paradoxically traverse the cell cycle at a slow rate; cancer growth occurs largely due to expansion of the stem cell pool, the unregulated proliferation of an amplifying population, and failure of apoptosis pathways (Fig. 72-7). Slow cell cycle progression and high levels of expression of antiapoptotic Bcl-2 family members and drug efflux pumps of the MDR family render cancer stem cells less vulnerable to cancer chemotherapy or radiation therapy. Implicit in the cancer stem cell hypothesis is the idea that failure to cure most human cancers is due to the fact that current therapeutic agents do not kill the stem cells. Identification and isolation of cancer stem cells will allow determination of the aberrant signaling pathways that distinguish these cells from

normal tissue stem cells. These would serve as potential therapeutic targets. Evidence that cells with stem cell properties can arise from other epithelial cells within the cancer by processes such as epithelial-mesenchymal transition also implies that it is essential to treat all of the cancer cells, and not just those with current stem cell-like properties, in order to eliminate the self-renewing cancer cell population. The exact nature of cancer stem cells remains an area of investigation. One of the unanswered questions is the exact origin of cancer stem cells for the different cancers.

PLASTICITY AND RESISTANCE

Cancer cells, and especially stem cells, have the capacity for significant plasticity allowing them to alter multiple aspects of cell biology in response to external factors (e.g., chemotherapy, radiation therapy, inflammation, immune response). In addition, heterogeneity between the different clones of cells within the tumor population and their interactions with each other and the tumor microenvironment provides the tumor with the capacity for significant plasticity in dealing with both internal and external stresses. Thus, a major problem in cancer therapy is that malignancies have a wide spectrum of mechanisms for both initial and adaptive resistance to treatments. These include inhibiting drug delivery to the cancer cells, blocking drug uptake and retention, increasing drug metabolism, altering levels of target proteins making them less sensitive to drugs, acquiring mutations in target proteins making them no longer sensitive to the drug, modifying metabolism and cell signaling pathways, using alternate signaling pathways, adjusting the cell replication process including mechanisms

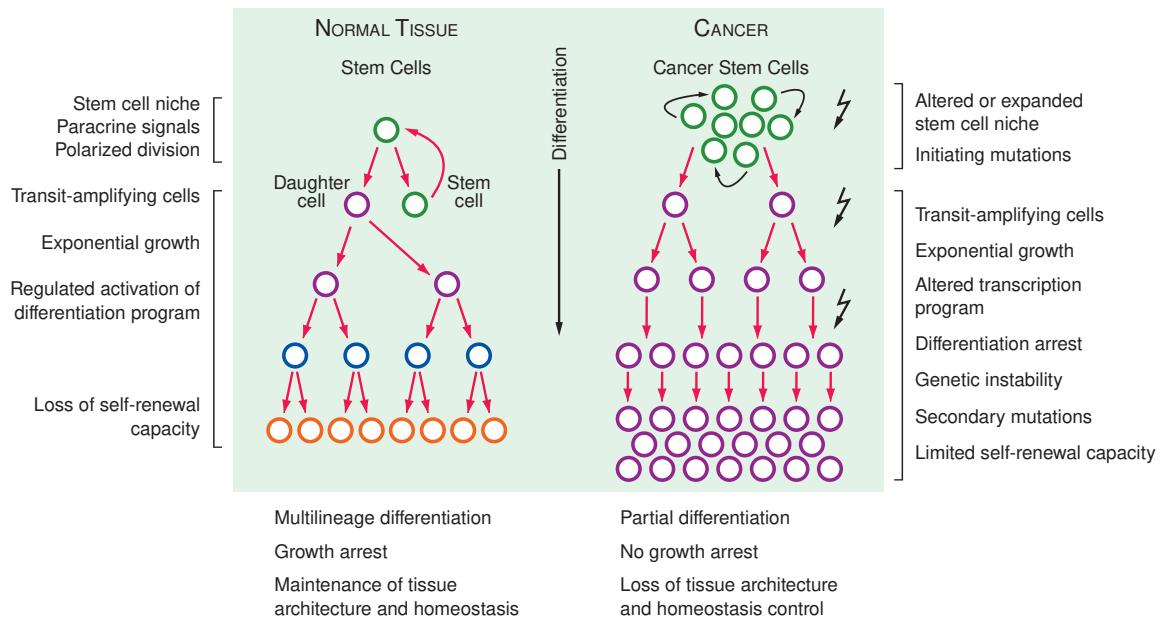


FIGURE 72-7 Cancer stem cells play a critical role in the initiation, progression, and resistance to therapy of malignant neoplasms. In normal tissues (*left*), homeostasis is maintained by asymmetric division of stem cells leading to one progeny cell that will differentiate and one cell that will maintain the stem cell pool. This occurs within highly specific niches unique to each tissue, such as in close apposition to osteoblasts in bone marrow, or at the base of crypts in the colon. Here, paracrine signals from stromal cells, such as sonic hedgehog or Notch ligands, as well as upregulation of β -catenin and telomerase, help to maintain stem cell features of unlimited self-renewal while preventing differentiation or cell death. This occurs in part through upregulation of the transcriptional repressor Brm-1 and inhibition of the p16^{Ink4a}/Arf and p53 pathways. Daughter cells leave the stem cell niche and enter a proliferative phase (referred to as *transit-amplifying*) for a specified number of cell divisions, during which time a developmental program is activated, eventually giving rise to fully differentiated cells that have lost proliferative potential. Cell renewal equals cell death, and homeostasis is maintained. In this hierarchical system, only stem cells are long-lived. The hypothesis is that cancers harbor stem cells that make up a small fraction (i.e., 0.001–1%) of all cancer cells. These cells share several features with normal stem cells, including an undifferentiated phenotype, unlimited self-renewal potential, and a capacity for some degree of differentiation; however, due to initiating mutations (mutations are indicated by lightning bolts), they are no longer regulated by environmental cues. The cancer stem cell pool is expanded, and rapidly proliferating progeny, through additional mutations, may attain stem cell properties, although most of this population is thought to have a limited proliferative capacity. Differentiation programs are dysfunctional due to reprogramming of the pattern of gene transcription by oncogenic signaling pathways. Within the cancer transit-amplifying population, genomic instability generates aneuploidy and clonal heterogeneity as cells attain a fully malignant phenotype with metastatic potential. The cancer stem cell hypothesis has led to the idea that current cancer therapies may be effective at killing the bulk of tumor cells but do not kill tumor stem cells, leading to a regrowth of tumors that is manifested as tumor recurrence or disease progression. Research is in progress to identify unique molecular features of cancer stem cells that can lead to their direct targeting by novel therapeutic agents.

by which the cell deals with DNA damage, inhibiting apoptosis, and evading the immune system. Thus, most metastatic cancers (except those curable with chemotherapy such as germ cell tumors) eventually become resistant to the therapy being utilized. Overcoming resistance is a major area of research.

CANCER METABOLISM

One of the distinguishing characteristics of cancer cells is that they have altered metabolism as compared with normal cells in supporting survival, their high rates of proliferation, and ability to metastasize. Complicating studies evaluating metabolic differences between normal and malignant cells is that there is heterogeneity in metabolism between different cells within a cancer. Malignant cells must focus a significant fraction of their energy resources into synthesis of proteins and other molecules (building blocks required for the production of new cells) while still maintaining sufficient ATP production to survive and grow. Although normal proliferating cells also have similar needs, there are differences in how cancer cells metabolize glucose and a number of other compounds including the amino acid glutamine as compared to normal cells in part because of genetic and epigenetic changes within cancer cells but also likely due to differences in the environments of cancer and normal cells. Many cancer cells utilize aerobic glycolysis (the Warburg effect) (Fig. 72-8) to metabolize glucose, leading to increased lactic acid production, whereas normal cells utilize oxidative phosphorylation in mitochondria under aerobic conditions, a much more efficient process for generating ATP for energy utilization but one that does not produce the same level of building blocks needed for new cells. One consequence is increased glucose uptake and utilization by cancer cells, a fact utilized in fluorodeoxyglucose (FDG)-positron emission tomography (PET) scanning to detect tumors. A number of proteins in cancer cells, including cMYC, HIF1, RAS, p53, pRB, and AKT, are involved in modulating glycolytic processes and controlling the Warburg effect. Although these pathways remain difficult to target therapeutically, both the PI3K pathway with signaling through mTOR and the AMP-activated kinase (AMPK) pathway that inhibits mTORC1 (a protein complex that includes mTOR) are important in controlling the glycolytic process and thus provide potential targets for inhibiting this process. An inhibitor of mTOR is approved for use against RCC (temsirolimus), and another inhibitor (everolimus) has activity against breast and neuroendocrine cancer and RCC. Other mTOR inhibitors

are in trials, and modulators of AMPK are being investigated. The inefficient utilization of glucose by malignant cells also leads to a need for alternative metabolic pathways for other compounds as well, one of which is glutamine. Similar to glucose, this provides both a source for structural molecules as well as energy production. Similarly to glucose, glutamine is also inefficiently utilized by cancer cells. Cancer cells can also take up nutrients released by surrounding cells and tissues, increasing the complexity of successfully therapeutically inhibiting metabolism in cancer.

Mutations in genes involved in the metabolic process occur in a number of cancers. Among the most frequently found to date are mutations in isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2). These have been most commonly seen in gliomas, AMLs, and intrahepatic cholangiocarcinomas. These mutations lead to the production of an oncometabolite (2-hydroxyglutarate [2HG]) instead of the normal product α -ketoglutarate. Although the exact mechanisms of oncogenesis by 2HG are still being elucidated, α -ketoglutarate is a key cofactor for a number of dioxygenases involved in controlling DNA methylation. 2HG can act as a competitive inhibitor for α -ketoglutarate, leading to alterations in methylation status (primarily hypermethylation) of genes (leading to epigenetic changes) that can have profound effects on a number of cellular processes including differentiation. Inhibitors of mutant IDH1 and IDH2 are approved for treating IDH mutant AML and are in clinical trials for glioblastomas and cholangiocarcinomas.

Much needs to be learned about the specific differences in metabolism between cancer cells and normal cells; however, even with the currently limited state of knowledge, modulators of metabolism are being tested clinically. The first of these is the antidiabetic agent metformin, both alone and in combination with chemotherapeutic agents. Metformin inhibits gluconeogenesis and may have direct effects on tumor cells by activating AMPK, a serine/threonine protein kinase that is downstream of the LKB1 tumor suppressor, and thus inhibiting mTOR complex 1 (mTORC1). This leads to decreased protein synthesis and proliferation. Studies to date have not yet established metformin to have a clear role as an anticancer agent.

TUMOR MICROENVIRONMENT, ANGIOGENESIS, AND IMMUNE EVASION

Tumors consist not only of malignant cells but also of a complex microenvironment including many other types of cells (including lymphocytes,

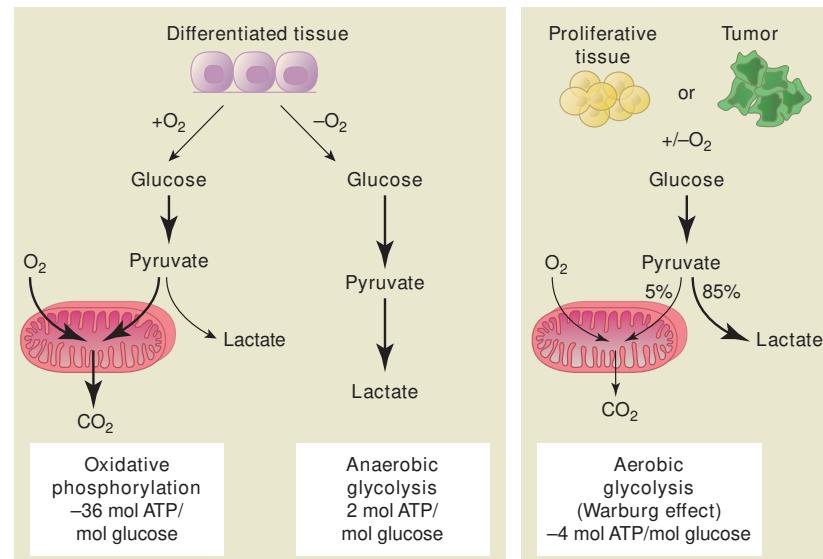


FIGURE 72-8 Warburg versus oxidative phosphorylation. In most normal tissues, the vast majority of cells are differentiated and dedicated to a particular function within the organ in which they reside. The metabolic needs are mainly for energy and not for building blocks for new cells. In these tissues, ATP is generated by oxidative phosphorylation that efficiently generates about 36 molecules of ATP for each molecule of glucose metabolized. By contrast, proliferative tumor tissues, especially in the setting of hypoxia, a typical condition within tumors, use aerobic glycolysis to generate energy for cell survival and generation of building blocks for new cells.

macrophages, myeloid cells, other inflammatory cells, fibroblasts, and fat cells), ECM, secreted factors (including growth factors and hormones), reactive oxygen and nitrogen species, mechanical factors, blood vessels, and lymphatics. This microenvironment is not static but rather is dynamic and continually evolving. Both the complexity and dynamic nature of the microenvironment enhance the difficulty of treating tumors. The microenvironment can contribute to resistance to anticancer therapies through a number of mechanisms.

OBESITY AND CANCER

Significant evidence links obesity and the increased risk of developing certain cancers including postmenopausal breast, colorectal, ovarian, endometrial, esophageal, gallbladder, thyroid, and kidney cancers, among others. Less certain are the mechanisms responsible for this risk. As outlined above, cancers arise in an environment with multiple factors, many of which can stimulate cell proliferation. Obesity impacts a variety of factors including hormonal factors, altered metabolism (especially adipose metabolism), and mediators of inflammatory response that all can impact the development of malignancy. Obesity is associated with a number of hormonal changes including high insulin, glucagon, and leptin levels that can stimulate growth of cells. It also leads to insulin resistance, which may contribute to cancer cell development, in part by increasing insulin-like growth factor-1 (IGF-1) levels. Obesity also leads to alterations in adipose, including fatty acid, metabolism, with production of compounds important for energy metabolism as well as for membrane function within cells that may contribute to carcinogenic process. Obesity contributes to an inflammatory environment in a variety of ways including increased levels of inflammatory proteins such as IL-6 and TNF- α . In terms of impact on survival with cancer, data primarily from breast cancer suggest that obesity is associated with decreased survival likely due, at least in part, to the impact of obesity on hormonal factors in development of certain breast cancers, although this may be limited to subsets of breast cancer patients. Some studies have suggested, paradoxically, that obesity may be associated with improved survival in some patients such as those with advanced-stage colorectal cancer. Clearly, the biology of the association between obesity and cancer and its impact on disease outcome is complex, and additional studies are necessary to better define the mechanisms involved.

MECHANISMS OF TUMOR VESSEL FORMATION

One of the critical elements of tumor cell proliferation is delivery of oxygen, nutrients, and circulating factors important for growth and survival. Thus, a critical element in growth of primary tumors and formation of metastatic sites is the *angiogenic switch*: the ability of the tumor to promote the formation of new blood vessels, including the recruitment of vascular endothelial cells (ECs). The angiogenic switch is a phase in tumor development when the dynamic balance of pro- and antiangiogenic factors is tipped in favor of vessel formation by the effects of the tumor on its immediate environment. Stimuli for tumor angiogenesis include hypoxemia, inflammation, and genetic lesions in oncogenes or tumor suppressors that alter tumor cell gene expression. Angiogenesis consists of several steps, including the stimulation of ECs by growth factors, degradation of the ECM by proteases, proliferation and migration of ECs into the tumor, and the eventual formation of new capillary tubes.

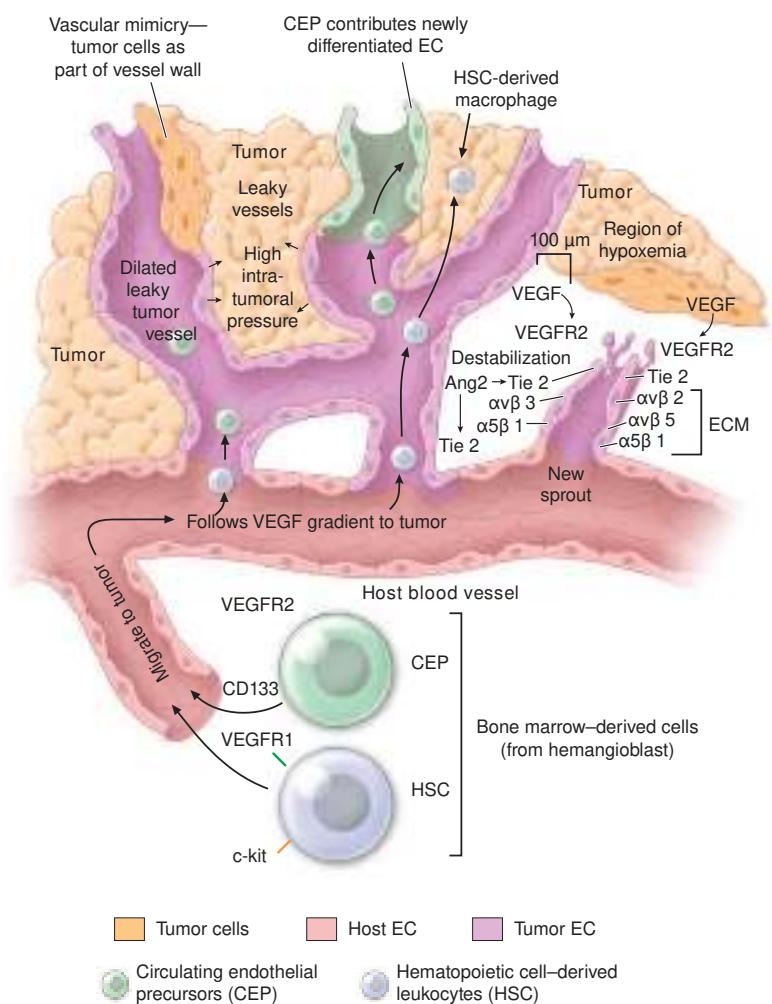


FIGURE 72-9 Tumor angiogenesis is a complex process involving many different cell types that must proliferate, migrate, invade, and differentiate in response to signals from the tumor microenvironment. Endothelial cells (ECs) sprout from host vessels in response to VEGF, bFGF, Ang2, and other proangiogenic stimuli. Sprouting is stimulated by VEGF/VEGFR2, Ang2/Tie-2, and integrin/extracellular matrix (ECM) interactions. Bone marrow-derived circulating endothelial precursors (CEPs) migrate to the tumor in response to VEGF and differentiate into ECs, while hematopoietic stem cells differentiate into leukocytes, including tumor-associated macrophages that secrete angiogenic growth factors and produce matrix metalloproteinases (MMPs) that remodel the ECM and release bound growth factors. Tumor cells themselves may directly form parts of vascular channels within tumors. The pattern of vessel formation is haphazard: vessels are tortuous, dilated, leaky, and branch in random ways. This leads to uneven blood flow within the tumor, with areas of acidosis and hypoxemia (which stimulate release of angiogenic factors) and high intratumoral pressures that inhibit delivery of therapeutic agents.

Tumors use a number of mechanisms to promote vascularization, subverting normal angiogenic processes for this purpose (Fig. 72-9). Primary or metastatic tumor cells sometimes arise in proximity to host blood vessels and grow around these vessels, parasitizing nutrients by co-opting the local blood supply. However, most tumor blood vessels arise by the process of sprouting, in which tumors secrete trophic angiogenic molecules, the most potent being VEGFs, that induce the proliferation and migration of host ECs into the tumor. Sprouting in normal and pathogenic angiogenesis is regulated by three families of transmembrane RTKs expressed on ECs and their ligands (VEGFs, angiopoietins, ephrins; Fig. 72-10), which are produced by tumor cells, inflammatory cells, or stromal cells in the tumor microenvironment.

Central to the angiogenic response are hypoxia-inducible factors (HIFs; especially 1 and 2), which are transcription factors that normally, in response to hypoxia, stimulate the transcription of a large number of genes responsive to hypoxia, including genes involved in metabolism as

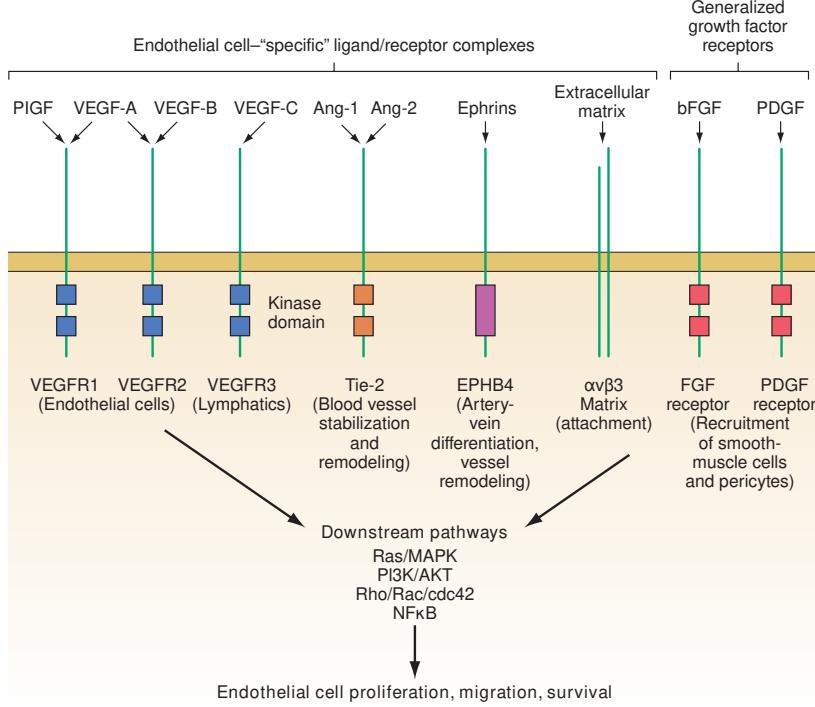


FIGURE 72-10 Critical molecular determinants of endothelial cell biology. Angiogenic endothelium expresses a number of receptors not found on resting endothelium. These include receptor tyrosine kinases (RTKs) and integrins that bind to the extracellular matrix and mediate endothelial cell (EC) adhesion, migration, and invasion. ECs also express RTKs (i.e., the fibroblast growth factor [FGF] and platelet-derived growth factor [PDGF] receptors) that are found on many other cell types. Critical functions mediated by activated RTK include proliferation, migration, and enhanced survival of endothelial cells, as well as regulation of the recruitment of perivascular cells and bloodborne circulating endothelial precursors and hematopoietic stem cells to the tumor. Intracellular signaling via EC-specific RTK utilizes molecular pathways that may be targets for future antiangiogenic therapies.

well as angiogenesis. HIF1 has a bigger role in stimulating metabolism (glycogenesis), whereas HIF2 plays a bigger role in angiogenesis. HIF protein function can also be enhanced in a number of ways in cancer not involving hypoxia, including mutations in the von Hippel-Lindau tumor suppressor gene (an E3 ubiquitin ligase that controls HIF levels by targeting it for degradation), such as occurs in some RCCs. Among the genes stimulated by HIF are VEGF and VEGF receptors. VEGFs and their receptors are required for embryonic vasculogenesis (development of new blood vessels when none preexist) and normal (wound healing, corpus luteum formation) and pathologic angiogenesis (tumor angiogenesis, inflammatory conditions such as rheumatoid arthritis). VEGF-A is a heparin-binding glycoprotein with at least four isoforms (splice variants) that regulates blood vessel formation by binding to the RTKs VEGFR1 and VEGFR2, which are expressed on all ECs in addition to a subset of hematopoietic cells (Fig. 72-9). VEGFR2 plays a more direct role in regulating EC proliferation, migration, and survival, whereas VEGFR1 appears to have more nuanced functions with a less direct role in stimulating EC processes in the normal adult (even acting as a decoy protein for VEGFA to decrease binding to VEGFR2) but with important effects during embryogenesis and on tumor angiogenesis. Tumor vessels may be more dependent on VEGFR signaling for growth and survival than normal ECs.

While VEGF signaling is a critical initiator of angiogenesis, this is a complex process regulated by additional signaling pathways (Fig. 72-10). The angiopoietin, Ang1, produced by stromal cells, binds to the EC RTK Tie-2 and promotes the interaction of ECs with the ECM and perivascular cells, such as pericytes and smooth-muscle cells, to form tight, nonleaky vessels. PDGF and basic fibroblast growth factor (bFGF) help to recruit these perivascular cells. Ang1 is required for maintaining the quiescence and stability of mature blood vessels

and prevents the vascular permeability normally induced by VEGF and inflammatory cytokines.

For tumor cell-derived VEGF to initiate sprouting from host vessels, the stability conferred by the Ang1/Tie2 pathway must be perturbed; this occurs by the secretion of Ang2 by ECs that are undergoing active remodeling. Ang2 binds to Tie2 and is a competitive inhibitor of Ang1 action: under the influence of Ang2, preexisting blood vessels become more responsive to remodeling signals, with less adherence of ECs to stroma and associated perivascular cells and more responsiveness to VEGF. Therefore, Ang2 is required at early stages of tumor angiogenesis for destabilizing the vasculature by making host ECs more sensitive to angiogenic signals. In the presence of Ang2, there is no stabilization by the Ang1/Tie2 interaction, and tumor blood vessels are leaky, hemorrhagic, and have poor association of ECs with underlying stroma. Sprouting tumor ECs express high levels of the transmembrane protein ephrin-B2 and its receptor, the RTK EPH, whose signaling appears to work with the angiopoietins during vessel remodeling. During embryogenesis, EPH receptors are expressed on the endothelium of primordial venous vessels while the transmembrane ligand ephrin-B2 is expressed by cells of primordial arteries; the reciprocal expression may regulate differentiation and patterning of the vasculature.

A number of additional ubiquitously expressed host molecules play critical roles in normal and pathologic angiogenesis. Proangiogenic cytokines, chemokines, and growth factors secreted by stromal cells or inflammatory cells make important contributions

to neovascularization, including bFGF, transforming growth factor- β (TGF- β), TNF- α , and IL-8. In contrast to normal endothelium, angiogenic endothelium overexpresses specific members of the integrin family of ECM-binding proteins that mediate EC adhesion, migration, and survival. Specifically, expression of integrins $\alpha v\beta 3$, $\alpha v\beta 5$, and $\alpha 5\beta 1$ mediates spreading and migration of ECs and is required for angiogenesis induced by VEGF and bFGF, which in turn can upregulate EC integrin expression. The $\alpha v\beta 3$ integrin physically associates with VEGFR2 in the plasma membrane and promotes signal transduction from each receptor to promote EC proliferation (via focal adhesion kinase, src, PI3K, and other pathways) and survival (by inhibition of p53 and increasing the Bcl-2/Bax expression ratio). In addition, $\alpha v\beta 3$ forms cell-surface complexes with matrix metalloproteinases (MMPs), zinc-requiring proteases that cleave ECM proteins, leading to enhanced EC migration and the release of heparin-binding growth factors, including VEGF and bFGF. EC adhesion molecules can be upregulated (i.e., by VEGF, TNF- α) or downregulated (by TGF- β); this, together with chaotic blood flow, explains poor leukocyte-endothelial interactions in tumor blood vessels and may help tumor cells avoid immune surveillance.

Tumor blood vessels are not normal; they have chaotic architecture and blood flow. Due to an imbalance of angiogenic regulators such as VEGFs and angiopoietins (see below), tumor vessels are tortuous and dilated with an uneven diameter, excessive branching, and shunting. Tumor blood flow is variable, with areas of hypoxemia and acidosis leading to the selection of variants that are resistant to hypoxemia-induced apoptosis (often involving the loss of p53 expression). Tumor vessel walls have numerous openings, widened interendothelial junctions, and discontinuous or absent basement membrane. This contributes to the high permeability of these vessels and, together with lack of functional intratumoral lymphatics, causes increased interstitial

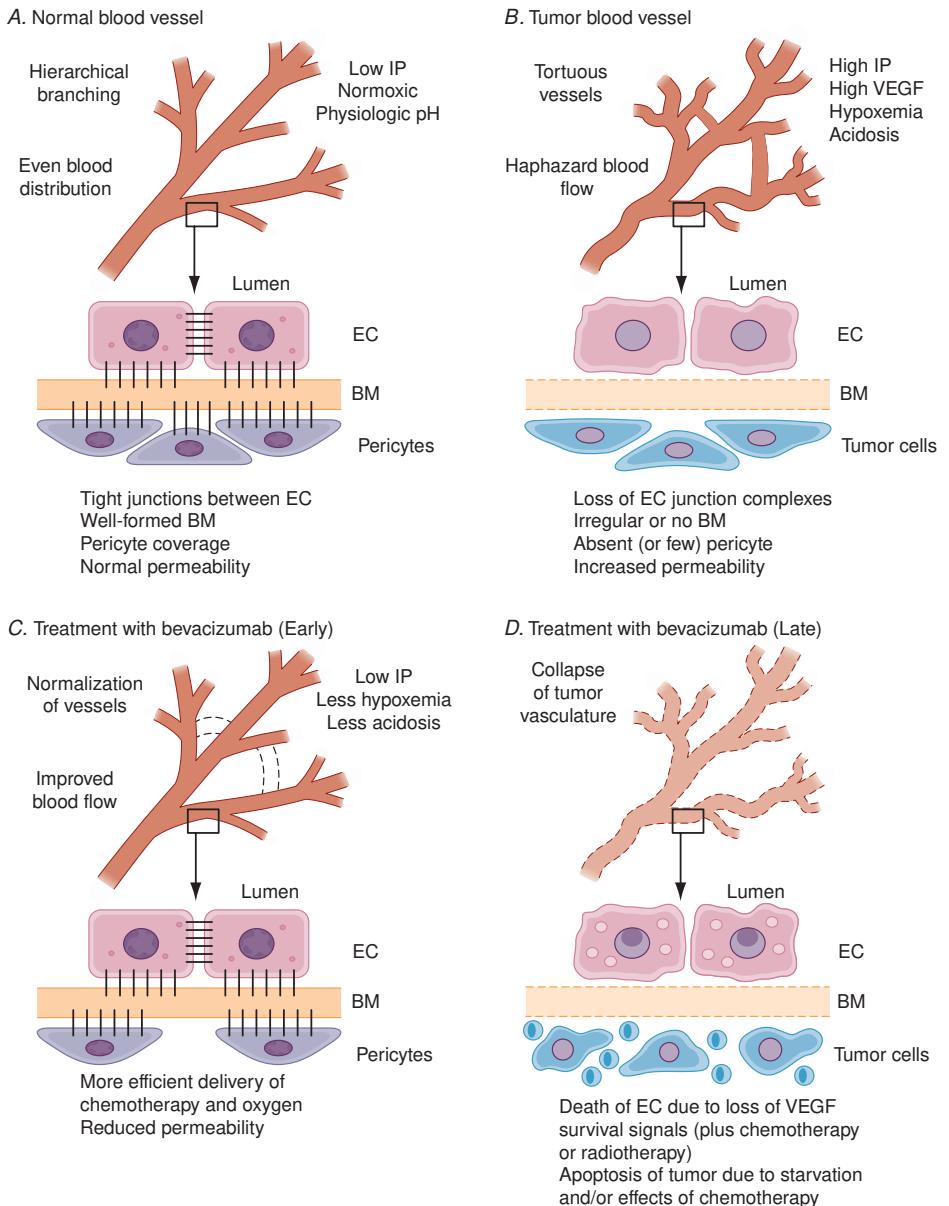


FIGURE 72-11 Normalization of tumor blood vessels due to inhibition of VEGF signaling. **A.** Blood vessels in normal tissues exhibit a regular hierarchical branching pattern that delivers blood to tissues in a spatially and temporally efficient manner to meet the metabolic needs of the tissue (top). At the microscopic level, tight junctions are maintained between endothelial cells (ECs), which are adherent to a thick and evenly distributed basement membrane (BM). Pericytes form a surrounding layer that provides trophic signals to the EC and helps maintain proper vessel tone. Vascular permeability is regulated, interstitial fluid pressure (IP) is low, and oxygen tension and pH are physiologic. **B.** Tumors have abnormal vessels with tortuous branching and dilated, irregular interconnecting branches, causing uneven blood flow with areas of hypoxemia and acidosis. This harsh environment selects genetic events that result in resistant tumor variants, such as the loss of p53. High levels of VEGF (secreted by tumor cells) disrupt gap junction communication, tight junctions, and adherens junctions between EC via src-mediated phosphorylation of proteins such as connexin 43, zonula occludens-1, VE-cadherin, and α/β -catenins. Tumor vessels have thin, irregular BM, and pericytes are sparse or absent. Together, these molecular abnormalities result in a vasculature that is permeable to serum macromolecules, leading to high tumor interstitial pressure, which can prevent the delivery of drugs to the tumor cells. This is made worse by the binding and activation of platelets at sites of exposed BM, with release of stored VEGF and microvessel clot formation, creating more abnormal blood flow and regions of hypoxemia. **C.** In experimental systems, treatment with bevacizumab or blocking antibodies to VEGFR2 leads to changes in the tumor vasculature that have been termed *vessel normalization*. During the first week of treatment, abnormal vessels are eliminated or pruned (dotted lines), leaving a more normal branching pattern. ECs partially regain features such as cell-cell junctions, adherence to a more normal BM, and pericyte coverage. These changes lead to a decrease in vascular permeability, reduced interstitial pressure, and a transient increase in blood flow within the tumor. Note that in murine models, this normalization period lasts only for ~5–6 days. **D.** After continued anti-VEGF/VEGFR therapy (which is often combined with chemo- or radiotherapy), ECs die, leading to tumor cell death (either due to direct effects of the chemotherapy or lack of blood flow).

pressure within the tumor (which also interferes with the delivery of therapeutics to the tumor; Figs. 72-9, 72-10, and 72-11). Tumor blood vessels have a deficit of perivascular cells such as pericytes and smooth-muscle cells that normally regulate flow in response to tissue metabolic needs.

Unlike normal blood vessels, the vascular lining of tumor vessels is not a homogeneous layer of ECs but often consists of a mosaic of ECs and tumor cells, which, because of their plasticity, can upregulate expression of genes normally only seen in ECs under hypoxic conditions. These cancer cell-derived vascular channels, which may be

lined by ECM secreted by the tumor cells, are referred to as *vascular mimicry*. During tumor angiogenesis, ECs are highly proliferative and express a number of plasma membrane proteins that are characteristic of activated endothelium, including growth factor receptors and adhesion molecules such as integrins. These abnormalities in tumor vasculature provide potential differential sensitivities from normal vessels to approaches to inhibit the process, allowing for the use of antiangiogenic agents in cancer treatment.

Lymphatic vessels also exist within tumors. Development of tumor lymphatics is associated with expression of VEGFR3 and its ligands VEGF-C and VEGF-D. The role of these vessels in tumor cell metastasis to regional lymph nodes remains to be determined. However, VEGF-C levels correlate significantly with metastasis to regional lymph nodes in lung, prostate, and colorectal cancers.

■ ANTIANGIOGENIC THERAPY

Angiogenesis inhibitors function by targeting the critical molecular pathways involved in EC proliferation, migration, and/or survival, many of which are highly expressed in the activated endothelium in tumors. Inhibition of growth factor and adhesion-dependent signaling pathways can induce EC apoptosis with concomitant inhibition of tumor growth. Different types of tumors can use distinct combinations of molecular mechanisms to activate the angiogenic switch. Therefore, it is doubtful that a single antiangiogenic strategy will suffice for all human cancers; rather, a number of agents or combinations of agents will be needed, depending on distinct programs of angiogenesis used by different human cancers. Despite this, experimental data indicate that for some tumor types, blockade of a single growth factor (e.g., VEGF) may inhibit tumor-induced vascular growth.

Bevacizumab, an antibody that binds VEGF, potentiates the effects of a number of different types of active chemotherapeutic regimens

used to treat a variety of different tumor types including colon, lung, ovarian, and cervical cancers. It also has activity in combination with interferon against RCCs and alone for glioblastomas. Other protein inhibitors of the VEGF signaling pathway approved for anticancer therapy include ramucirumab (a monoclonal antibody directed against VEGFR2, approved for use against gastric/gastroesophageal, colon, and lung cancers) and ziv-aflibercept (a recombinant protein inhibitor of VEGF, approved for colorectal cancer). Hypertension is the most common side effect of inhibitors of VEGF (or its receptors) but can be treated with antihypertensive agents and uncommonly requires discontinuation of therapy. Rare but serious potential risks include arterial thromboembolic events, including stroke and myocardial infarction, hemorrhage, bowel perforation, and inhibition of wound healing.

Several small-molecule inhibitors (SMIs) that target VEGF RTK activity but are also inhibitory to other kinases have also been approved to treat certain cancers. Sunitinib (see above and Table 72-2) has activity directed against mutant c-Kit receptors (approved for GIST), but also targets VEGFR and PDGFR, and has antitumor activity against pancreatic neuroendocrine and metastatic RCCs, presumably on the basis of its antiangiogenic activity. Similarly, sorafenib, originally developed as a Raf kinase inhibitor but with potent activity against VEGFR and PDGFR, has activity against RCC, differentiated thyroid and hepatocellular cancers, and desmoid tumors. A closely related molecule to sorafenib, regorafenib, has activity against colorectal cancer, GIST, and hepatocellular cancer. Other inhibitors of the VEGF pathway approved for the treatment of various cancers include axitinib, pazopanib, lenvatinib, and cabozantinib.

The success in targeting tumor angiogenesis has led to enhanced enthusiasm for the development of drugs that target other aspects of the angiogenic process; some of these therapeutic approaches are outlined in Fig. 72-12. Recently, an inhibitor of HIF2- α has shown

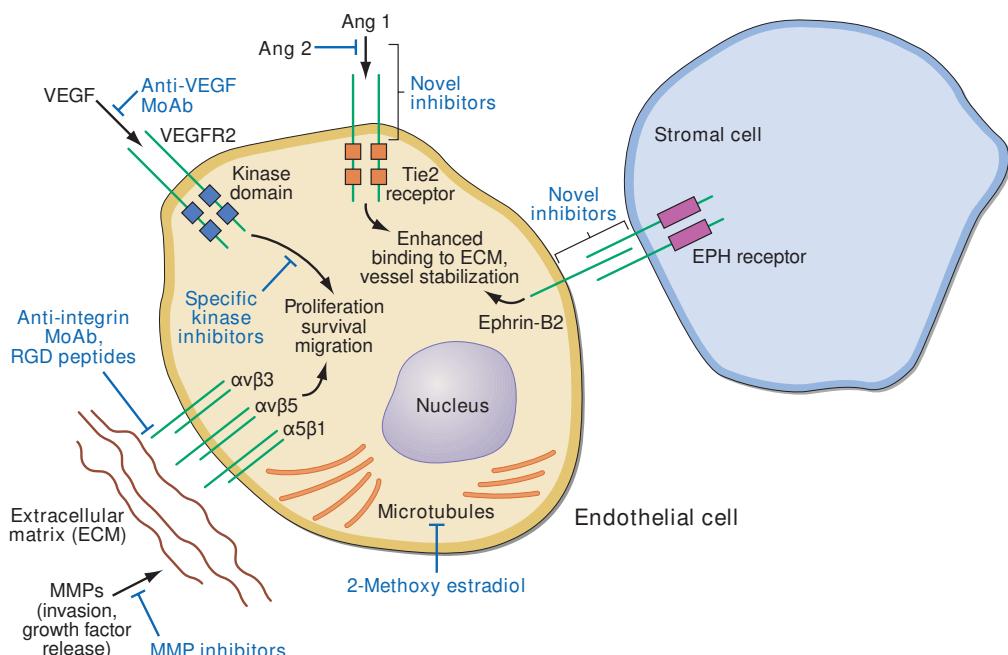


FIGURE 72-12 Knowledge of the molecular events governing tumor angiogenesis has led to a number of therapeutic strategies to block tumor blood vessel formation. The successful therapeutic targeting of VEGF and its receptors VEGFR is described in the text. Other endothelial cell-specific receptor tyrosine kinase pathways (e.g., angiopoietin/Tie2 and ephrin/EPH) are likely targets for the future. Ligation of the $\alpha_v\beta_3$ integrin is required for EC survival. Integrins are also required for EC migration and are important regulators of matrix metalloproteinase (MMP) activity, which modulates EC movement through the ECM as well as release of bound growth factors. Targeting of integrins includes development of blocking antibodies, small peptide inhibitors of integrin signaling, and arg-gly-asp-containing peptides that prevent integrin:ECM binding. Peptides derived from normal proteins by proteolytic cleavage, including endostatin and tumstatin, inhibit angiogenesis by mechanisms that include interfering with integrin function. Signal transduction pathways that are dysregulated in tumor cells indirectly regulate EC function. Inhibition of EGF-family receptors, whose signaling activity is upregulated in a number of human cancers (e.g., breast, colon, and lung cancers), results in downregulation of VEGF and IL-8, while increasing expression of the antiangiogenic protein thrombospondin-1. The Ras/MAPK, PI3K/Akt, and Src kinase pathways constitute important antitumor targets that also regulate the proliferation and survival of tumor-derived EC. The discovery that ECs from normal tissues express tissue-specific "vascular addressins" on their cell surface suggests that targeting specific EC subsets may be possible.

preliminary evidence of antitumor activity against RCC in a clinical trial. There is also evidence suggesting potential enhanced activity when anti-VEGF agents are used in combination with immunomodulators including immune checkpoint inhibitors. However, it is not yet known whether this will produce a clinically meaningful enhancement of antitumor activity.

EVASION OF THE IMMUNE SYSTEM BY CANCERS

The immune system plays a critical role in maintaining organismal integrity including by defending against pathogens as well as preventing and limiting the growth of cancers. There is a complex interaction between cancer and the host from the development of the first malignant cell to the establishment of a clinical cancer and its subsequent growth, invasion, and metastasis. The immune system plays a critical role in the prevention of cancer development. This is exemplified by the increased risk for cancer development in individuals who are significantly immunosuppressed, such as by inherited defects in mechanisms important for immune function, the immunosuppression necessary to maintain allogeneic organ transplants, and immunosuppression seen from certain infections such as human immunodeficiency virus. There are two components of the immune system. The first is innate immunity (present in the organism and not dependent on prior exposure to a specific antigen, such as those present in a pathogen or malignant cell), which tends to be general and not specific. The second is the adaptive immune component, which depends on the innate immune component for activation and provides the specificity to the response with significant expansion of cells to target the specific antigens present on the pathogen or malignant cell. Thus, while the innate process provides the first line of defense, the adaptive process is necessary for the specificity of response and providing memory to more rapidly attack cells should the pathogen infection recur or the malignant cells grow. The immune system has to be tightly regulated to allow for clearance of unwanted antigens while preventing an immune-mediated attack on the self. (See Chap. 349 for details on the function of the immune system).

Not surprisingly, since cancers arise from normal cells within the body that have a variety of processes to prevent destruction by the immune system, they have a variety of mechanisms that allow them to evade detection and elimination by the immune system (Fig. 72-13). These include downregulation of cell surface proteins involved in

immune recognition (including MHC proteins and tumor-specific antigens), expression of other cell surface proteins that inhibit immune function (including members of the B7 family of proteins such as PD-L1), secretion of proteins and other molecules that are immunosuppressive, recruitment and expansion of immunosuppressive cells such as regulatory T cells (which are important for maintaining tolerance against self-antigens), induction of T cell tolerance, and downregulation of death receptors (Fig. 72-14). Due to the marked heterogeneity of cells within a cancer, a variety of immune-suppressive mechanisms are continuously occurring and changing. In addition, the inflammatory effects of some of the immune mediator cells in the tumor microenvironment (especially tissue-associated macrophages and myeloid-derived suppressor cells) can suppress T cell responses to the tumor as well as stimulate inflammation that can enhance tumor growth.

There are marked differences in the way different malignancies respond to current immunotherapeutic approaches. For example, melanomas, RCC, Merkel cell carcinomas, cancers with defects in DNA repair associated with microsatellite instability with accumulation of gene mutations, and lymphomas respond well to current immunotherapeutic approaches, whereas pancreatic and microsatellite-stable colon cancers do not. While there is not a complete understanding of why these differences exist and there are many factors both within the cancer cells and in the microenvironment that play a role, several factors have been identified that appear to be important. These include the number of mutations present in the tumor (tumor mutational burden), presence of new or neoantigens, expression of immune checkpoint proteins (e.g., PD-L1 for anti-PD-1 or anti-PD-L1 therapy), density of tumor-infiltrating lymphocytes, and host genetic factors. One of these (PD-L1 expression by the tumor) has sufficient predictive value for certain tumors (e.g., non-small-cell lung cancer) to be used in making treatment decisions regarding the use of antibodies targeting PD-1 or PD-L1. However, neither PD-L1 expression nor any other marker can predict responsiveness of most tumors to immunotherapy. Better biomarkers that define potential responsiveness of specific cancers to immunotherapy are badly needed. A major area of research is to try to identify approaches that would convert cancers that are not responsive to immunotherapy to being responsive.

Immunotherapy approaches to treat cancer can be divided into those aimed at activating the immune response and those designed to

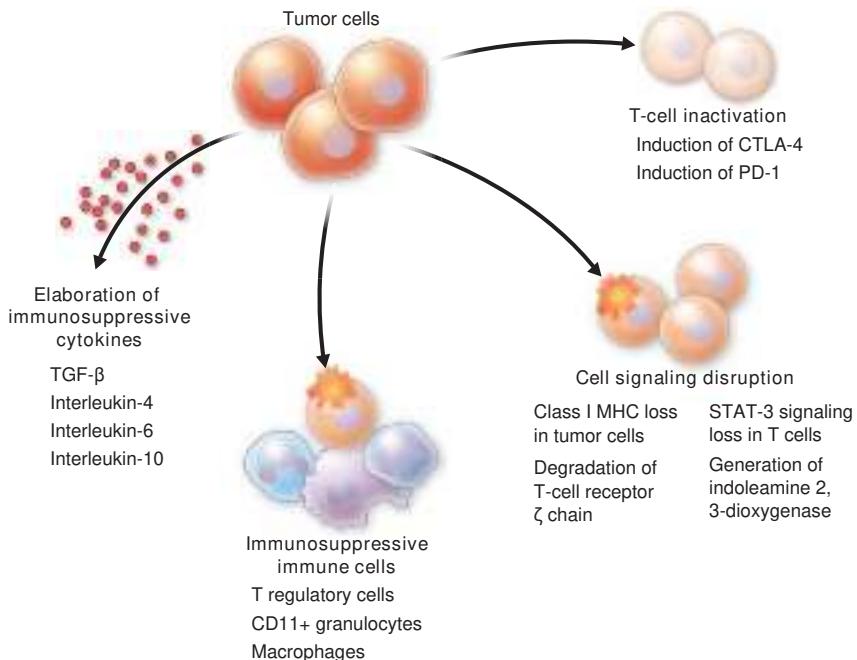


FIGURE 72-13 Tumor-host interactions that suppress the immune response to the tumor.

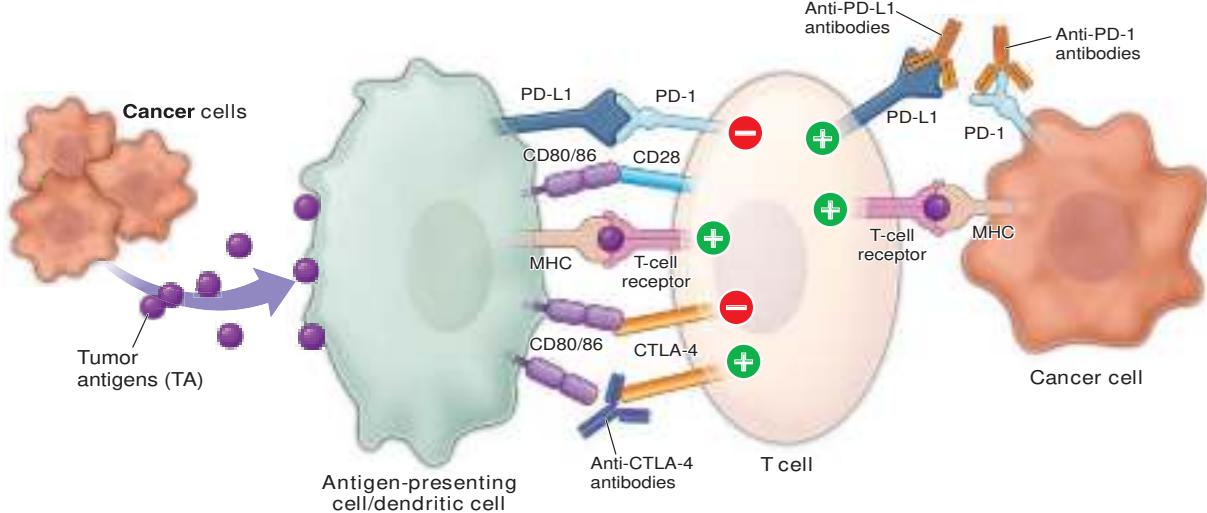


FIGURE 72-14 Inhibition of T-cell activation against cancer cells by engagement of co-inhibitory molecules including PD-1, PD-L1, and CTLA-4 and reversal of this inhibition by antibodies against these proteins. The red ovals in the T cell indicate inhibitory signals, and the green oval indicates stimulatory signals.

release the brakes that prevent an effective immune response against tumors. Approaches at activating the immune response against cancer including using immunostimulatory molecules such as interferons, IL-2, and especially monoclonal antibodies have had some success.

A more direct approach to enhance the activity of T cells directed against specific tumors involves isolating T cells from patients and re-engineering the cells to express chimeric antigen receptors (CAR-T cells) that recognize antigens present on the cells of that individual's tumor. The most commonly used approach to date has been to engineer the cells to express receptors targeting the CD19 antigen on acute lymphocytic leukemia (ALL) and diffuse large B-cell lymphoma (DLBCL) cells. These have been shown to have significant antitumor activity in the treatment of patients with ALL and DLBCL, including durable remissions in patients refractory to standard therapies, and are approved for these malignancies. However, there have also been significant issues with toxicity including cytokine release syndrome, organ toxicity felt to be due to inadvertent targeting of antigens present in the organ, and neurotoxicity. These patients often require aggressive supportive care by individuals experienced in the delivery of CAR-T cells. In addition, as is true for most anticancer therapies, mechanisms of resistance have developed, most commonly the outgrowth of tumor cells no longer expressing the antigen. Mechanisms for preventing the development of resistant cells are being explored. CAR-T cells are undergoing clinical investigation against other hematologic malignancies (e.g., multiple myeloma) and solid tumors. Approaches to develop allogeneic CAR-T-cell therapies are also being explored.

The other approach to enhancing the immune response against cancers is releasing the brakes that inhibit a response by targeting of proteins or cells (e.g., regulatory T cells) involved in normal homeostatic control to prevent autoimmune damage to the host but that malignant cells and their stroma can also utilize to inhibit the immune response directed against them. The approach that is furthest along clinically has involved targeting CTLA-4, PD-1, and PD-L1 (and others)—co-inhibitory molecules that are expressed on the surface of cancer cells, cells of the immune system, and/or stromal cells and are involved in inhibiting the immune response against cancer (Figs. 72-13 and 72-14). This approach has had clinical activity against a variety of cancers. A monoclonal antibody directed against CTLA-4 is approved for the treatment of melanoma, and antibodies targeting PD-1 or PD-L1 are approved for use against many cancers, including melanoma, RCC, lung cancer (both non-small-cell lung and small-cell lung), head and neck cancer, urothelial cancer, cervical cancer, hepatocellular carcinoma, gastric cancer, esophageal cancer, microsatellite instability (MSI)-high cancers, cancers with high tumor mutational burden

(TMB), Merkel cell cancer, primary B-cell mediastinal lymphoma, and Hodgkin's lymphoma. They continue to be evaluated against other malignancies as well. The combination of anti-CTLA-4 and anti-PD-1 antibodies has been approved for treatment of a number of cancers including melanoma, RCC, lung cancer, pleural mesothelioma, and MSI-high metastatic colorectal cancers. Immune checkpoint inhibitors are being used singly, in pairs, and in combination with chemotherapy in many ongoing clinical trials. Specific determinants of response to immune checkpoint inhibitors are still being defined, but in addition to high PD-L1 expression, the presence of increased neoantigens in the tumor, such as seen in patients with MSI-high and TMB-high cancers, may be one important determinant of better responses.

A number of other proteins are involved in controlling the immune response (both ones that enhance activity [e.g., CD27 and CD40] as well as ones involved in inhibiting response [e.g., LAG3, TIM-3, TIGIT]). Antibodies have been developed to modulate function of these proteins, and many are in clinical development for cancer therapy. In addition, various combinations targeting more than one protein involved in potentially enhancing the immune response against cancers or with other anticancer approaches (targeted agents, chemotherapy, radiation therapy) that may lead to enhanced antitumor activity are also being explored. An important aspect of these approaches is balancing sufficient release of the negative control of the immune response to allow immune-mediated attack on the tumors while not allowing too much of an immune response against normal tissues and thus inducing severe autoimmune effects (e.g., against lung, liver, skin, thyroid, pituitary gland, or the gastrointestinal tract). As is true for other immunotherapeutic approaches against cancer, major efforts are ongoing to better understand the mechanism of immune toxicity from these approaches and, therefore, ways of controlling this while not abrogating the antitumor effects.

Improved knowledge of the biology of the interactions between the immune system and cancers continues to be rapid with the promise for additional significant improvements in use of immunotherapy to treat cancer.

SUMMARY

Although each of the biological aspects of cancers and examples of targeting them has been addressed individually, clearly there is complicated cross-talk between these that occurs in all cancers that needs to be better understood to optimally treat different cancers. The explosion of information on tumor cell biology, metastasis, and tumor-host interactions (including angiogenesis, other tumor-stromal interactions, and immune evasion by tumors) has ushered in a new era of rational

targeted therapy for cancer. Furthermore, it has become clear that specific molecular factors detected in individual tumors (specific gene mutations, gene expression profiles, miRNA expression, overexpression of specific proteins) can be used to tailor therapy and maximize antitumor effects. Potentially of greater impact on decreasing deaths from cancer, better understanding of the biology of early cancer development and technologic development to improve sensitivity and specificity in detecting cancer-specific molecules (e.g., mutated genes) provide hope that approaches for earlier detection of cancer can be developed.

A

Robert G. Fenton contributed to this chapter in prior editions, and important material from those prior chapters has been included here.

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TABLE 73-1 Spectrum of Cancer-Related Interventions

Asymptomatic patient (breast, cervix, colon, some lung) screening
Consideration of cancer in a differential diagnosis
Physical examination, imaging, or endoscopy to define a possible tumor
Phlebotomy for molecular studies and circulating tumor cell characterization
Diagnosis of cancer by biopsy or removal:
Routine histology
Specialized histology: immunohistochemistry
Molecular studies
Cytogenetic studies
Staging the cancer: Where has it spread?
Treatment
Localized (surgical removal with or without local radiation therapy and/or topical therapy may be curative)
Systemic (prevent or reverse organ compromise)
Supportive care
During treatment: related to tumor effects on patient
During treatment: to counteract side effects of treatment
After treatment: to ameliorate the adverse effects of treatment
Palliative and end of life
When useful treatments are not feasible or desired

of hollow viscera, but also may reflect altered platelet number or blood coagulation. Tumors may also present with a “paraneoplastic syndrome” owing to the effects of substances they secrete. Although statistically the fraction of patients with cancer underlying a particular presenting sign or symptom may be low, the implications of missing an early-stage tumor call for vigilance in considering cancer as the basis for persistent signs or symptoms.

Evidence of a tumor’s existence can come from careful physical examination, e.g., enlarged lymph nodes in lymphomas or palpable mass in a breast or soft tissue site. A mass may also be detected or confirmed by an imaging modality, such as plain x-ray, computed tomography (CT) scan, ultrasound, positron emission tomography (PET) imaging, or nuclear magnetic resonance approaches. Endoscopy may directly visualize a tumor.

ESTABLISHING A CANCER DIAGNOSIS

Once a potential tumor is defined, establishing the diagnosis is the next step in the intervention spectrum. This requires a biopsy procedure in most circumstances and pathologic confirmation that cancer is present; very rarely, where biopsy would be definitely injurious and imaging modalities are unequivocal, such as with a likely brainstem glioma, treatment might be reasonably considered based on clinical and imaging evidence without biopsy. In addition to light microscopy, biopsied tissue also allows definition of genetic abnormalities and protein expression patterns (Table 73-2).

The extent of specialized testing needs to be tailored to an individual patient’s case. Global DNA sequencing of genes expressed in tumors has not been shown to convey conclusive advantage in terms of survival. But the aggregate “mutational burden” present in tumors and the intactness of DNA repair genes (e.g., breast cancer susceptibility 1 and 2 [*BRCA1/2*], microsatellite instability, homologous recombination pathway-associated genes) may suggest valuable treatment courses in tumors without curative potential. Testing for certain abnormalities in Table 73-2 can be the basis for use of specific U.S. Food and Drug Administration (FDA)-approved therapeutic agents.

Optimally, an *excisional biopsy* occurs, in which the entire tumor mass is removed with a margin of normal tissue surrounding it. If an excisional biopsy cannot be performed, *incisional biopsy* is the procedure of second choice: a wedge of tissue is removed, trying to include the majority of the cross-sectional diameter to minimize sampling error. Biopsy techniques that involve cutting into tumor risk facilitating the spread of the tumor, and consideration with a surgeon of whether the biopsy approach is a potential prelude to a curative surgery

73

Principles of Cancer Treatment

Edward A. Sausville, Dan L. Longo



CANCER PRESENTATION

Localized or systemic cancer is frequent in the differential diagnosis of a variety of common complaints. Affording patients the greatest opportunity for cure or meaningful prolongation of life is greatly aided by cancer diagnosis early in its natural history. The spectrum of possible cancer-related interventions to make cure possible are shown in Table 73-1.

DETECTION OF A CANCER

The term *cancer*, as used here, is synonymous with the term *tumor*, whose original derivation from Latin simply meant “swelling,” not otherwise specified. Swelling reflects increased interstitial fluid pressure and increased cellular and stromal mass, compared to normal tissue. Leukemias, a cancer of the blood-forming tissues, presents in a disseminated form frequently without tumor masses. Tumors can also present by organ dysfunction, such as dyspnea on exertion from anemia caused by leukemia replacing normal marrow, cough from lung cancers, jaundice from tumors blocking bile ducts, or neurologic signs from gliomas. Hemorrhage frequently results from involvement

TABLE 73-2 Diagnostic Biopsy: Standard-of-Care Molecular and Special Studies to Be Considered

All solid tumors:
Tumor mutational burden
Microsatellite instability DNA repair pathway intactness
Homologous recombination DNA repair pathway intactness
Breast cancer: primary and suspected metastatic
Breast cancer susceptibility 1 and 2 (<i>BRCA1/2</i>) gene mutations
Hormone receptor expression: estrogen, progesterone
<i>HER2/neu</i> oncoprotein
<i>PI3KA</i> mutation status
Lung cancer: primary and suspected metastatic
If nonsquamous non-small-cell:
Epidermal growth factor receptor (<i>EGFR</i>) mutation
<i>ALK</i> gene fusion
<i>BRAFV600E</i> mutation
Programmed cell death ligand 1 (PD-L1) expression
Colon cancer: suspected metastatic
<i>KRAS</i> mutation
<i>BRAFV600E</i> mutation
Gastrointestinal stromal tumor
<i>KIT</i> mutation
Melanoma
<i>BRAF</i> mutation
c-kit expression and <i>KIT</i> mutation if present
Pancreatic cancer
<i>BRCA1/2</i> mutation
Prostate cancer
<i>BRCA1/2</i> mutation
Thyroid cancer
<i>RET</i> gene alterations (mutations, translocations, amplification)
Gliomas
1p/19q co-deletion
Alkylguanine alkyltransferase promoter methylation
Isocitrate dehydrogenase 1 and 2 mutation
Leukemia (peripheral blood mononuclear cells and/or bone marrow)
Cytogenetics
Flow cytometry
Treatment-defining chromosomal translocations/mutations
Bcr-Abl fusion protein
t(15;17)
inversion 16
t(8;21)
FMS-associated tyrosine kinase (<i>FLT3</i>) mutation
Nucleophosmin gene mutational status
Isocitrate dehydrogenase 1 and 2 mutation
Lymphoma
Immunohistochemistry for CD20, CD30, T-cell markers
Treatment-defining chromosomal translocations:
t(14;18)
t(8;14)
Translocations involving <i>ALK</i> gene

accounting for possible diagnoses may best inform the approach taken. *Core-needle biopsy* usually obtains considerably less tissue but can provide information to plan a treatment. *Fine-needle aspiration* generally yields a suspension of cells from a mass. If positive for cancer, it may allow inception of systemic treatment, or it can provide a basis for planning a more extensive surgical procedure. It is unreliable as a sole diagnostic method to make a cancer diagnosis in most cases. A “negative” fine-needle aspiration cannot be taken as definitive evidence that a tumor is absent. In some instances, features of diagnostic imaging are sufficient to make a reliable diagnosis without obtaining tissue, usually

with presence of a tumor associated circulating diagnostic marker, e.g., alpha fetoprotein in hepatocellular carcinoma.

CANCER STAGING

An essential component of correct patient management in many cancer types is defining the extent of disease to determine whether localized treatments, “combined-modality” approaches, or systemic treatments should initially be considered. Radiographic and other imaging tests can be helpful in defining the *clinical stage*; *pathologic staging* documents the histologic presence of tumor in tissue biopsies obtained through a surgical procedure. Lymph node sampling in breast cancer, melanoma, lung, head and neck, colon, and other intra-abdominal cancers may provide crucial information.

Staging systems have evolved to define a “T” component related to the size of the tumor or its invasion into local structures, an “N” component related to the number and nature of lymph node groups adjacent to the tumor with evidence of tumor spread, and an “M” component, based on the presence of local or distant metastatic sites. The various TNM components are then aggregated to stages, usually stage I to III or IV, depending on the anatomic site. The numerical stages reflect similar long-term survival outcomes of the aggregated TNM groupings in a numeric stage after treatment tailored to the stage. In general, stage I tumors are T1 (reflecting small size), N0 or N1 (reflecting no or minimal node spread), and M0 (no metastases). Such early-stage tumors are usually amenable to curative approaches with local treatments. On the other hand, stage IV tumors have metastasized to distant sites or locally invaded viscera in a nonresectable way. They are treated with palliative intent, except for those diseases with exceptional sensitivity to systemic treatments such as chemotherapy or immunotherapy. Also, the TNM staging system is not useful in diseases such as leukemia, where bone marrow infiltration is never localized, or central nervous system (CNS) tumors, where tumor histology and the extent of feasible resection are more important in driving prognosis.

CANCER TREATMENT

The goal of cancer treatment is first to eradicate the cancer; if not possible, the goal shifts to palliation: amelioration of symptoms and preservation of quality of life while striving to extend life. When cure of cancer is possible, cancer treatments may be undertaken despite the certainty of severe toxicities, and these may produce toxicity with no benefit. Conversely, when the clinical goal is palliation, careful attention to minimizing the toxicity of treatments becomes a significant goal.

Cancer treatments are divided into two main types: *local* and *systemic*. Local treatments include surgery, radiation therapy (including photodynamic therapy), and ablative approaches, including radiofrequency and thermal or cryosurgical approaches. Systemic treatments include chemotherapy (including hormonal therapy and molecular targeted therapy) and biologic therapy (including immunotherapy). The modalities are often used in combination. *Oncology*, the study of tumors including treatment approaches, is a multidisciplinary effort with surgical, radiation, and internal medicine-related areas of oncologic expertise.

Normal organs and cancers share the property of having a population of cells actively progressing through the cell cycle, with their division providing a basis for organ or tumor growth, and a population of cells not in cycle; these include *stem cells*, whose properties are being elucidated. Cancer stem cells serve as a basis for tumor initiating or repopulating cells. Tumors follow a Gompertzian growth curve (Fig. 73-1), with the growth fraction of a neoplasm high with small tumor burdens and declining until, at the time of diagnosis, with a tumor burden of $1-5 \times 10^9$ tumor cells, the growth fraction is usually 1–4% for many solid tumors. By this view, the most rapid growth rate occurs before the tumor is detectable. An alternative explanation for such growth properties may also emerge from the ability of tumors at metastatic sites to recruit circulating tumor cells from the primary tumor or other metastases. Key features of tumor growth are the ability to stimulate new supporting stroma through angiogenesis and ingrowth of fibroblasts and immune cells (Chap. 72).

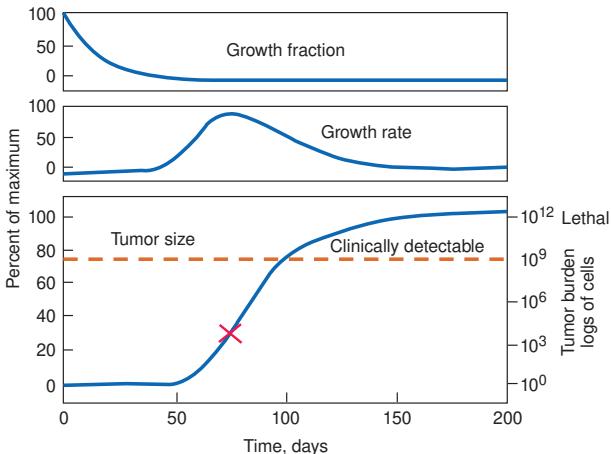


FIGURE 73-1 Gompertzian tumor growth. The growth fraction of a tumor declines exponentially over time (*top*), peaking before it is clinically detectable (*middle*). Tumor size increases slowly, goes through an exponential phase, and slows again as the tumor has limitation of nutrients or host regulatory influences occur. The maximum growth rate occurs at $1/e$, the point at which the tumor is about 37% of its maximum size (marked with an *X*). Tumor becomes detectable at a burden of about 10^8 (1 cm^3) cells and kills the patient at a tumor cell burden of about 10^{12} (1 kg).

LOCALIZED CANCER TREATMENTS

SURGERY

Surgery is unquestionably the most effective means of treating cancer. At least 40% of cancer patients are cured by surgery. Unfortunately, a large fraction of patients with solid tumors have metastatic disease not accessible for removal. Even when cancer is not curable by surgery alone, the removal of tumor can afford local control of tumor, preserve organ function, achieve debulking that permits more effective subsequent therapy, and allow more detailed staging. Cancer surgery aiming for cure is usually planned to excise the tumor completely with an adequate margin of normal tissue (the margin varies with the tumor and the anatomy), touching the tumor as little as possible to prevent vascular and lymphatic spread, and minimizing operative risk. Such a resection is defined as an R0 resection. R1 and R2 resections, in contrast, are imprecisely defined pathologically as having microscopic or macroscopic, respectively, tumor at resection margins. Such outcomes may be the basis for reoperation to obtain optimal margins if feasible and of likely clinical utility. Extending the procedure to resect draining lymph nodes obtains prognostic information and may, in some anatomic locations, improve survival.

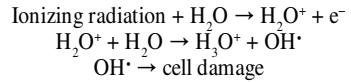
Laparoscopic approaches are being used for primary abdominal and pelvic tumors, although with certain tumors (e.g., uterine and cervix), controversy exists as to the desirability of laparoscopic tissue removal. Lymph node spread may be assessed using the sentinel node approach, in which the first draining lymph node is defined by injecting a dye or radioisotope into the tumor site at operation and then resecting the first node to turn blue or collect isotope. The sentinel node assessment appears to provide information without the risks (lymphedema, lymphangiosarcoma) associated with resection of all regional nodes. Advances in adjuvant chemotherapy (chemotherapy given systemically after removal of all local disease surgically without evidence of active metastatic disease) and radiation therapy following surgery have permitted a substantial decrease in the extent of primary surgery necessary to obtain the best outcomes. Thus, “lumpectomy” with radiation therapy is as effective as modified radical mastectomy for breast cancer, and limb-sparing surgery followed or preceded by adjuvant radiation therapy and chemotherapy has replaced amputation for most childhood rhabdomyosarcomas and osteosarcomas. More limited surgery spares organ function, as in larynx and bladder cancer. In some settings (e.g., bulky testicular cancer or stage III breast cancer), surgery is not the first treatment modality used. After diagnostic biopsy, chemotherapy and/or radiation therapy is delivered, followed by a surgical procedure

to remove residual masses; this is called *neoadjuvant therapy*. Coordination among the surgical oncologist, radiation oncologist, and medical oncologist is crucial.

Surgery may be curative in a subset of patients with metastatic disease. Patients with limited lung metastases from osteosarcoma may be cured by resection of the lung lesions. In patients with colon cancer who have fewer than five liver metastases restricted to one lobe and no extrahepatic metastases, hepatic lobectomy may produce long-term disease-free survival in 25% of selected patients. In the setting of hormonally responsive tumors, oophorectomy may eliminate estrogen production, and orchietomy may reduce androgen production, hormones that drive metastatic breast and all prostate cancers, respectively. In selecting a surgeon or center for primary cancer treatment, consideration must be given to the volume of cancer surgeries undertaken by the site. Studies in a variety of cancers have shown that increased annual procedure volume appears to correlate with outcome. Surgery is used in a number of ways for palliative or supportive care of the cancer patient. These include insertion and care of central venous catheters, control of pleural and pericardial effusions and ascites, caval interruption for recurrent pulmonary emboli, stabilization of cancer-weakened weight-bearing bones, and control of hemorrhage, among others. Surgical bypass of gastrointestinal, urinary tract, or biliary tree obstruction can alleviate symptoms and prolong survival. Surgical procedures may provide relief of pain or neurologic dysfunction (spinal cord decompression). Splenectomy may relieve symptoms and reverse hypersplenism. Intrathecal or intrahepatic therapy relies on surgical placement of appropriate infusion portals. Surgery may correct other treatment-related toxicities such as adhesions or strictures. Plastic and reconstructive surgery can correct the effects of disfiguring primary treatment. Surgery is also a tool valuable in the prevention of cancers in high-risk populations. Prophylactic mastectomy, colectomy, oophorectomy, and thyroidectomy are mainstays of prevention of genetic cancer syndromes.

RADIATION

Radiation Biology and Medicine Therapeutic radiation is ionizing, causing breaks in DNA and generation of free radicals from cell water that damage cancer cell membranes, proteins, and organelles. Radiation damage is augmented by oxygen; hypoxic cells are more resistant.



X-ray and gamma-ray photons are the forms of ionizing radiation most commonly used to treat cancer. Particulate ionizing radiation using protons has also become available.

Radiation dose is quantitated based on the amount of energy absorbed by the tumor, not on radiation generated by the machine. The International System (SI) unit for radiation dose is the Gray (Gy): 1 Gy refers to 1 J/kg of tissue; 1 Gy equals 100 centigrays (cGy) of absorbed dose. A historically used unit appearing in the oncology literature, the *rad* (radiation absorbed dose), is defined as 100 ergs of energy absorbed per gram of tissue and is equivalent to 1 cGy. Radiation dose is measured by placing detectors at the body surface or in irradiated phantoms that resemble human form and substance. The features that make a particular cell more or less sensitive to radiation involve DNA repair proteins that, in their physiologic role, protect against environmentally related DNA damage.

Localized Radiation Therapy Radiation effect is influenced by three determinants: total absorbed dose, number of fractions, and time of treatment. A typical course of radiation therapy should be described as 4500 cGy delivered to a particular target (e.g., mediastinum) over 5 weeks in 180-cGy fractions. Most curative radiation treatment programs are delivered once a day, 5 days a week, in 150- to 200-cGy fractions. Nondividing cells are more resistant than dividing cells; delivering radiation in repeated fractions is done to expose a larger

number of tumor cells that have entered the division cycle. The energy of the radiation determines its ability to penetrate tissue. Low-energy x-rays (150–400 kV) scatter when they strike the body, resulting in more damage to adjacent normal tissues and less radiation delivered to the tumor. Megavoltage radiation (>1 MeV) has very low lateral scatter; this produces a skin-sparing effect, more homogeneous distribution of the radiation energy, and greater deposit of the energy in the tumor, or *target volume*. The *transit volume* includes the tissues through which the beam passes to the target volume. Computational approaches and delivery of many beams to converge on a target volume are the basis for “gamma knife” and related approaches to deliver high doses to tumor, sparing normal tissue.

Therapeutic radiation is delivered in three ways: (1) *teletherapy*, with focused beams of radiation generated at a distance and aimed at the tumor within the patient; (2) *brachytherapy*, with encapsulated sources of radiation implanted directly into or adjacent to tumor tissues; and (3) *systemic therapy*, with radionuclides administered, for example, intravenously but perhaps targeted by some means to a tumor site. Teletherapy with x-ray or gamma-ray photons is the most commonly used form of radiation therapy and also delivers particulate forms of radiation such as proton beams. The difference between photons and protons relates to volume with greatest delivery of energy: protons have a narrow range of energy deposition. Electron beams are a particulate form of radiation that, in contrast to photons and protons, have a very low tissue penetrance and are used to treat cutaneous tumors. Certain drugs used in cancer treatment may also act as radiation sensitizers. For example, compounds that incorporate into DNA (e.g., halogenated pyrimidines, cisplatin) augment radiation effects at local sites and are important adjuncts to radiation of certain tumors, e.g., squamous head and neck, uterine cervix, and rectal cancers.

Toxicity of Radiation Therapy Although radiation therapy is most often administered to a local region, systemic effects, including fatigue, anorexia, nausea, and vomiting, may develop that are related in part to the volume of tissue irradiated, dose fractionation, radiation fields, and individual susceptibility. Injured tissues release cytokines that act systemically to produce these effects. Bone is among the most radio-resistant organs, with radiation effects being manifested mainly in children through premature fusion of the epiphyseal growth plate. By contrast, the male testis, female ovary, and bone marrow are the most sensitive organs. Any bone marrow in a radiation field will be eradicated by therapeutic irradiation. Organs with less need for cell renewal, such as heart, skeletal muscle, and nerves, are more resistant to immediate radiation effects. In radiation-resistant organs, the vascular endothelium is the most sensitive component. Acute toxicities include mucositis, skin erythema (ulceration in severe cases), and bone marrow toxicity. Often these can be alleviated by periodic interruption of treatment.

Chronic toxicities are more serious. Radiation of the head and neck region produces thyroid failure; cataracts and retinal damage can lead to blindness; salivary glands stop making saliva, which leads to dental caries and poor dentition. Mediastinal irradiation increases myocardial vascular disease. Other late vascular effects include chronic constrictive pericarditis, lung fibrosis, viscous stricture, spinal cord transection, and radiation cystitis or enteritis.

A serious late toxicity is the development of second solid tumors in or adjacent to the radiation fields. Such tumors can develop in any organ or tissue and occur at a rate of ~1% per year beginning in the second decade after treatment.

■ OTHER LOCALIZED CANCER TREATMENTS

Endoscopy allows placement of stents to unblock viscera by mechanical means, palliating, for example, gastrointestinal or biliary obstructions. Radiofrequency ablation (RFA) refers to focused microwave nonionizing radiation to induce thermal injury within a volume of tissue. RFA can be useful in the control of metastatic lesions, particularly in liver, that may threaten biliary drainage (as one example in patients with otherwise unresectable disease). Cryosurgery uses extreme cold to sterilize lesions in certain sites, such as prostate and kidney, at a very

early stage, eliminating the need for modalities with more side effects such as surgery.

Some chemicals (porphyrins, phthalocyanines) are preferentially taken up by cancer cells. When intense light, delivered by a laser, is shone on cells containing these compounds, free radicals are generated and the cells die. Such phototherapy is used to treat skin cancer; ovarian cancer; and cancers of the lung, colon, rectum, and esophagus. Palliation of recurrent locally advanced disease can sometimes be dramatic and last many months.

Infusion of chemotherapeutic or biologic agents or radiation-bearing delivery devices such as isotope-coated glass spheres into local sites through catheters have been used to treat disease limited to that site; in selected cases, prolonged control of truly localized disease has been possible.

SYSTEMIC CANCER TREATMENTS

The concept that systemically administered chemicals might have a useful effect on cancers was historically derived from three sets of observations. Paul Ehrlich in the nineteenth century observed that different dyes reacted with different cell and tissue components. He hypothesized the existence of “magic bullets” that might bind to tumors, owing to the affinity of the agent for the tumor. Observation of the toxic effects of certain mustard gas derivatives on the bone marrow during World War I suggested that smaller doses of these agents might be used to treat tumors of marrow-derived cells. Finally, the fact that tumors from hormone-responsive tissues, e.g., breast tumors, could shrink after oophorectomy led to the idea that endogenous or exogenous substances might modulate tumor growth by altering its regulatory biology. Chemicals achieving each of these goals are currently used as cancer chemotherapy agents.

Anecdotal reports of tumor regression following intratumoral injection of bacterial extracts raised the possibility of immune system-mediated tumor regression. Serotherapy of infectious disease in the preantibiotic era encouraged analogous efforts to develop vaccine- and antibody-based treatments for cancer. Administration of autologous immune cells obtained by pheresis procedures from a patient or purified from a patient's removed tumor, activated by cytokines *ex vivo*, achieved durable disease control in a small fraction of patients. These observations provided the rationale for more modern efforts to treat tumors using cell-mediated immunity.

Systemic cancer treatments are of three broad types. *Cytotoxic chemotherapy agents* are “small molecules” (generally with molecular mass <1500 Da) that cause major regression of experimental tumors growing in animals. These agents mainly target DNA structure or segregation of chromosomes in mitosis. *Cancer molecular target therapies* refer to small molecules designed and developed to interact with a defined macromolecule important in maintaining the malignant state. As described in Chap. 72, successful tumors have activated biochemical pathways that lead to uncontrolled proliferation through the action of hormone receptor proteins, oncogene products, loss of cell cycle inhibitors, or loss of cell death regulation, and have acquired the capacity to replicate chromosomes indefinitely, invade, metastasize, and evade the immune system. *Cancer biologic therapies* are most frequently macromolecules, cells, or cell extracts that have a particular target (e.g., anti-growth factor receptor, cytokine, or immunomodulatory antibodies) or may have the capacity to induce a host immune response to kill tumor cells. Most recent additions to cancer biologic therapies include genetically modified cells that directly attack tumor cells and tumor-infecting viruses that can kill tumor cells but also elicit host antitumor immune responses.

■ SYSTEMIC CANCER THERAPY OVERVIEW

General Principles The *therapeutic index* of any drug is the degree of separation between toxic and therapeutic doses. Really useful drugs have large therapeutic indices, and this usually occurs when the drug target is expressed in the disease-causing compartment as opposed to the normal compartment. Cytotoxic chemotherapeutic agents have the unfortunate property that their main targets, DNA and microtubules,

are present in both normal and tumor tissues. Therefore, they have relatively narrow therapeutic indices. Targeted agents can also cause effects on their target in normal tissues, or “off-target” effects on unrelated targets in organs experiencing damage. Biologic therapies may elicit misdirected immune responses on normal organ function. A key activity in oncology drug development is striving to administer a dose of agent that can convey benefit with a minimal or tolerable side effect profile.

Figure 73-2 illustrates steps in cancer drug development. Following demonstration of antitumor activity in animal models, potentially useful anticancer agents are further evaluated to define an optimal schedule of administration and suitable drug formulation. Safety testing in two animal species on an analogous schedule of administration defines the starting dose for a phase I trial in humans, usually but not always in patients with cancer who have exhausted “standard” (already approved) treatments. The initial dose is usually one-sixth to one-tenth of the dose just causing easily reversible toxicity in the more sensitive animal species. If the agent is not intrinsically toxic, doses of drug achieving fractions of the useful concentration from model systems are studied. Escalating doses of drug are then given during the human phase I trial until reversible toxicity is observed or the desired drug concentration is achieved. Dose-limiting toxicity (DLT) defines a dose that conveys greater toxicity than would be acceptable in routine practice, allowing definition of a lower maximum-tolerated dose (MTD). The occurrence of toxicity is, if possible, correlated with plasma drug concentrations. The MTD or a dose just lower than the MTD is usually the dose suitable for phase 2 trials, where a fixed dose is administered to a relatively homogeneous set of patients with a particular tumor type. If no toxicity has emerged in phase 1 trials, administration of the optimal biologic dose to achieve effective drug concentrations is undertaken. A partial response (PR) historically was defined as a decrease of at least 50% in a tumor’s bidimensional area obtained by imaging; more recent response criteria (e.g., Response Evaluation Criteria in Solid Tumors

[RECIST]) may use a 30% decrease in aggregate unidimensional areas of target lesions. Response criteria for immunologically directed agents may allow a substantial transient increase in tumor volume as long as a patient’s clinical status is stable, as these agents may evoke inflammatory responses in tumors with subsequent shrinkage or stabilization of lesions then occurring subsequently. A complete response (CR) connotes disappearance of all tumor; progression of disease signifies an increase in size of existing lesions by >25% from baseline or best response or development of new lesions; stable disease fits into none of the above categories.

In a phase 3 trial, evidence of improved overall survival or improvement in the time to progression of disease on the part of the new drug is sought in comparison to an appropriate control population. Data from the entire process are the basis for application to a regulatory agency to approve the new agent for commercial marketing.

Cancer drug clinical trials conventionally use a toxicity grading scale where grade 1 toxicities do not require treatment, grade 2 toxicities may require symptomatic treatment but are not life-threatening, grade 3 toxicities are potentially life-threatening if untreated, grade 4 toxicities are actually life-threatening, and grade 5 toxicities are those that result in the patient’s death. Active efforts to quantitate effects of anticancer agents on quality of life also frequently occur in early development of oncology drugs.

Development of targeted agents may proceed differently. While phase 1–3 trials are still conducted, focus on a particular tumor type even in phase 1 may be enabled by molecular analysis to define target expression in a patient’s tumor necessary for or relevant to the drug’s action. Ideally, pharmacodynamic studies would also assess whether the target has been hit. The failure of a targeted therapy can be either because the drug missed the target or it hit the target but the target was not central to the tumor’s growth and survival. Within the past decade, agents have been approved for clinical use not in relation to an

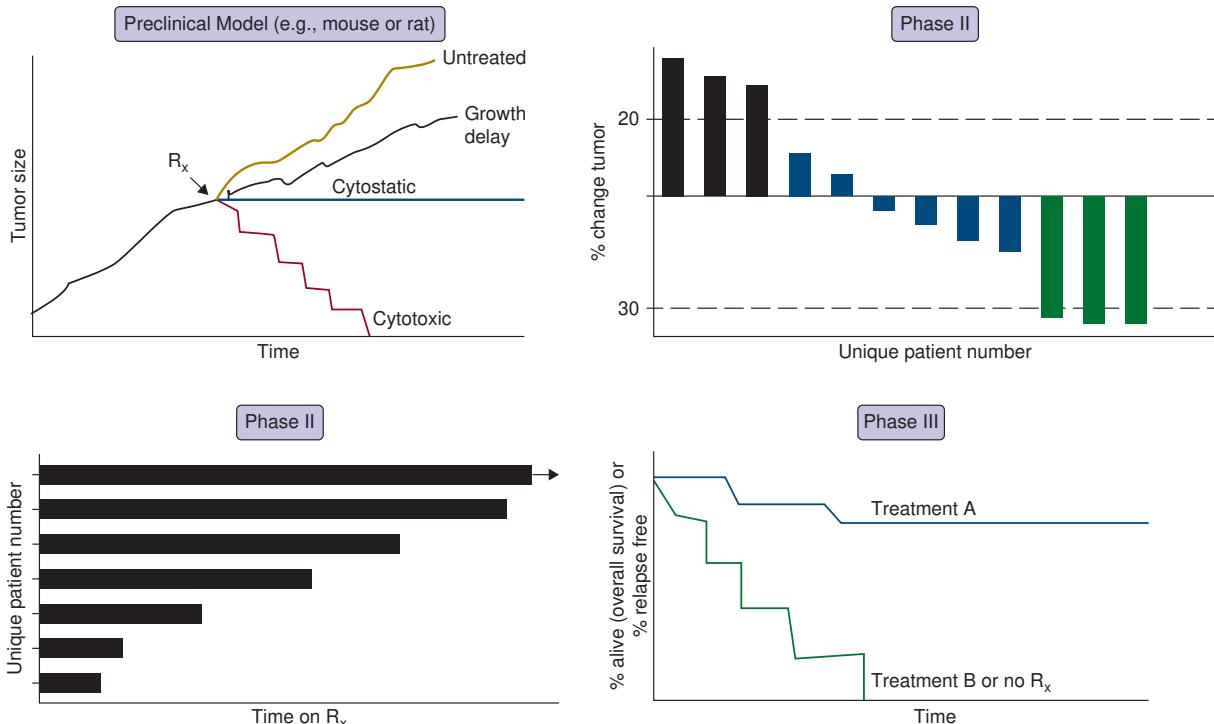


FIGURE 73-2 Steps in cancer drug discovery and development. Preclinical activity (top left) in animal models of cancers may be used as evidence to support the entry of the drug candidate into phase 1 trials in humans to define a correct dose and observe any clinical antitumor effect. The drug may then be advanced to phase 2 trials directed against specific cancer types, with rigorous quantitation of antitumor effects. *Waterfall plots* are a standard representation of how patients’ tumor sizes change in relation to treatment, with predefined cutoffs defining progression of disease (20% increase in size) or partial response (30% decrease in size) serving as benchmarks of potential valuable effect (top right). *Swimmer plots* (bottom left) allow the delineation of patients with especially long (or short) times on treatment even without response, another basis in the former case for potential perceived clinical benefit of the treatment. *Kaplan-Meier plots* (bottom right) of survival indices in phase 3 comparative trials may allow definition of superiority, inferiority, or no difference of treatment effect compared to standard or no treatment.

originating organ site of disease but across all organ types possessing certain molecular or biologic features.

Useful cancer drug treatment strategies using conventional chemotherapy agents, targeted agents, hormonal treatments, or biologicals all have one of two valuable outcomes. They can induce cancer cell death, resulting in tumor shrinkage with corresponding clinical benefit evidenced by improvement in patient survival, or increase in time until the disease progresses. Another potential outcome is induction

of cancer cell *differentiation* or *dormancy* with loss of tumor cell replicative potential and reacquisition of phenotypic properties resembling normal cells. Interaction of a chemotherapeutic drug with its target induces a “cascade” of further signaling steps. These signals ultimately lead to cell death by triggering an “execution phase,” where proteases, nucleases, and endogenous regulators of the cell death pathway are activated (Fig. 73-3), or differentiation by alteration of cancer genome function.

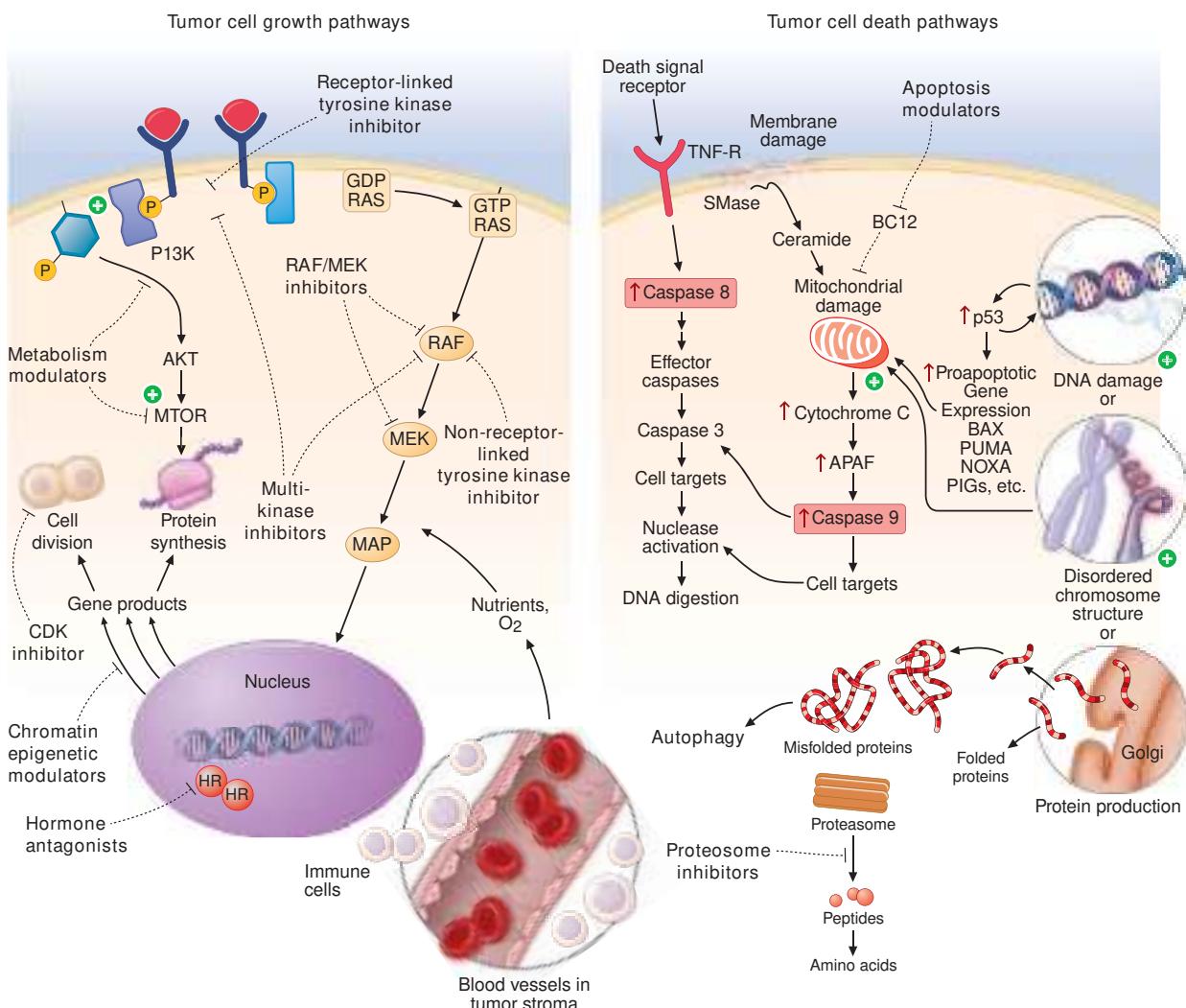


FIGURE 73-3 Tumor growth and death pathways affected by targeted and cytotoxic agents. After a growth factor binds to its receptor (*left side of figure*), in the most commonly activated cell proliferation pathway, increased tyrosine kinase activity occurs, either by autophosphorylation of receptor-linked kinases or through recruitment of non-receptor-linked tyrosine kinases, which may also be active constitutively, without requiring a growth factor. This leads to docking of “adaptor” proteins to the phosphorylated tyrosines. One important pathway activated occurs after exchange of GDP for GTP in the RAS family of protooncogene products. GTP-RAS activates the RAF kinase, leading to a phosphorylation cascade of MEK and MAP kinases that ultimately alters gene function to produce transcripts that activate cell cycle progression through cyclin-dependent kinases (CDKs). Another route to gene activation utilizes hormone receptors (HRs) interacting with tissue-specific growth regulators such as steroid hormones to alter gene function leading to cell cycle activation. Tyrosine phosphorylation can lead to activation of phosphatidylinositol-3-kinase (PI3K) to produce the phosphorylated lipid phosphatidylinositol-3-phosphate, which activates the AKT kinase to act downstream on the mammalian target of rapamycin kinase (mTOR), directly increasing translation of key mRNAs for gene products regulating cell growth. Cytotoxic agents cause cell death (*right side of figure*) through apoptosis and/or induction of autophagy. Apoptosis is also stimulated by interruption of growth factor (GF) cytokine death signals (e.g., tumor necrosis factor receptor [TNF-R]), which activate “upstream” cysteine aspartyl proteases (caspases) to directly digest cytoplasmic and nuclear proteins, resulting in activation of “downstream” caspases; these activate nucleases to cause DNA fragmentation, a hallmark of apoptosis. Chemotherapy agents that create lesions in DNA or alter mitotic spindle function activate gene function to alter mitochondrial integrity. The antiapoptotic protein BCL2 attenuates mitochondrial toxicity, whereas proapoptotic gene products such as BAX, PUMA, etc., antagonize the action of BCL2. Damaged mitochondria release cytochrome C and apoptosis-activating factor (APAF), which activate caspase 9 to cause DNA fragmentation. In addition, membrane damage with activation of sphingomyelinases results in the production of ceramides that can cause direct damage to mitochondria. Protein translation is followed by a folding process in the Golgi apparatus. Misfolded proteins are processed through the proteasome for protease digestion and recycling of amino acids. Disruption of this process can contribute to autophagy, where the cell starves for critical nutrients, or itself induce apoptosis through a distinct pathway activated by misfolded protein accumulation. Antiangiogenic agents and immune therapies work in the tumor stroma (*lower left*) on supporting elements including blood vessels and host inflammatory cells.

Targeted agents differ from chemotherapy agents in that they do not indiscriminately cause macromolecular lesions but regulate the action of macromolecules to whose function tumors have been described as “addicted” in the sense that without the pathway’s continued action, the tumor cell cannot survive. In this way, targeted agents directed at such “oncogenic driver” molecules may alter the threshold for tumors driven by these molecules to undergo cell death.

Strategies in Systemic Cancer Management The past 30 years have witnessed a marked evolution in the systemic treatment of cancer not amenable to cure by locally applied treatments. Nonspecific cytotoxic agents of limited efficacy for most patients but highly active and curative in a minority disease types have been joined by targeted and biologic therapies. **Table 73-3, A** lists those tumors considered curable by conventionally available chemotherapeutic agents even when disseminated or metastatic. If a tumor is truly localized to a single site, consideration of surgery or primary radiation therapy should be given as well. Chemotherapy may be used as part of multimodality approaches to offer primary treatment to a clinically localized tumor (**Table 73-3, B**). Chemotherapy can be administered as an *adjuvant*, i.e., in addition to surgery or radiation (**Table 73-3, C**), even after all clinically apparent disease has been removed. This use of chemotherapy has curative potential in, e.g., lung, breast, and colorectal

neoplasms, as it eliminates clinically unapparent tumor that may have already disseminated. *Neoadjuvant* chemotherapy refers to administration of chemotherapy before any surgery or radiation to a local tumor in an effort to enhance the effect of subsequent local treatment.

Chemotherapy is routinely used in doses that produce reversible acute side effects, primarily consisting of transient myelosuppression with or without gastrointestinal toxicity (usually nausea). “High-dose” chemotherapy regimens can produce markedly increased therapeutic effect, although at the cost of potentially life-threatening complications that require intensive support, usually in the form of hematopoietic stem cell support from the patient (*autologous*) or from donors matched for histocompatibility loci (*allogeneic*), or pharmacologic “rescue” strategies to block the effect of the high-dose chemotherapy on normal tissues. High-dose regimens have curative potential in defined clinical settings (**Table 73-3, D**).

If cure is not possible, chemotherapy may be undertaken with the goal of palliating the tumor’s effect on the host (**Table 73-3, E**). In this usage, value is perceived by the demonstration of improved symptom relief, progression-free survival, or overall survival. The data result from a clinical research protocol used as a basis for FDA approval for commercial use of the agent. Patients treated with palliative intent should be aware of their diagnosis and the limitations of the proposed treatments, have access to supportive care, and have suitable “performance status,” according to assessment algorithms such as the one developed by Karnofsky (see **Table 69-4**) or by the Eastern Cooperative Oncology Group (ECOG) (see **Table 69-5**). ECOG performance status (PS) 0 patients are without symptoms; PS1 patients are ambulatory but restricted in strenuous physical activity; PS2 patients are ambulatory and active 50% or more of the time but unable to work; PS3 patients are capable of limited self-care but are active <50% of the time; and PS4 patients are totally confined to bed or chair and incapable of self-care. Only PS0, PS1, and PS2 patients are generally considered suitable for palliative (noncurative) treatment. If there is curative potential, even poor-PS patients may be treated (especially if their symptoms are directly related to a cancer that may respond to treatment), but their prognosis is usually inferior to that of good-PS patients treated with similar regimens. Assessment of physiologic reserve through use of the geriatric assessment tool can be helpful, but no measure of comorbidities or physiologic reserve is considered standard.

The turn of the millennium marked the arrival of alternative strategies for cancer treatment. Prominent among these are *cancer biologic therapy*, which harnesses the use of immune system-derived reagents or strategies, and *cancer targeted therapies*, which are directed at specific molecular targets differentially expressed in malignant as opposed to normal tissues.

CANCER BIOLOGIC THERAPY

Figure 73-4 presents the landscape of cancer biologic therapy agents and actions. The goal of biologic therapy is to manipulate the host-tumor interaction in favor of the host, potentially at an optimum biologic dose that might be different than MTD. As a class, biologic therapies may be distinguished from cytotoxic and molecularly targeted agents in that biologic therapies require activity (e.g., antigen expression or internalization) on the part of the tumor cell or on the part of the host (e.g., T-cell engagement or cytokine elaboration) to allow therapeutic effect.

Antibody-Mediated Therapeutic Approaches Figure 73-4 illustrates current antibody-based strategies in cancer treatment. The ability to grow very large quantities of high-affinity monoclonal antibodies directed at specific tumor antigens produced by animals allows the grafting of animal-derived antigen-combining sequences into human immunoglobulin genes (chimerized or humanized products) or derived de novo from mice bearing human immunoglobulin gene loci. Four general strategies have emerged using antibodies. *Anti-tumor cell antibodies* target tumor cells directly to inhibit intracellular functions or attract immune or stromal cells. *Bispecific tumor engaging (BiTe) antibodies* directly bind to a tumor cell and to a host immune cell. *Immunoregulatory antibodies* target antigens expressed on host

TABLE 73-3 Clinical Impact on Cancers with Cytotoxic Chemotherapy

A. Advanced Cancers with Possible Cure	D. Cancers Possibly Cured with High-Dose Chemotherapy with Stem Cell Support
Acute lymphoid and acute myeloid leukemia (pediatric/adult)	Relapsed leukemias, lymphoid and myeloid
Hodgkin’s disease (pediatric/adult)	Relapsed lymphomas, Hodgkin’s and non-Hodgkin’s
Lymphomas—certain types (pediatric/adult)	Chronic myeloid leukemia
Germ cell neoplasms	Multiple myeloma
Embryonal carcinoma	E. Cancers Responsive with Useful Palliation, But Not Cure, by Chemotherapy
Teratocarcinoma	Bladder carcinoma
Seminoma or dysgerminoma	Chronic myeloid leukemia
Choriocarcinoma	Hairy cell leukemia
Gestational trophoblastic neoplasia	Chronic lymphocytic leukemia
Pediatric neoplasms	Lymphoma—certain types
Wilms’ tumor	Multiple myeloma
Embryonal rhabdomyosarcoma	Gastric carcinoma
Ewing’s sarcoma	Cervix carcinoma
Peripheral neuroepithelioma	Endometrial carcinoma
Neuroblastoma	Soft tissue sarcoma
Small-cell lung carcinoma	Head and neck cancer
Ovarian carcinoma	Adrenocortical carcinoma
B. Advanced Cancers Possibly Cured by Chemotherapy and Radiation	Islet cell neoplasms
Squamous carcinoma (head and neck)	Breast carcinoma
Squamous carcinoma (anus)	Colorectal carcinoma
Breast carcinoma	Renal carcinoma
Carcinoma of the uterine cervix	F. Tumors Poorly Responsive in Advanced Stages to Chemotherapy
Non-small-cell lung carcinoma (stage III)	Pancreatic carcinoma
Small-cell lung carcinoma	Biliary tract neoplasms
C. Cancers Possibly Cured with Chemotherapy as Adjuvant to Surgery	Thyroid carcinoma
Breast carcinoma	Carcinoma of the vulva
Colorectal carcinoma ^a	Non-small-cell lung carcinoma
Osteogenic sarcoma	Prostate carcinoma
Soft tissue sarcoma	Melanoma (subsets)

^aRectum also receives radiation therapy.

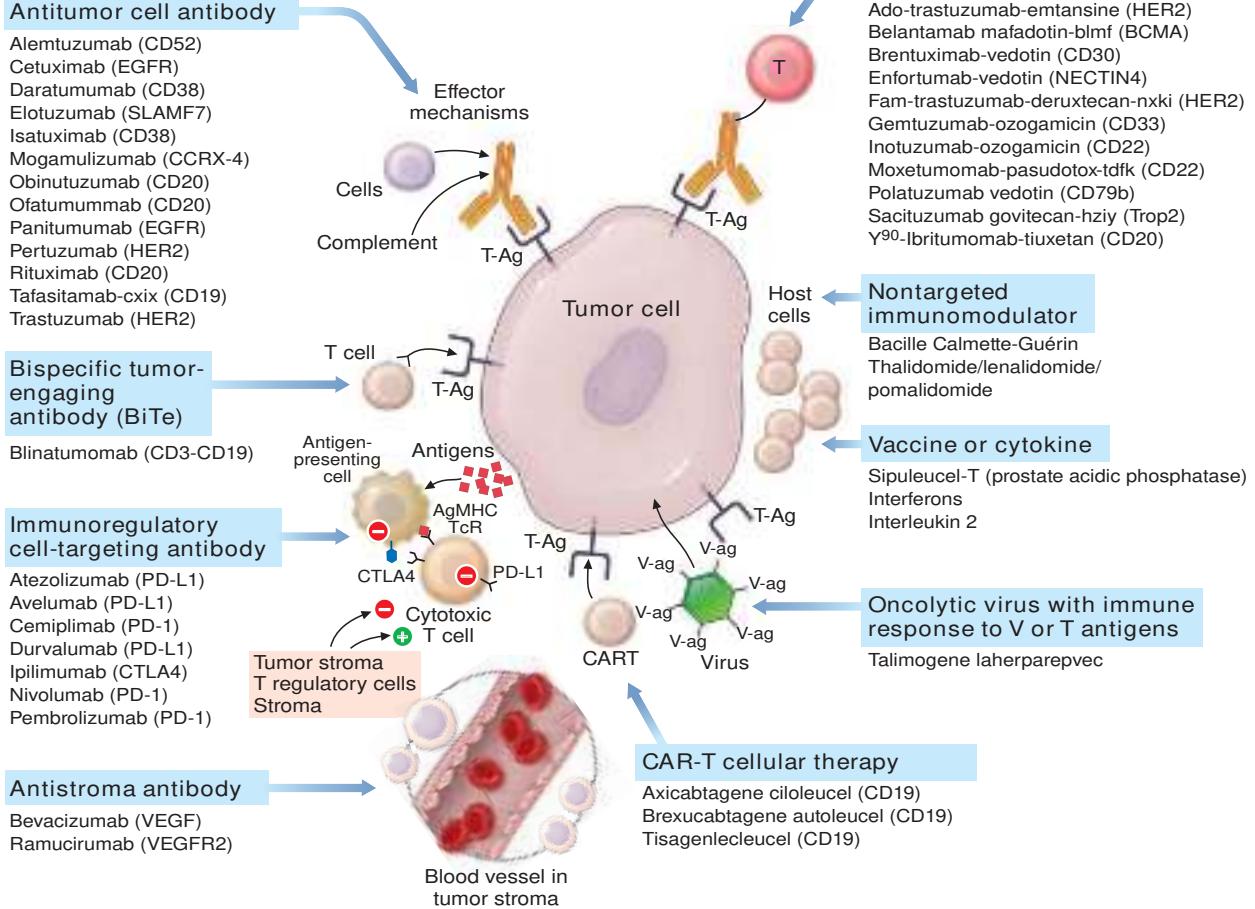


FIGURE 73-4 Immunologic treatments for cancer. Anti-tumor cell antibodies targeting antigens (T-Ag) expressed on tumor cells and indicated in parentheses for each antibody or antibody-derived construct (*upper left*) can either directly interfere with tumor cell function by, e.g., inhibiting growth-promoting pathways, or recruit host immune effector cells actively (especially through *bispecific tumor-engaging [BiTe] strategies*), Fc receptors, or cytotoxic mechanisms such as complement. Proceeding *clockwise* in the figure, *antibody conjugates* can also be engineered to deliver cytotoxic drugs, bacteria-derived toxins, or radios isotopes (T) to T-Ags (*upper right*). Relatively *nonspecific immunomodulators* include vaccines instilled directly into the tumor stroma, agents such as the “imids” that alter tumor and stromal cell cytokine production, and cytokines such as interferon or interleukin 2 (IL-2), which can affect tumor-infiltrating lymphocyte function or have direct antitumor effects. *Vaccines* targeting tumor cell antigens or live attenuated *oncolytic viruses* injected into tumors can cause tumor cell lysis with induction of a prominent host antitumor immune response to virus Ags and T-Ags. In the *left lower portion* of the figure, strategies to target stromal and immune cells include derivation of autologous T cells that are then infected with a lentivirus or other construct that targets antigens (T-Ags) expressed on tumor cells (*chimeric antigen receptor [CAR] T cells*), with the targeted antigen in parentheses. Alternatively, endogenous T cells can be activated by *immunomodulatory cell targeting antibodies*. Specifically, tumor cell-derived antigens are taken up by antigen-presenting cells (APCs), also in the stroma. Antigens are processed by the APCs to peptides presented by the major histocompatibility complex (MHC) to T-cell antigen receptors (TcRs), thus providing a positive (+) activation signal for the cytotoxic tumor cells to kill tumor cells bearing that antigen. Negative (-) signals inhibiting cytotoxic T-cell action include the CTLA4 receptor (on T cells), interacting with the B7 family of negative regulatory signals from APCs, and the PD receptor (on T cells), interacting with the PD ligand-1 (PD-L1) (-) signal coming from tumor cells expressing the PD-L1. Strategies that inhibit CTLA4 and PD-1 function are a means of stimulating cytotoxic T-cell activity to kill tumor cells. *Tumor stroma-directed antibodies* cause anti-vascular endothelial growth factor (VEGF)-mediated antiangiogenic and tumor interstitial pressure-modulating strategies.

immune cells to boost the host's immune response to the tumor. Finally, *antibody conjugates* link the antibody to drugs, toxins, or radioisotopes to target these “warheads” for delivery to the tumor. These will be considered with cytotoxic agents. *Stroma-directed antibodies* are currently available against tumor supporting vasculature.

ANTI TUMOR CELL ANTIBODIES FIG. 73-4 Humanized antibodies against the CD20 molecule expressed on B-cell lymphomas (rituximab and ofatumumab) are exemplary of antibodies that affect both signaling events driving lymphomagenesis as well as activating immune responses against B-cell neoplasms. They are used as single agents and in combination with chemotherapy and radiation in the treatment of B-cell neoplasms. Obinutuzumab is an antibody with altered

glycosylation that enhances its ability to activate killer cells; it is also directed against CD20 and is of value in chronic lymphocytic leukemia.

Unintended side effects of any antibody include infusion-related hypersensitivity reactions, usually limited to the first infusion, which can be managed with glucocorticoid and/or antihistamine prophylaxis, and prolonged infusion strategies.

Anti-B-cell-directed antibodies can have the unintended effect of exacerbating immunosuppression with the emergence of increased opportunistic infections. Reactivation of latent infections may also occur; an assessment of a patient's hepatitis B and C status is conventionally done before treatment. Concomitant use of antivirals directed against hepatitis may be indicated. Patients with HIV and lymphoma need antivirals optimized to minimize interaction with

anti-lymphoma treatments; consultation with infectious disease specialists is warranted. Anti-tumor cell antibodies also include approaches to activate complement and are exemplified by alemtuzumab directed against CD52; it is active in chronic lymphoid leukemia and T-cell malignancies. Tumor lysis syndrome prophylaxis may be warranted.

Epidermal growth factor receptor (EGFR)-directed antibodies (e.g., cetuximab and panitumumab) have activity in colorectal cancer refractory to chemotherapy, particularly when used to augment the activity of an additional chemotherapy program, and in the primary treatment of head and neck cancers treated with radiation therapy. Direct effects on the tumor may mediate an antiproliferative effect as well as stimulate the participation of host mechanisms involving immune cell or complement-mediated response to tumor cell-bound antibody. Anti-EGFR antibodies can cause an acneiform rash requiring topical antibiotic and glucocorticoid cream treatment; photosensitivity also occurs.

The HER2/neu receptor overexpressed on epithelial cancers, especially breast and certain gastrointestinal cancers, was initially targeted by trastuzumab, with activity in potentiating the action of chemotherapy in breast cancer as well as evidence of single-agent activity. Trastuzumab appears to interrupt intracellular signals derived from HER2/neu and to stimulate anti-tumor cell immune mechanisms. The anti-HER2 antibody pertuzumab, specifically targeting the domain of HER2/neu responsible for dimerization with other HER2 family members, is more specifically directed against HER2 signaling function and augments the action of trastuzumab. Both trastuzumab and pertuzumab can damage cardiac function, particularly in patients with prior exposure to anthracyclines, and left ventricular function should be checked pre-treatment and monitored during treatment.

The BiTe antibody blinatumomab was constructed to have an anti-CD19 antigen-combining site directed at a cancer cell as one valency with an anti-CD3 binding site as the other. This antibody can bring T cells (with their anti-CD3 activity) close to neoplastic B cells bearing the CD19 determinant. Blinatumomab is active in B-cell neoplasms such as acute lymphocytic leukemia. Unique toxicities include cytokine release syndrome (fever, hypotension, tachycardia) and neurologic deterioration manifest initially by deterioration in handwriting accuracy, which can proceed to more florid cortical dysfunction, suggesting a need for dose pausing and/or glucocorticoid use.

STROMA-DIRECTED ANTIBODIES (FIG. 73-4) The anti-vascular endothelial growth factor (VEGF) antibody bevacizumab shows some evidence of value in renal cancers, where activation of VEGF signaling occurs as part of disabled hypoxia-induced signaling in the tumor cells. When combined with chemotherapeutic agents, it may increase responses in colorectal and nonsquamous lung cancers. The mechanism for this effect may relate to improved delivery and tumor uptake of the active chemotherapeutic agent, owing to decreased tumor interstitial pressure. VEGF was originally isolated as a “tumor permeability factor” causing leakiness of tumor blood vessels. When used in gliomas, it may, by decreasing vascular permeability, allow replacement of steroids to decrease intracranial pressure. Bevacizumab is directed against VEGF, which is normally a secreted product and not attached to tumor cells. Bevacizumab has a number of side effects including hypertension, thrombosis, proteinuria, hemorrhage, and gastrointestinal perforations with or without prior surgeries; these adverse events also occur with small-molecule drugs modulating VEGFR function.

IMMUNOREGULATORY ANTIBODIES (FIG. 73-4) Purely immunoregulatory antibodies stimulate immune responses to mediate tumor-directed cytotoxicity. An understanding of the tumor-host interface has revealed that cytotoxic tumor-directed T cells are frequently inhibited by ligands upregulated in the tumor cells. The programmed death ligand 1 (PD-L1; also known as B7-homolog 1) was initially recognized as inducing T cell death through a receptor present on T cells, termed the programmed death (PD) receptor, which physiologically regulates the intensity of the immune response to any antigen. The PD family of ligands and receptors also regulates macrophage function, present in tumor stroma. These actions raised the hypothesis that antibodies directed against the PD signaling axis (both anti-PD-L1 and anti-PD)

might be useful in cancer treatment by allowing reactivation of the immune response against tumors.

Ipilimumab, an antibody directed against the anti-CTLA4 (cytotoxic T lymphocyte antigen 4), which is expressed on T cells (not tumor cells), responds to signals from antigen-presenting cells (Fig. 73-4) and also downregulates the intensity of the T-cell proliferative response to antigens derived from tumor cells. Indeed, manipulation of the CTLA4 axis was the first demonstration that purely immunoregulatory antibody strategies directed at T-cell physiology could be safe and effective in the treatment of cancer. Ipilimumab, alone or in combination with PD-1-directed antibodies, is approved for treatment of metastatic melanoma and lung cancers.

Nivolumab, directed against the PD-1 receptor, or atezolizumab (anti-PD-L1) are exemplary of anti-PD-1 directed immunoregulatory antibodies, with clinical benefits in many cancers (Table 73-4). Pembrolizumab is approved for first-line treatment of metastatic non-small-cell lung cancer tumors that express the PD-L1 ligand. This development was a milestone in cancer therapeutics, replacing chemotherapy in this patient subset.

Importantly, the clinical observation that tumors most amenable to treatment with immunoregulatory antibodies were in sites (lung, skin, genitourinary) exposed to environmental carcinogens or occurred in patients with known mutations in DNA repair pathways stimulated specific research as to whether the “mutational burden” of tumors could predict value from anti-PD strategies. Results to date confirm this hypothesis and led to the first regulatory approvals for immunomodulating antibodies in a “tissue agnostic” fashion. Specifically, detection of deficiencies in a tumor DNA mismatch repair system or with evidence of increased tumor mutation burden is a specific indication for use of certain immunoregulatory agents, irrespective of the disease site of origin. The increased efficacy in the setting of higher tumor mutational burden is thought to be due to the presence of more proteins in the tumor structurally altered by mutation that can be recognized as foreign by the immune system.

Prominent autoimmune hepatic, endocrine, cutaneous, neurologic, and gastrointestinal adverse events can occur with the use of ipilimumab as well as the PD-1-directed antibodies. Emergency use of glucocorticoids may be required to attenuate severe toxicities. Although theoretically such glucocorticoid use can attenuate the antitumor effect, response rates do not appear to be compromised by their use to abrogate serious organ toxicity attributable to use of immunomodulatory antibodies. Importantly for the general internist, immunologic toxicities can occur late after exposure to the modulators of PD and CTLA4 action, even while the patient may have sustained control of tumor growth.

Nontargeted Immunomodulators (Fig. 73-4) Bacille Calmette-Guérin, a killed mycobacterial product, invokes a useful immune response when instilled locally into the bladder in the setting of preinvasive bladder cancers. The “imids” thalidomide, lenalidomide, and pomalidomide alter cytokine elaboration in the tumor microenvironment and have antiangiogenic actions. They are a cornerstone in the management of multiple myeloma. Thromboses (warranting consideration of prophylactic anticoagulation), gastrointestinal and neuropathic adverse events, and prominent teratogenicity can occur as a consequence of their use.

Cytokines Only interferon α (IFN- α) and interleukin 2 (IL-2) are in routine clinical use. IFN is not curative for any tumor but can induce partial responses in follicular lymphoma, hairy cell leukemia, chronic myeloid leukemia, melanoma, and Kaposi’s sarcoma. It produces fever, fatigue, a flulike syndrome, malaise, myelosuppression, and depression and can induce clinically significant autoimmune disease.

IL-2 exerts its antitumor effects indirectly through augmentation of immune function. Its biologic activity is to promote the growth and activity of T cells and natural killer (NK) cells. High doses of IL-2 can produce tumor regression in certain patients with metastatic melanoma and renal cell cancer. About 2–5% of patients may experience complete remissions that are durable. Patients may require blood pressure support and intensive care to manage the toxicity. However, once

TABLE 73-4 Clinical Impact of Host T Lymphocyte–Modified Cells^a or Host T Lymphocyte–Directed Immunoregulatory Antibodies^c

A. Advanced Cancers With Positive Effect (at least 25% of treated patients have stable disease or progression-free survival of ≥27 weeks or better) or Frequent or Unexpected Prolonged Responders (efficacy may be limited to CD expression-dependent or PD-1 ligand-expressing subtypes)
Acute lymphoid leukemia ^b
Adrenocortical carcinoma ^c
Breast cancer, hormone receptor negative, HER2 negative (with chemotherapy) ^c
Colorectal cancer (microsatellite instability-high [MSI-H] or mismatch repair deficient, following treatment with fluoropyrimidine, oxaliplatin, and irinotecan) ^c
Cervix, squamous carcinoma ^c
Cutaneous, squamous carcinoma ^c
Diffuse large B-cell non-Hodgkin's lymphoma, not otherwise specified ^a
Diffuse large B-cell non-Hodgkin's lymphoma, primary mediastinal subtype ^b
Endometrial carcinoma (with lenvatinib, if microsatellite instability-stable [MSI-S] or mismatch repair wild-type) ^c
Esophageal squamous carcinoma ^c
Gastric/gastroesophageal adenocarcinoma ^c
Head and neck squamous carcinoma ^c
Hepatocellular cancer (after sorafenib) ^c
Hodgkin's disease ^c
Mantle cell lymphoma ^a
Melanoma ^c
Merkel cell carcinoma ^c
MSI-H or mismatch repair-deficient solid tumors without satisfactory alternative ^c
Mycosis fungoïdes ^c
Multiple myeloma ^a
Non-small-cell lung carcinoma ^c
Paraganglioma/pheochromocytoma ^c
Renal cell carcinoma ^c
Small-cell lung carcinoma ^c
Solid tumors with high tumor mutational burden (TMB) (≥10 mutations/megabase) that have progressed following prior therapy without satisfactory alternative treatment ^c
Thyroid carcinoma ^c
Urothelial carcinoma ^c (including bladder, ureter)
B. Advanced Cancers With Insufficient Data to Support Host T Lymphocyte or Immunoregulatory Antibodies
Acute myeloid leukemia
Anus, squamous carcinoma
Breast cancer, hormone receptor positive
Breast cancer, hormone receptor negative, HER2 positive
Biliary tract cancers (if MSI-S or mismatch repair wild-type)
Chronic lymphocytic leukemia
Chronic myeloid leukemia
Gastrointestinal neuroendocrine/islet cell carcinoma
Glioma, all grades including glioblastoma
Germ cell neoplasms
Ovarian cancer
Osteogenic sarcoma
Pancreas adenocarcinoma
Pediatric tumors (Wilms', rhabdomyosarcoma, Ewing's, neuroblastoma, osteosarcoma)
Prostate adenocarcinoma
Salivary gland carcinoma
Soft tissue sarcoma
T-cell non-Hodgkin's lymphoma (except mycosis fungoïdes)
Vulva, squamous carcinoma

^aChimeric antigen receptor (CAR)-modified autologous T cells in relapsed or refractory cases. ^bBoth CAR-modified autologous T cells or an immunoregulatory antibody. ^cT-cell directed immunoregulatory antibody strategies including anti-PD1 and/or anti-PD-L1 antibodies; or BiTe antibodies against a particular tumor cell antigen.

the agent is stopped, most of the toxicities reverse completely within 3–6 days.

T Cell–Mediated Therapies The strongest evidence that the immune system can exert clinically meaningful antitumor effects comes from allogeneic bone marrow transplantation. Adoptively transferred T cells from the donor expand in the tumor-bearing host, recognize the tumor as being foreign, and can mediate impressive antitumor effects (graft-versus-tumor effects). Three types of currently used cancer treatments take advantage of the ability of T cells to kill tumor cells.

1. *Transfer of allogeneic T cells.* This occurs in three major settings: in allogeneic bone marrow transplantation; as purified lymphocytic transfusions following bone marrow recovery after allogeneic bone marrow transplantation; and as pure lymphocyte transfusions following immunosuppressive (nonmyeloablative) therapy (also called reduced-intensity or minitransplants). In each of these settings, the effector cells are donor T cells that recognize the tumor as being foreign, probably through minor histocompatibility differences. The main risk of such therapy is the development of graft-versus-host disease because of the minimal difference between the cancer and the normal host cells. This approach has been highly effective in certain hematologic cancers refractory to chemotherapeutic strategies.

2. *Transfer of autologous T cells.* In this approach, the patient's own T cells are removed from the tumor-bearing host, manipulated in several ways *in vitro*, and given back to the patient. Tumor antigen-specific T cells can be developed after retroviral transduction of the desired T-cell antigen receptor and expanded to large numbers over many weeks *ex vivo* before administration. These chimeric antigen receptor (CAR) T cells (Fig. 73-4) have evidence of sustained value in patients with refractory hematopoietic neoplasms such as diffuse large B-cell lymphoma and mantle cell lymphoma. Prominent adverse effects include cytokine release (fever, tachycardia, hypotension) and neurologic manifestations. Clinical investigations are seeking to develop solid-tumor antigen-directed CAR strategies, as well as to utilize different immune cell populations such as NK cells to deliver the antigen receptor construct in ways that may allow “off-the-shelf” products not requiring manipulation and purification of patients' autologous cells.

A second autologous T-cell strategy uses activation of the patient's T cells to polyclonal stimulators such as anti-CD3 and anti-CD28 after a short period *ex vivo* and then amplification in the host after transfer by stimulation with IL-2. Short periods removed from the patient permit the cells to overcome the tumor-induced T-cell defects, and such cells traffic and home to sites of disease better than cells that have been in culture for many weeks.

3. *Tumor vaccines aimed at boosting T-cell immunity.* Two types of vaccine approaches are currently approved. Purified autologous antigen-presenting cells can be pulsed with tumor, its membranes, or particular tumor antigens and delivered as a vaccine. Vaccine adjuvants such as granulocyte-macrophage colony-stimulating factor (GM-CSF) may be co-administered. One such vaccine, sipuleucel-T (Fig. 73-4), is approved for use in patients with hormone-independent prostate cancer. In this approach, the patient undergoes leukapheresis, wherein mononuclear cells (that include antigen-presenting cells) are removed from the patient's blood. The cells are pulsed in a laboratory with an antigenic fusion protein comprising a protein frequently expressed by prostate cancer cells, prostate acid phosphatase, fused to GM-CSF, and matured to increase their capacity to present the antigen to immune effector cells. The cells are then returned to the patient in a well-tolerated treatment. Although no objective tumor response was documented in clinical trials, median survival was increased by about 4 months.

Another important vaccine strategy is directed at infectious agents whose action ultimately is tied to the development of human cancer. Hepatitis B vaccine in an epidemiologic sense prevents hepatocellular carcinoma, and a tetravalent human papillomavirus vaccine prevents infection by virus types currently accounting for 70% of cervical cancer. Unfortunately, these vaccines are ineffective at treating patients who have developed a virus-induced cancer.

Oncolytic Viruses (Fig. 73-4) Laboratory studies in animals have utilized viruses to destroy tumors because tumor cells lack endogenous host mechanisms, e.g., IFN elaboration or recognition strategies of viral nucleic acids, that limit virus spread. Viral infection of tumors also can stimulate a prominent host response to viral and tumor cell antigens, leading to immune effects against local tumor cells. Talimogene laherparepvec is a clinically approved attenuated herpes virus that acts to stimulate immune responses when instilled locally into melanoma deposits. Systemic effects are minimal in this application. This general strategy is being considered particularly in tumors not amenable to useful effects of currently approved immunoregulatory antibodies or in conjunction with immunoregulatory antibodies.

CANCER CYTOTOXIC THERAPY

Table 73-5 lists commonly used cytotoxic cancer chemotherapy agents and pertinent clinical aspects of their use, with particular reference to adverse effects that might be encountered by the generalist in the care of patients. The drugs were initially discovered through screening of chemicals and natural product extracts to define evidence of antitumor activity in animals or were designed with knowledge of biochemical pathways affecting nucleic acid synthesis. They may be usefully grouped into two general categories: those affecting DNA and those affecting microtubules.

As illustrated in Fig. 73-3, disruption of DNA or microtubule integrity is a major trigger of cellular apoptosis pathways. An additional factor in drug effect stems from recent observations that tumor cells have increased tolerance of specific types of DNA damage owing to defects in DNA repair pathways. This state is thought to facilitate the survival of the neoplastic clone as it experiences DNA mutations during the course of carcinogenesis. DNA-directed cytotoxic agents can interact with certain DNA repair mutations in a “synthetic lethal” fashion: the DNA repair mutation enhances lethality of the chemotherapy agent. Examples of a potential “synthetic lethal effect” will be pointed out in relation to clinical applications below.

DNA-Interactive Agents DNA replication occurs during the synthesis or S-phase of the cell cycle, with chromosome segregation of the replicated DNA in the M, or mitosis, phase. The G₁ and G₂ “gap phases” precede S and M, respectively. Chemotherapeutic agents have been divided into “phase-nonspecific” agents, which can act in any phase of the cell cycle, and “phase-specific” agents, which require the cell to be at a particular cell cycle phase to cause greatest effect.

Alkylating agents (Table 73-5) as a class are cell cycle phase-nonspecific agents. They break down, either spontaneously or after normal organ or tumor cell metabolism, to reactive intermediates that covalently modify bases in DNA. This leads to cross-linkage of DNA strands or the appearance of breaks in DNA as a result of repair efforts. Damaged DNA cannot complete normal cell division; in addition, it activates apoptosis. Alkylating agents share similar toxicities: myelosuppression, alopecia, gonadal dysfunction, mucositis, and pulmonary fibrosis. They also share the capacity to cause “second” neoplasms, particularly leukemia, years after use, particularly when used in low doses for protracted periods.

Cyclophosphamide is inactive unless metabolized by the liver to 4-hydroxy-cyclophosphamide, which decomposes into an alkylating species, as well as to chloroacetaldehyde and acrolein. The latter causes chemical cystitis; therefore, excellent hydration must be maintained while using cyclophosphamide. If severe, the cystitis may be attenuated or prevented altogether (if expected from the dose of cyclophosphamide to be used) by mesna (2-mercaptopethanesulfonate). Liver disease impairs cyclophosphamide activation. Sporadic interstitial pneumonitis leading to pulmonary fibrosis can accompany the use of cyclophosphamide, and high doses used in conditioning regimens for bone marrow transplant can cause cardiac dysfunction. Ifosfamide is a cyclophosphamide analogue also activated in the liver, but more slowly, and it requires co-administration of mesna to prevent bladder injury. CNS effects, including somnolence, confusion, and psychosis, can follow ifosfamide use; the incidence appears related to low body surface area or decreased creatinine clearance.

Several alkylating agents are less commonly used. Bendamustine has activity in chronic lymphocytic leukemia and certain lymphomas. Busulfan can cause profound myelosuppression, alopecia, and pulmonary toxicity but is relatively “lymphocyte sparing.” It is used in transplant preparation regimens. Melphalan shows variable oral bioavailability and undergoes extensive binding to albumin and α_1 -acidic glycoprotein. Mucositis appears more prominently; however, it has prominent activity in multiple myeloma.

Nitrosoureas break down to carbamylating species that not only cause a distinct pattern of DNA base pair-directed reactivity but also can covalently modify proteins. They share the feature of causing relatively delayed bone marrow toxicity, which can be cumulative and long-lasting. Procarbazine is metabolized in the liver and possibly in tumor cells to yield a variety of free radical and alkylating species. In addition to myelosuppression, it causes hypnotic and other CNS effects, including vivid nightmares. It can cause a disulfiram-like syndrome on ingestion of ethanol. Dacarbazine (DTIC) is activated in the liver to yield the highly reactive methyl diazonium cation. It causes only modest myelosuppression 21–25 days after a dose but causes prominent nausea on day 1. Temozolomide is structurally related to dacarbazine but is activated by nonenzymatic hydrolysis in tumors and is bioavailable orally. Brain tumors with alkylguanine alkyl transferase deficiency are selectively susceptible to temozolomide, which alkylates the O⁶ position of guanine.

Cisplatin was discovered fortuitously by observing that bacteria present in electrolysis solutions with platinum electrodes could not divide. Only the *cis* diamine configuration is active as an antitumor agent. In tumor cells, a chloride is lost from each position. The resulting positively charged species is an efficient DNA interactor, forming Pt-based cross-links. Cisplatin is administered with abundant hydration, including forced diuresis with mannitol to prevent kidney damage; even with the use of hydration, gradual decrease in kidney function is common, along with noteworthy anemia. Hypomagnesemia frequently attends cisplatin use and can lead to hypocalcemia and tetany. Other common toxicities include neurotoxicity with stocking-and-glove sensorimotor neuropathy. Hearing loss occurs in 50% of patients treated with conventional doses. Cisplatin is intensely emetogenic, requiring prophylactic antiemetics. Myelosuppression is less evident than with other alkylating agents. Chronic vascular toxicity (Raynaud’s phenomenon, coronary artery disease) is a more unusual toxicity. Carboplatin displays less nephro-, oto-, and neurotoxicity. However, myelosuppression is more frequent, and because the drug is exclusively cleared through the kidney, adjustment of dose for creatinine clearance must be accomplished through use of various dosing nomograms. Oxaliplatin is a platinum analogue with noteworthy activity in colon cancers refractory to other treatments. It is prominently neurotoxic.

Trabectedin binds to DNA through the “DNA minor groove” with covalent interaction with the N2 position of certain guanines. Uniquely among the DNA interactors, this can lead to the disruption of the selective FUS-CHOP transcription factor action, important in the pathogenesis of certain liposarcomas. Transient altered liver function can occur, as well as cytopenias. Lurbinectedin is an analogue of trabectedin and also alters RNA polymerase function after binding to the minor groove of DNA, but has a distinct pharmacologic profile.

Antitumor Antibiotics and Topoisomerase Poisons Antitumor antibiotics are substances produced by bacteria that provide a chemical defense against hostile microorganisms. They bind to DNA directly and can frequently undergo electron transfer reactions to generate free radicals in close proximity to DNA, leading to DNA damage in the form of single-strand breaks or cross-links. Topoisomerase poisons include natural products or semisynthetic derivatives that modify enzymes that allow DNA to unwind during replication or transcription. These include topoisomerase I, which creates single-strand breaks that then rejoin following the passage of the other DNA strand through the break. Topoisomerase II creates double-strand breaks through which another segment of DNA duplex passes before rejoining. Owing to the role of topoisomerase I in the replication fork, topoisomerase I poisons cause lethality if the topoisomerase I-induced lesions occur in S-phase.

TABLE 73-5 Cytotoxic Chemotherapy Agents^a

DRUG	TOXICITY	INTERACTIONS, ISSUES
Direct DNA-Interacting Agents^a		
Alkylator		
Bendamustine	Contraindicated with prior sensitivity to polyethylene glycol 400, propylene glycol, or monothioglycerol; cytopenias, infections, cutaneous eruptions, hepatotoxicity	Monitor for tumor lysis syndrome, extravasation, anaphylaxis, and infusion reactions
Carboplatin	Marrow: platelets > WBCs; nausea, renal (high dose)	Reduce dose according to CrCl to AUC of 5–7 mg/mL per min [AUC = dose/(CrCl + 25)]
Carmustine (BCNU)	Myeloid (delayed nadir), GI, liver (high dose), renal	Pulmonary toxicity especially after >1400 mg/m ² cumulative dose; can be delayed in appearance
Cisplatin	Nausea, neuropathy, auditory, marrow: platelets > WBCs; renal, ↓ Mg ²⁺ , ↓ Ca ²⁺	Maintain high urine flow; osmotic diuresis, monitor intake/output, K ⁺ , Mg ²⁺ ; emetogenic—prophylaxis needed; full dose if CrCl >60 mL/min and tolerate fluid push
Chlorambucil	Common alkylator ^b	Liver metabolism required to activate to phosphoramide mustard + acrolein; mesna protects against “high-dose” bladder damage
Cyclophosphamide	Marrow (relative platelet sparing), cystitis, common alkylator ^b , fulilike symptoms, cardiac (high dose)	Metabolic activation
Dacarbazine (DTIC)	Myelosuppressive, cystitis, neurologic, metabolic acidosis	Analog of cyclophosphamide, must use concomitant mesna
Ifosfamide	Marrow, bladder, CNS	
Lomustine (CCNU)	Marrow (delayed nadir)	
Lurbinectedin	Marrow, hepatotoxicity, nausea, vomiting	CYP3A4
Melphalan	Marrow (delayed nadir), GI (high dose)	Decreased renal function delays clearance
Oxaliplatin	Nausea, anemia	Acute reversible neurotoxicity; chronic sensory neurotoxicity cumulative with dose; reversible laryngopharyngeal spasm
Procarbazine	Marrow, nausea, neurologic, common alkylator ^b	Liver and tissue metabolism required, disulfiram-like effect with alcohol, acts as MAOI. HBP after tyrosinase-rich foods
Temozolomide	Nausea, vomiting, headache, fatigue, constipation	Myelosuppression
Trabectedin	Neutropenia, risk of fever; thrombocytopenia; rhabdomyolysis, reversible hepatic toxicity but dose reduce with liver impairment	Unusual capillary leak risk; CYP3A4
Antitumor Antibiotics and Topoisomerase Poisons		
Bleomycin	Pulmonary, skin, Raynaud's, hypersensitivity	Monitor DLCO during treatment (inactivate by bleomycin hydrolase; decreased in lung/skin); O ₂ enhances pulmonary toxicity; cisplatin-induced decrease in CrCl may increase skin/lung toxicity; reduce dose if CrCl <60 mL/min
Dactinomycin	Marrow, nausea, mucositis, vesicant, alopecia	Radiation recall
Doxorubicin and daunorubicin	Marrow, mucositis, alopecia, cardiac acute/chronic, vesicant	Heparin aggregate; coadministration increases clearance; acetaminophen, BCNU increase liver toxicity; radiation recall; dose reduce with increased bilirubin
Epirubicin	Marrow, mucositis, alopecia, cardiac acute/chronic, vesicant	Dose reduce with increased bilirubin, decreased CrCl
Etoposide (VP16-213)	Marrow (WBCs > platelet), alopecia, hypotension, hypersensitivity with rapid IV, nausea, mucositis	Hepatic metabolism and renal excretion (30%); reduce doses with liver and kidney failure; accentuate antimetabolite action
Idarubicin	Marrow, mucositis, alopecia, cardiac acute/chronic, vesicant	Dose reduce with increased bilirubin, decreased CrCl
Irinotecan	Diarrhea: “early onset” with cramping, flushing, vomiting; “late onset” after several doses; marrow, alopecia, nausea, vomiting, pulmonary	Prodrug requires enzymatic clearance to active drug SN-38; early diarrhea due to acetylcholine release, can counter with atropine; late diarrhea, use loperamide 4 mg with first stool then 2 mg q2h until 12 h without stool up to 16 mg/24 h
Mitoxantrone	Marrow, cardiac (less than doxorubicin), vesicant; blue urine, nails, and sclerae	Interacts with heparin; less alopecia, nausea than doxorubicin; radiation recall; less alopecia, nausea than doxorubicin
Topotecan	Marrow, mucositis, nausea, alopecia	Reduce dose with renal failure; rare interstitial pneumonitis
Indirectly DNA-Interacting Agents		
Antimetabolites		
Asparaginase	Decrease protein synthesis; indirect inhibition of DNA synthesis by decreased histone synthesis; clotting factors; glucose; albumin hypersensitivity; CNS; pancreatitis; hepatic	Blocks methotrexate action
Capecitabine	Diarrhea, hand-foot syndrome	Prodrug of 5-fluorouracil due to intratumoral metabolism
2-Chlorodeoxyadenosine	Marrow, renal, fever	Notable use in hairy cell leukemia
Cytosine arabinoside	Marrow, mucositis, neurologic (high dose), conjunctivitis (high dose; use steroid eyedrops until 72 h after last dose), noncardiogenic pulmonary edema	Metabolized in tissues by deamination but renal excretion prominent at doses >500 mg; therefore, dose reduce in “high-dose” regimens in patients with decreased CrCl
Fudarabine phosphate	Marrow, neurologic, lung	Dose reduction with renal failure; metabolized to F-ara, converted to F-ara ATP in cells by deoxycytidine kinase
5-Fluorouracil	Marrow, mucositis, neurologic, skin changes	Toxicity enhanced by leucovorin by increasing “ternary complex” with thymidylate synthase; dihydropyrimidine dehydrogenase deficiency increases toxicity; metabolism in tissue
Gemcitabine	Marrow, nausea, hepatic, fever/“flu syndrome”	Rare pulmonary/capillary leak syndrome; rare hemolytic-uremic syndrome; rare posterior reversible encephalopathy syndrome; radiosensitization

(Continued)

TABLE 73-5 Cytotoxic Chemotherapy Agents^a (Continued)

DRUG	TOXICITY	INTERACTIONS, ISSUES
Hydroxyurea	Marrow, nausea, mucositis, skin, rare renal, liver, lung, CNS	Decrease dose with renal failure; augments antimetabolite effect
6-Mercaptopurine (6-MP)	Marrow, liver, nausea	Variable bioavailability, metabolized by xanthine oxidase, decrease dose with allopurinol; increased toxicity with thiopurine methyltransferase deficiency
Methotrexate	Marrow, liver, lung, renal tubular, mucositis	Toxicity lessened by “rescue” with leucovorin; excreted in urine; decrease dose in renal failure; NSAIDs increase renal toxicity
Pemetrexed	Anemia; neutropenia	Supplement folate/B ₁₂ Caution in renal failure
Pralatrexate	Thrombocytopenia, myelosuppression, mucositis	Active in peripheral T-cell lymphoma
6-Thioguanine	Marrow, liver, nausea	Variable bioavailability; increased toxicity with thiopurine methyltransferase deficiency
Trifluridine/tipiracil	Marrow, mucositis, nausea, vomiting, unusual hand/foot	Trifluridine directly inhibits thymidylate synthase and is incorporated into DNA; tipiracil inhibits thymidine phosphorylase, which degrades trifluridine
Antimitotic Agents		
Docetaxel	Hypersensitivity to vehicle; fluid retention syndrome; marrow; dermatologic; peripheral neuropathy; nausea infrequent; some stomatitis	Premedicate with steroids, H ₁ and H ₂ blockers; may require lengthened infusions to avoid hypersensitivity
Eribulin	Marrow; peripheral neuropathy; QT prolongation	Dose modify in liver and kidney impairment
Ixabepilone	Myelosuppression, neuropathy, hypersensitivity to infusion	Premedicate with steroids, H ₁ and H ₂ blockers; may require lengthened infusions to avoid hypersensitivity; dose modification for liver impairment; CYP3A4
Nab-paclitaxel (protein bound)	Neuropathy, anemia, marrow	Dose adjust with liver dysfunction; caution with inhibitors or inducers of either CYP2C8 or CYP3A4
Paclitaxel	Hypersensitivity to vehicle; marrow; alopecia, mucositis, peripheral neuropathy, CV conduction, infrequent nausea	Premedicate with steroids, H ₁ and H ₂ blockers; hepatic clearance with dose reduction with increased bilirubin; caution with inhibitors or inducers of either CYP2C8 or CYP3A4
Vinblastine	Vesicant; marrow; peripheral neuropathy (less common but similar spectrum to other vincas); hypertension, Raynaud's, ileus/constipation (use prophylactic stool softeners)	Hepatic clearance; dose reduction for bilirubin >1.5 mg/dL
Vincristine	Vesicant, marrow (less than vinblastine), neurologic, GI; ileus/constipation (use prophylactic stool softeners); SIADH; rare CV	Hepatic clearance; dose reduction for bilirubin >1.5 mg/dL
Vinorelbine	Vesicant, marrow, allergic bronchospasm (immediate), dyspnea/cough (subacute), neuropathic (less prominent but similar spectrum to other vincas)	Hepatic clearance; dose reduction for bilirubin >1.5 mg/dL

^aAll agents in this category should be regarded as potentially fetotoxic, and use during pregnancy is either contraindicated or undertaken with clear understanding of risk of fetal harm; likewise not recommended for use during lactation. ^bCommon alkylator: alopecia, pulmonary, infertility, plus teratogenesis.

Abbreviations: ATP, adenosine triphosphate; AUC, area under the curve; CNS, central nervous system; CrCl, creatinine clearance; CV, cardiovascular; GI, gastrointestinal; CYP3A4, avoid concomitant strong CYP3A inhibitors and avoid concomitant strong CYP3A inducers; DLCO, diffusing capacity of carbon monoxide; F-ara, fludarabine; HBP, high blood pressure; MAOI, monoamine oxidase inhibitor; NSAID, nonsteroidal anti-inflammatory drug; SIADH, syndrome of inappropriate antidiuretic hormone secretion; WBC, white blood cells.

Doxorubicin intercalates into DNA, thereby altering DNA structure, replication, and topoisomerase II function. It can also undergo reduction of its quinone ring system, with reoxidation to form reactive oxygen radicals. It causes predictable myelosuppression, alopecia, nausea, and mucositis. In addition, it causes acute cardiotoxicity in the form of atrial and ventricular dysrhythmias, but these are rarely of clinical significance. In contrast, cumulative doses >550 mg/m² are associated with a 10% incidence of chronic cardiomyopathy. The incidence of cardiomyopathy appears to be related to peak serum concentration, with low-dose, frequent treatment or continuous infusions better tolerated than intermittent higher-dose exposures. Cardiotoxicity has been related to iron-catalyzed oxidation and reduction of doxorubicin in the heart. Dexrazoxane is an intracellular chelating agent that can act as a cardio-protectant. Doxorubicin's cardiotoxicity is increased when given together with trastuzumab, the anti-HER2/neu antibody. Radiation recall or interaction with concomitantly administered radiation to cause local site complications is frequent. The drug is a powerful vesicant, with necrosis of tissue apparent 4–7 days after an extravasation; therefore, it should be administered into a rapidly flowing intravenous line. Dexrazoxane also can mitigate doxorubicin extravasation. Doxorubicin is metabolized by the liver, so doses must be reduced by 50–75% in the presence of liver dysfunction. Daunorubicin is closely related to doxorubicin and is preferable to doxorubicin owing to less mucositis and colonic damage with frequent high doses used in the

curative treatment of leukemia. Idarubicin is also used in leukemia treatment and may have somewhat less cardiotoxicity. Encapsulation of daunorubicin into a liposomal formulation has attenuated cardiac toxicity with antitumor activity in Kaposi's sarcoma, other sarcomas, multiple myeloma, and ovarian cancer.

Mitoxantrone is a synthetic topoisomerase II-directed agent with a mechanism similar to the anthracyclines, with less but not absent cardiotoxicity, comparing the ratio of cardiotoxic to effective doses; it is still associated with a 10% incidence of cardiotoxicity at cumulative doses of >150 mg/m². Etoposide binds directly to topoisomerase II and DNA in a reversible ternary complex. It stabilizes the covalent intermediate in the enzyme's action where the enzyme is covalently linked to DNA. Prominent clinical effects include myelosuppression, nausea, and transient hypotension related to the speed of administration of the agent. Etoposide is a mild vesicant but is relatively free from other large-organ toxicities.

Camptothecins target topoisomerase I. Topotecan is a camptothecin derivative approved for use in gynecologic tumors and small-cell lung cancer. Toxicity is limited to myelosuppression and mucositis. Irinotecan is a camptothecin with evidence of activity in colon carcinoma. Irinotecan is a prodrug, metabolized in the liver to SN-38, its active metabolite. Levels of SN-38 are particularly high in the setting of Gilbert's disease, characterized by defective uridine diphosphate glucuronosyl transferase (UGT) 1A1 and indirect hyperbilirubinemia, a

condition that affects about 10% of the white population in the United States. In addition, irinotecan's myelosuppression is clearly influenced by the patient's genotype for UGT1As. Irinotecan causes a delayed (48–72 h) secretory diarrhea related to the toxicity of SN-38. The diarrhea can be treated effectively with loperamide or octreotide; immediate diarrhea when it occurs is responsive to atropine.

Fam-trastuzumab deruxtecan and sacituzumab govitecan are antibody-drug conjugates (Fig. 73-4) that allow specific targeting of camptothecin and SN-38, respectively, to HER2-positive and triple-negative breast cancers, respectively. Adverse events are driven by off-target effects of the chemotherapy agent and include cytopenia, nausea, vomiting, and, in the case of fam-trastuzumab deruxtecan, severe interstitial pneumonitis.

Bleomycin forms complexes with Fe^{2+} while also bound to DNA. It remains an important component of curative regimens for Hodgkin's disease and germ cell neoplasms. Oxidation of Fe^{2+} gives rise to superoxide and hydroxyl radicals, causing DNA damage. The drug causes little, if any, myelosuppression. Bleomycin is cleared rapidly, but augmented skin and pulmonary toxicity in the presence of renal failure necessitates dose reduction in renal failure. Bleomycin is not a vesicant and can be administered intravenously, intramuscularly, or subcutaneously. Common side effects include fever and chills, facial flush, and Raynaud's phenomenon. The most feared complication of bleomycin treatment is pulmonary fibrosis, which increases in incidence at >300 cumulative units administered and is minimally responsive to treatment (e.g., glucocorticoids). The earliest indicator of an adverse effect is usually a decline in the carbon monoxide diffusing capacity (DLCO) or coughing, although cessation of drug immediately upon documentation of a decrease in DLCO may not prevent further decline in pulmonary function. Bleomycin is inactivated by a bleomycin hydrolase, whose concentration is diminished in skin and lung. Because bleomycin-dependent electron transport is dependent on O_2 , bleomycin toxicity may become apparent after exposure to transient very high fraction of inspired oxygen (FIO_2) even late after treatment. Thus, during surgical procedures, patients with prior exposure to bleomycin should be maintained on the lowest FIO_2 consistent with maintaining adequate tissue oxygenation.

Dactinomycin interacts directly with DNA to inhibit RNA transcription. It is important in the curative treatment of pediatric neoplasms, some of which also occur in young adults. Prominent myelosuppression, mucositis, alopecia, radiation recall, and nausea require management.

Calicheamicins are DNA-interacting antitumor antibiotics too toxic for clinical use but, when used as antibody-drug conjugates, can be useful in the treatment of CD33+ acute myeloid leukemia (gemtuzumab ozogamicin) and CD22+ acute lymphocytic leukemia (inotuzumab ozogamicin). Patients must be monitored for hypersensitivity reactions and for hepatotoxicity due to veno-occlusive disease of hepatic veins, resulting from release of the calicheamicin or metabolites in the liver.

Antimetabolites A broad definition of antimetabolites would include compounds that interfere with purine or pyrimidine synthesis. Some antimetabolites also cause DNA damage indirectly, through misincorporation into DNA. They tend to convey greatest toxicity to cells in S-phase, and the degree of toxicity increases with duration of exposure. Common toxic manifestations include stomatitis, diarrhea, and myelosuppression.

Methotrexate inhibits dihydrofolate reductase, which regenerates reduced folates from the oxidized folates produced when thymidine monophosphate is formed from deoxyuridine monophosphate. Without reduced folates, cells die a "thymine-less" death. *N*5-Tetrahydrofolate or *N*5-formyltetrahydrofolate (leucovorin) can bypass this block and rescue cells from methotrexate, which is retained in cells by polyglutamylation. Methotrexate is transported into cells by a membrane carrier, and high concentrations of drug can bypass this carrier and allow diffusion of drug directly into cells. These properties have suggested the design of "high-dose" methotrexate regimens with leucovorin rescue of normal marrow and mucosa as part of curative approaches to osteosarcoma in the adjuvant setting and hematopoietic

neoplasms of children and adults. Methotrexate is cleared by the kidney via both glomerular filtration and tubular secretion, and toxicity is augmented by renal dysfunction and drugs such as salicylates, probenecid, and nonsteroidal anti-inflammatory agents that undergo tubular secretion. With normal renal function, 15 mg/m^2 leucovorin will rescue 10^{-8} – 10^{-6} M methotrexate in 3–4 doses. However, with decreased creatinine clearance, doses of 50 – 100 mg/m^2 are continued until methotrexate levels are $<5 \times 10^{-8} \text{ M}$. In addition to bone marrow suppression and mucosal irritation, methotrexate can cause renal failure itself at high doses owing to crystallization in renal tubules; therefore, high-dose regimens require alkalinization of urine with increased flow by hydration. Methotrexate can be sequestered in third-space collections and diffuse back into the general circulation, causing prolonged myelosuppression. Less frequent adverse effects include reversible increases in transaminases and hypersensitivity-like pulmonary syndrome. Chronic low-dose methotrexate can cause hepatic fibrosis. When administered to the intrathecal space, methotrexate can cause chemical arachnoiditis and CNS dysfunction.

Pemetrexed is a folate-directed antimetabolite that inhibits the activity of several enzymes, including thymidylate synthetase (TS), dihydrofolate reductase, and gycinamide ribonucleotide formyltransferase. To avoid toxicity to normal tissues, pemetrexed is given with low-dose folate and vitamin B₁₂ supplementation. Pemetrexed has notable activity against certain lung cancers and, in combination with cisplatin, also against mesotheliomas.

5-Fluorouracil (5-FU) represents an early example of "rational" drug design in that tumor cells incorporate radiolabeled uracil more efficiently into DNA than normal cells. 5-FU is metabolized in cells to 5'-FdUMP, which inhibits TS. In addition, misincorporation can lead to single-strand breaks, and RNA can aberrantly incorporate FUMP. 5-FU is metabolized by dihydropyrimidine dehydrogenase, and deficiency of this enzyme can lead to excessive toxicity from 5-FU. Oral bioavailability varies unreliable, but prodrugs such as capecitabine have been developed that allow at least equivalent activity to parenteral 5-FU-based approaches. Intravenous administration of 5-FU leads to bone marrow suppression after short infusions but to stomatitis after prolonged infusions. Leucovorin augments the activity of 5-FU by promoting formation of the ternary covalent complex of 5-FU, the reduced folate, and TS. Less frequent toxicities include CNS dysfunction, with prominent cerebellar signs, and endothelial toxicity manifested by thrombosis, including pulmonary embolus and myocardial infarction. Trifluridine is a fluorinated pyrimidine that as the triphosphate is directly incorporated into DNA, evoking DNA damage, and as the monophosphate can inhibit TS. It is administered as a fixed-dose combination with tipiracil, an inhibitor of trifluridine degradation by thymidine phosphorylase.

Cytosine arabinoside (ara-C) is incorporated into DNA after formation of ara-CTP, resulting in S-phase-related toxicity. Continuous infusion schedules allow maximal efficiency, with uptake maximal at 5–7 μM . Ara-C can be administered intrathecally. Adverse effects include nausea, diarrhea, stomatitis, chemical conjunctivitis, and cerebellar ataxia. Gemcitabine is a cytosine derivative that is similar to ara-C in that it is incorporated into DNA after anabolism to the triphosphate, rendering DNA susceptible to breakage and repair synthesis, which differs from that in ara-C in that gemcitabine-induced lesions are very inefficiently removed. In contrast to ara-C, gemcitabine appears to have useful activity in a variety of solid tumors, with limited nonmyelosuppressive toxicities.

6-Thioguanine and 6-mercaptopurine (6MP) are used in the treatment of acute lymphoid leukemia. Although administered orally, they display variable bioavailability. 6MP is metabolized by xanthine oxidase and therefore requires dose reduction when used with allopurinol. 6MP is also metabolized by thiopurine methyltransferase; genetic deficiency of thiopurine methyltransferase results in excessive toxicity.

Fludarabine phosphate is a prodrug of F-adenine arabinoside (F-ara-A), which in turn was designed to diminish the susceptibility of ara-A to adenosine deaminase. F-ara-A is incorporated into DNA and can cause delayed cytotoxicity even in cells with low growth fraction, including chronic lymphocytic leukemia and follicular B-cell

lymphoma. CNS and peripheral nerve dysfunction and T-cell depletion leading to opportunistic infections can occur in addition to myelosuppression. 2-Chlorodeoxyadenosine is a similar compound with activity in hairy cell leukemia. Hydroxyurea inhibits ribonucleotide reductase, resulting in S-phase block. It is orally bioavailable and useful for the acute management of myeloproliferative states.

Asparaginase is a bacterial enzyme that causes breakdown of extracellular asparagine required for protein synthesis in certain leukemic cells. This effectively stops tumor cell DNA synthesis, as DNA synthesis requires concurrent protein synthesis. The outcome of asparaginase action is therefore very similar to the result of the small-molecule antimetabolites. Because asparaginase is a foreign protein, hypersensitivity reactions are common, as are effects on organs such as pancreas and liver that normally require continuing protein synthesis. This may result in decreased insulin secretion with hyperglycemia, with or without hyperamylasemia and clotting function abnormalities. Close monitoring of clotting functions should accompany use of asparaginase. Paradoxically, owing to depletion of rapidly turning over anticoagulant factors, thromboses particularly affecting the CNS may also be seen with asparaginase.

Mitotic Spindle Inhibitors Microtubules form the mitotic spindle, and in interphase cells, they are responsible for the cellular “sculpturing” along which various motile and secretory processes occur. Microtubules are composed of repeating heterodimers of α and β isoforms of the protein tubulin. Vincristine binds to the tubulin heterodimer with the result that microtubules are disaggregated. This results in the block of growing cells in M-phase, where a structurally disordered mitotic spindle apparatus is a powerful proapoptotic signal (Fig. 73-3). Vincristine is metabolized by the liver, and dose adjustment in the presence of hepatic dysfunction is required. It is a powerful vesicant, and infiltration can be treated by local heat and infiltration of hyaluronidase. At clinically used intravenous doses, neurotoxicity in the form of glove-and-stocking neuropathy is frequent. Acute neuropathic effects include jaw pain, paralytic ileus, urinary retention, and the syndrome of inappropriate antidiuretic hormone secretion. Myelosuppression is not seen at conventional doses. Vinblastine is similar to vincristine, except that it tends to be more myelotoxic, with more frequent thrombocytopenia and also mucositis and stomatitis. Vinorelbine is a vinca alkaloid that appears to have differences in resistance patterns in comparison to vincristine and vinblastine; it may be administered orally.

The taxanes include paclitaxel and docetaxel. These agents differ from the vinca alkaloids in that the taxanes stabilize microtubules against depolymerization. The “stabilized” microtubules function abnormally and are not able to undergo the normal dynamic changes of microtubule structure and function necessary for cell cycle completion. Taxanes are among the most broadly active antineoplastic agents for use in solid tumors, with evidence of activity in ovarian cancer, breast cancer, Kaposi’s sarcoma, and lung tumors. They are administered intravenously, and their vehicles cause hypersensitivity reactions. Premedication with dexamethasone (8–16 mg orally or intravenously 12 and 6 h before treatment) and diphenhydramine (50 mg) and cimetidine (300 mg), both 30 min before treatment, decreases but does not eliminate the risk of hypersensitivity reactions to the paclitaxel vehicle. A protein-bound formulation of paclitaxel (called *nab-paclitaxel*) has at least equivalent antineoplastic activity and decreased risk of hypersensitivity reactions. Paclitaxel may also cause myelosuppression, neurotoxicity in the form of glove-and-stocking numbness, and paresthesia. Docetaxel causes comparable degrees of myelosuppression and neuropathy. Docetaxel uses a different vehicle that can cause fluid retention in addition to hypersensitivity reactions; dexamethasone premedication with or without antihistamines is also frequently used. Cabazitaxel is a taxane with somewhat better activity in prostate cancers than earlier generations of taxanes, perhaps due to superior delivery to sites of disease.

Epothilones represent a class of microtubule-stabilizing agents optimized for activity in taxane-resistant tumors. Ixabepilone has clear evidence of activity in breast cancers resistant to taxanes and

anthracyclines such as doxorubicin. Side effects include myelosuppression and peripheral sensory neuropathy. Eribulin is a microtubule-directed agent with activity in patients who have had progression of disease on taxanes. It alters dynamics of microtubule remodeling in cells.

Ado-trastuzumab emtansine is an antibody conjugate of the HER2/neu-directed trastuzumab and a highly toxic microtubule targeted drug (emtansine), which by itself is too toxic for human use; the antibody-drug conjugate shows valuable activity in patients with breast cancer who have developed resistance to the “naked” antibody. Brentuximab vedotin is an anti-CD30 antibody drug conjugate with the distinct microtubule poison dolastatin with activity in neoplasms such as Hodgkin’s lymphoma where the tumor cells frequently express CD30. Polatuzumab vedotin analogously targets CD79a in B-cell lymphomas. Enfortumab vedotin uses an antibody to NECTIN4 to target the vedotin “warhead” to urothelial neoplasms expressing that target. Belantamab mafodotin targets BCMA (B-cell maturation) expressed myeloma but using a distinct microtubule toxin, auristatin. Toxicity from these agents is driven by off-target effects of the microtubule agent and include myelosuppression and neuropathy, but belantamab mafodotin can cause ocular keratopathy, which requires monitoring.

CANCER MOLECULAR TARGETED THERAPY

Agents in this class share the characteristic that they are directed at specific cancer cell molecular targets important in the proliferation of tumors. While these agents can ultimately lead to tumor cell death, this occurs by altered regulation of a specific biochemical pathway affecting tumor cell susceptibility to apoptosis or growth arrest (Fig. 73-3).

Hormone Receptor-Directed Therapy Steroid hormone receptor-related molecules were arguably the first “molecular target” classes of anticancer drugs. When bound to their ligands, these receptors can alter gene transcription in hormone-responsive tissues. While in some cases, such as breast cancer, demonstration of the target hormone receptor is necessary for their use, in other cases such prostate cancer (androgen receptor) and lymphoid neoplasms (glucocorticoid receptor), the relevant receptor is always present in the tumor.

Glucocorticoids are generally given in “pulsed” high doses in leukemias and lymphomas, where they induce cell death in tumor cells. Cushing’s syndrome and inadvertent adrenal suppression on withdrawal from high-dose glucocorticoids can be significant complications, along with infections common in immunosuppressed patients, in particular *Pneumocystis* pneumonia, which classically appears a few days after completing a course of high-dose glucocorticoids.

Tamoxifen is a partial estrogen receptor antagonist; it antagonizes in breast tumors, mirroring its effect on breast tissue, but owing to agonistic activities in vascular and uterine tissue, side effects include increased risk of thromboembolic phenomena and a small increased incidence of endometrial carcinoma, which appears after chronic use (usually >5 years). Progestational agents—including medroxyprogesterone acetate, androgens including fluoxymesterone (Halotestin), and, paradoxically, estrogens—have approximately the same degree of activity in primary hormonal treatment of breast cancers that have elevated expression of estrogen receptor protein. Estrogen itself is not used often due to prominent cardiovascular and uterotrophic activity.

Aromatase refers to a family of enzymes that catalyze the formation of estrogen in various tissues, including ovary, peripheral adipose tissue, and some tumor cells. Aromatase inhibitors are of two types: irreversible steroid analogues such as exemestane and the reversible inhibitors such as anastrozole and letrozole. Anastrozole is superior to tamoxifen in the adjuvant treatment of breast cancer in postmenopausal patients with estrogen receptor-positive tumors. Letrozole treatment affords benefit following tamoxifen treatment. Adverse effects of aromatase inhibitors may include an increased risk of osteoporosis, fatigue, and altered serum lipids.

Metastatic prostate cancer is treated primarily by androgen deprivation. Orchiectomy causes responses in 80% of patients. If not accepted by the patient, testicular androgen suppression can also be induced by luteinizing hormone-releasing hormone (LHRH) agonists such as

leuprolide and goserelin. These agents cause tonic stimulation of the LHRH receptor, with loss of normal pulsatile activation resulting in net decreased output of luteinizing hormone (LH) by the anterior pituitary. Therefore, as primary hormonal manipulation in prostate cancer, one can choose orchectomy or an LHRH agonist, but not both. This pathway can also be blocked by relugolix, an oral gonadotropin-releasing hormone antagonist.

The addition of androgen receptor blockers, including flutamide or bicalutamide, is of uncertain additional benefit in extending overall response duration, although pretreatment with these agents before LHRH agonists is important to avoid a surge in testosterone after initial LH release. Enzalutamide also binds to the androgen receptor and antagonizes androgen action in a mechanistically distinct way. Somewhat analogous to inhibitors of aromatase, agents have been derived that inhibit testosterone and other androgen synthesis in the testis, adrenal gland, and prostate tissue. Abiraterone inhibits 17 α -hydroxylase/C17,20 lyase (CYP17A1) and has been shown to be active in prostate cancer patients experiencing progression despite androgen blockade.

Tumors that respond to a primary hormonal manipulation may frequently respond to second and third hormonal manipulations. Thus, breast tumors that had previously responded to tamoxifen have, on relapse, notable response rates to withdrawal of tamoxifen itself or to subsequent addition of an aromatase inhibitor or progestin. Likewise, initial treatment of prostate cancers with leuprolide plus flutamide may be followed after disease progression by response to withdrawal of flutamide. These responses may result from the removal of antagonists from mutant steroid hormone receptors that have come to depend on the presence of the antagonist as a growth-promoting influence.

Non-Receptor-Linked Tyrosine Kinase Antagonists Table 73-6 lists currently approved non-hormone receptor pathway-directed molecularly targeted chemotherapy agents, with features of their use of import to the generalist, particularly in recognizing potential drug-induced morbidities and interactions with other classes of drugs. The basis for discovery of drugs of this type was the prior knowledge of oncogene-directed pathways driving tumor growth (Fig. 73-3). In most cases, non-receptor tyrosine kinases ultimately activate signaling through the RAF/M κ EK/MAP kinase cascade, in common with the receptor-linked tyrosine kinases. Diagnostic demonstration of an active non-receptor tyrosine kinase may guide selection of an agent. A repeated preclinical and clinical observation in a variety of tumor types is that mutational activation of the tyrosine kinase target induces a state of “oncogene addiction” on the part of the tumor. This then is the basis for a “synthetic lethal” effect of the kinase inhibitor with respect to tumor viability.

In hematologic tumors, the prototypic agent of this type is imatinib, which targets the ATP binding site of the p210^{bcr-abl} protein tyrosine kinase that is formed as the result of the chromosome 9;22 translocation producing the Philadelphia chromosome in chronic myeloid leukemia (CML). It has lesser activity in the blast phase of CML, where the cells may have acquired additional mutations in p210^{bcr-abl} itself or other genetic lesions. Its side effects are relatively tolerable in most patients and include hepatic dysfunction, diarrhea, and fluid retention. Rarely, patients receiving imatinib have decreased cardiac function, which may persist after discontinuation of the drug. The quality of response to imatinib enters into the decision about when to refer patients with CML for consideration of stem cell transplant approaches. Nilotinib is a tyrosine protein kinase inhibitor with activity against p210^{bcr-abl} but with increased potency and perhaps better tolerance by certain patients. Dasatinib, another inhibitor of the p210^{bcr-abl} oncoproteins, also has activity against certain mutant variants of p210^{bcr-abl} that are refractory to imatinib and arise during therapy or are present de novo. Dasatinib also has inhibitory action against kinases belonging to the src tyrosine protein kinase family; this activity may contribute to its effects. The T315I mutant of p210^{bcr-abl} is resistant to imatinib, nilotinib, bosutinib, and dasatinib; ponatinib has activity in patients with this T315I/p210^{bcr-abl}, but ponatinib has noteworthy associated thromboembolic toxicity. Use of this class of targeted agents is thus critically guided

not only by the presence of the p210^{bcr-abl} tyrosine kinase, but also by the presence of specific mutations in the ATP binding site.

Janus kinases (JAK) 1 and 2 are mutated in certain myeloproliferative states; cytopenias and infrequent arrhythmias infrequently complicate the use of ruxolitinib, the prototypic JAK inhibitor. Bruton's tyrosine kinase (BTK) is an intrinsic component of B-cell antigen receptor signaling and therefore is activated in many types of proliferating B cells. Inhibitors of BTK, including ibrutinib, acalabrutinib, and zanubrutinib, have noteworthy activity in certain lymphomas. Cytopenias and cardiac arrhythmias can occur, along with propensity to infection (indeed, the BTK was discovered as deficient in congenital hypogammaglobulinemia, presenting with repeated infections in childhood). Initial use of the BTK inhibitors requires consideration of prophylaxis against tumor lysis syndrome in case of a robust lympholytic effect of the agent.

Receptor-Linked Tyrosine Kinase Antagonists Mutated EGFR drives a significant fraction of non-small-cell lung cancers (NSCLCs). Erlotinib and gefitinib are the prototypic EGFR antagonists that, in early clinical trials, showed evidence of responses in a small fraction of patients with NSCLC. Subsequent studies by clinical oncologists in an effort to understand the basis of these excellent responses found that the probability of response to the agents was markedly increased in patients with an activating EGFR mutation, and current practice now routinely profiles patients with NSCLC for the presence of sensitizing mutations of EGFR. Side effects were generally acceptable, consisting mostly of acneiform rash (treated with glucocorticoid creams and clindamycin gel) and diarrhea. Patients with activating mutations who initially responded to gefitinib or erlotinib but who then had progression of the disease then acquired additional mutations in the enzyme, analogous to the mutational variants responsible for imatinib resistance in CML. Subsequent generations of EGFR antagonists have activity against more uncommon mutants (osimertinib) or a biochemically irreversible mechanism (dacomitinib).

Mutated anaplastic lymphoma kinase (ALK) and activated RET oncogene likewise drive distinct fractions of NSCLCs. Crizotinib, alectinib, and lorlatinib target ALK, but have prominent adverse cardiac, metabolic, and, in the case of lorlatinib, pulmonary events. Selpercatinib targets RET in NSCLCs (and thyroid cancers) but also with the chance of cardiac and liver toxicity.

Steel factor, a blood cell precursor-related bone marrow growth factor, uses the KIT receptor tyrosine kinase. KIT and variants of the platelet-derived growth factor receptor (PDGFR) are expressed in gastrointestinal stromal sarcoma (GIST). In addition to anti-p210^{bcr-abl} kinase activity, imatinib also inhibits mutants of KIT and PDGFR. Imatinib has found clinical utility in GIST, a tumor previously notable for its refractoriness to chemotherapeutic approaches. Imatinib's degree of activity varies with the specific mutational variant of KIT or PDGFR present in a particular patient's tumor.

HER2-driven breast cancers may be usefully treated with lapatinib; diarrhea and cardiac dysfunction can occur. Neratinib or tucatinib may also be useful in HER2-positive breast cancers after trastuzumab has ceased to be of value; diarrhea and liver toxicity also require monitoring and management.

Alteration of fibroblast growth factor (FGF) signaling can contribute to the growth of urothelial carcinomas and cholangiocarcinomas. Erdafitinib and pemigatinib, respectively, may be of utility with careful attention to ocular toxicity and hyperphosphatemia; the latter is an “on-target” toxicity of disrupting FGF receptor signaling in the kidney. Likewise, gilteritinib is active against the FMS-like tyrosine kinase-3 (FLT3) mutated in a fraction of poor-prognosis (treated by conventional chemotherapy) acute myeloid leukemias (AMLS). Cardiac, hepatic, gastrointestinal, and neurologic adverse events can occur, along with “differentiation” of the AML cells with cytokine elaboration and pulmonary side effects, requiring management with steroids and potentially hydroxyurea.

The neurotropic tyrosine kinase receptor (NRTK) undergoes translocation with fusion to a variety of different partners to produce a family of chimeric proteins in a small fraction of a variety of solid

TABLE 73-6 Molecularly Targeted Agents^a

DRUG	TARGET/INDICATION	ADVERSE EVENTS	NOTES
Non-Receptor Tyrosine Kinase Antagonists			
Acalabrutinib	Bruton's tyrosine kinase; mantle cell lymphoma after one prior treatment; CLL/SLL	Cytopenias, opportunistic infections, atrial fibrillation/flutter	CYP3A4, avoid proton pump inhibitors (PPIs); stagger administration with H ₂ blockers
Bosutinib	Bcr-Abl fusion protein (CML); wild-type and imatinib-resistant mutants	Myelosuppression, hepatic, QTc prolongation	CYP3A4; avoid PPIs; stagger administration with H ₂ blockers
Dasatinib	Bcr-Abl fusion protein (CML/ALL); wild-type and imatinib-resistant mutants	Myelosuppression (bleeding, infection); pulmonary hypertension, CHF, fluid retention; QTc prolongation; caution with hepatic impairment	CYP3A4; avoid PPIs; stagger administration with H ₂ blockers
Ibrutinib	Bruton's tyrosine kinase; CLL/SLL; mantle cell lymphoma after CD20-directed therapy; Waldenström's	Nausea, anemia, neutropenia, thrombocytopenia, fatigue, musculoskeletal pain, stomatitis, hypertension, cardiac arrhythmias, tumor lysis syndrome	CYP3A4
Imatinib	Bcr-Abl fusion protein (CML/ALL); c-kit mutants, PDGFR variants (GI stromal tumor [GIST]; eosinophilic syndromes)	Nausea, periorbital edema, rare CHF, QTc prolongation, hypothyroid	Myelosuppression not frequent in solid tumor indications; co-administration with CYP3A4 inducers/inhibitors may require dose adjustment; if need anticoagulation, no warfarin; heparinoids favored
Nilotinib	Bcr-Abl fusion protein (CML) and some imatinib-resistant variants	CHF, hepatic, QTc, electrolyte abnormalities, increased lipase, hypothyroidism	Interaction with CYP3A4-metabolized drugs; also CYP2C8, CYP2C9, CYP2D6, and CYP2B6; avoid food 2 h before and 1 h after a dose
Ponatinib	T315I mutation of Bcr-Abl fusion protein (CML)	Clotting, hepatic, CHF, pancreatitis, neuropathy, rash, arrhythmia, tumor lysis, reversible posterior leukoencephalopathy, wound healing altered	CYP3A4
Ruxolitinib	Janus kinase 1,2; intermediate- or high-risk myelofibrosis, including primary myelofibrosis, post-polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis	Thrombocytopenia, anemia, dizziness, headache	Adjust dose in renal and hepatic impairment, strong CYP3A4 inhibitors, or with fluconazole >200 mg doses except with GVHD
Zanubrutinib	Bruton's tyrosine kinase; mantle cell lymphoma after one prior therapy	Cytopenia, cardiac arrhythmia	Avoid with CYP3A4 interacting agents
Receptor-Linked Tyrosine Kinase Antagonists			
Afatinib	First-line treatment of NSCLC with nonresistant ATP site mutation of EGFR	Diarrhea; rash; ocular keratitis; interstitial lung disease; hepatic failure	Dose adjustment with PgP inhibitors
Alectinib	Anaplastic lymphoma kinase (ALK)-positive metastatic NSCLC	Hepatotoxicity; interstitial lung disease; renal impairment; bradycardia	Myalgia and CPK elevations with muscle pain, tenderness, weakness
Avapritinib	GIST unresectable or metastatic with a PDGFRA exon 18 mutation, including PDGFRA D842V mutations	Edema, nausea, fatigue, CNS effects including altered cognition, sleep and mood disorders, hallucinations	Monitor for intracranial hemorrhage; avoid CYP3A4 inducer/inhibitors
Ceritinib	ALK-positive NSCLC: advanced or metastatic	GI adverse reactions, may require dose adjustment; hepatotoxicity, hyperglycemia, interstitial lung disease (permanently discontinue); QT interval prolongation (monitor with concomitant drugs known to prolong QT)	CYP3A, CYP2C9
Crizotinib	ALK-positive NSCLC: advanced or metastatic	Interstitial pneumonitis; hepatic; QTc prolongation; bradycardia; visual loss	Avoid CYP3A4 inducer/inhibitor
Dacomitinib	Advanced or metastatic NSCLC with epidermal growth factor receptor (EGFR) exon 19 deletion or exon 21 L858R via irreversible mechanism	Diarrhea, cutaneous: hold and/or dose reduce; interstitial lung disease (permanently discontinue)	Avoid with PPIs; use locally acting antacids or H ₂ receptor antagonist and administer at least 6 h before or 10 h after H ₂ receptor antagonist; CYP2D6
Erdafitinib	Target FGFR; advanced or metastatic urothelial cancer with an FGFR3 or FGFR2 alteration that has progressed beyond traditional platinum-based therapies	Stomatitis, fatigue, cutaneous changes, diarrhea; uncommon central serous retinopathy; retinal detachment; therefore, monitor with ophthalmologic exams during treatment; hyperphosphatemia a pharmacodynamic effect due to FGF23/Klotho signaling disruption	CYP2C9, CYP3A4 interactors; OCT2 substrates; separate dosing by at least 6 h before or after administration of PgP substrates
Erlotinib	First-line treatment of NSCLC with ATP site mutation of EGFR; second-line treatment of wild-type EGFR NSCLC; pancreatic cancer with gemcitabine	Rash, diarrhea, renal failure, interstitial pneumonitis, liver	Administer at least 1 h before or 2 h after meals; CYP3A4; avoid with PPIs and space dosing with antacids; can alter warfarin effect; microangiopathic hemolytic anemia especially in pancreatic cancer, rare
Gefitinib	First-line treatment of NSCLC with ATP site mutation of EGFR	Rash, diarrhea, rare interstitial pneumonitis, ocular keratitis, GI perforation	CYP3A4; avoid with PPIs; monitor warfarin effect with gefitinib. In the United States, only with prior documented benefit in second-line treatment of NSCLC if not EGFR mutated

(Continued)

TABLE 73-6 Molecularly Targeted Agents^a (Continued)

DRUG	TARGET/INDICATION	ADVERSE EVENTS	NOTES
Gilteritinib	Relapsed or refractory AML with an <i>FLT3</i> mutation	Hepatotoxicity, myalgia/arthritis, fatigue/malaise, mucositis, edema, rash, noninfectious diarrhea, dyspnea, nausea, cough, constipation, eye disorders, hypotension, vomiting, and renal impairment	Also inhibits AXL; unusual AML differentiation syndrome, requiring corticosteroids and consideration of hydroxyurea; posterior reversible encephalopathy syndrome possible (discontinue); prolonged QT interval: interrupt and/or reduce dose with a QTcF >500 ms (correct hypokalemia or hypomagnesemia prior to and during administration); pancreatitis: interrupt and/or reduce dosage; Pgp substrates; CYP3A
Lapatinib	Breast cancer: with capecitabine in HER2/neu advanced/metastatic after trastuzumab and chemotherapy; with aromatase inhibition if ER positive, HER2/neu positive	↓ LVEF; liver; rash, nausea; diarrhea, palmar-plantar erythrodynesthesia	Interstitial lung disease and pneumonitis (discontinue if severe); QTc: monitor ECG and electrolytes, CYP3A4, CYP2C8, Pgp substrate interactions
Larotrectinib	Targets TRKA, TRKB, and TRKC fusion proteins; indicated in any adult or pediatric solid tumor with a neurotrophic receptor tyrosine kinase (NTRK) gene fusion without a known acquired resistance mutation, with no satisfactory alternative treatments, or that has progressed following treatment	Neurotoxicity with potential cognitive impairment; hepatotoxicity, modify dose or withhold depending on severity	CYP3A4
Lorlatinib	NSCLC: ALK-positive metastatic NSCLC that has progressed on crizotinib and at least one other ALK inhibitor; or with progression on alectinib or ceritinib as the first ALK inhibitor therapy for metastatic disease	Hyperlipidemia: initiate or increase the dose of lipid-lowering agents and withhold and resume or dose reduce based on severity; AV block: withhold and resume or dose modify; CNS effects including seizures, hallucinations, altered cognitive function, altered mood, suicidal ideation, altered speech, mental status, and sleep	Targets ALK and also anti-ROS activity but FDA label limited to ALK indications; CYP3A4 (NB severe hepatotoxicity with strong CYP3A inducers; discontinue strong CYP3A inducers for 3 plasma half-lives prior to use); interstitial lung disease (ILD): immediately withhold and consider discontinuance with suspected ILD/pneumonitis
Neratinib	Breast cancer: with capecitabine in HER2/neu advanced metastatic disease after two prior HER2/neu agents; extended adjuvant treatment after early-stage adjuvant trastuzumab	Diarrhea; nausea; vomiting; abdominal pain; increased ALT/AST	Aggressive diarrhea prophylaxis with loperamide; avoid concomitant PPI antacids; separate from administration of other antacids; avoid CYP3A4 concomitant medications
Osimertinib	First-line treatment of metastatic NSCLC with <i>EGFR</i> exon 19 deletions or exon 21 L858R mutations; <i>EGFR</i> T790M mutation-positive NSCLC, progressed on or after EGFR TKI therapy	Interstitial lung disease, QTc prolongation, cardiomyopathy, ocular keratitis	Avoid or adjust dose with strong CYP3A4 inducers
Pemigatinib	Cholangiocarcinoma: previously treated, unresectable, locally advanced or metastatic cholangiocarcinoma with a fibroblast growth factor receptor 2 (FGFR2) fusion or other rearrangement	Hyperphosphatemia as pharmacodynamic effect: adjust dose if needed; stomatitis, nausea, diarrhea.	Retinal detachment; perform ocular exam with ocular coherence tomography prior to and every 2–3 months during treatment; CYP3A4
Selpercatinib	NSCLC: advanced or metastatic and <i>RET</i> fusion positive; medullary thyroid cancer: advanced or metastatic <i>RET</i> -mutant medullary thyroid cancer; advanced or metastatic <i>RET</i> fusion-positive thyroid cancer that requires systemic therapy and that is radioactive iodine refractory (if appropriate)	Hepatotoxicity: monitor liver functions every 2 weeks during first 3 months, then monthly; hypertension, wound healing effects: withhold 1 week prior to surgery and at least 2 weeks after surgery; hemorrhage	QT interval prolongations: assess QTc at baseline, maintain electrolytes; avoid with QTc-prolonging drugs, avoid with antacids, but if not avoidable, take with food (with PPI) or modify administration time (with H ₂ receptor antagonist or locally acting antacid); CYP3A, CYP2C8 interaction
Tucatinib	Breast cancer: with trastuzumab and capecitabine after one or more HER2/neu regimens in the metastatic setting	Diarrhea, hepatotoxicity	CYP3A4, CYP2C8 interaction
RAF/MEK Inhibitors			
Binimetinib	In combination with encorafenib, for the treatment of patients with unresectable or metastatic melanoma with a <i>BRAF</i> V600E or V600K mutation	Cardiomyopathy, venous thromboembolism; ocular, interstitial lung disease, hepatotoxicity, rhabdomyolysis	Targets MEK; dose modify with liver disease
Cobimetinib	In combination with vemurafenib for unresectable or metastatic melanoma with a <i>BRAF</i> V600E or V600K mutation	New primary malignancies, cutaneous and noncutaneous; hemorrhage, retinal vein occlusion, cardiomyopathy: evaluate LVEF before and during treatment, severe dermatologic reactions, rhabdomyolysis, hepatotoxicity, photosensitivity	CYP3A interaction

(Continued)

TABLE 73-6 Molecularly Targeted Agents^a (Continued)

DRUG	TARGET/INDICATION	ADVERSE EVENTS	NOTES
Dabrafenib	<i>BRAFV600E</i> in melanoma; both alone and in combination with trametinib; may be useful in other tumors with <i>BRAFV600E</i>	As a single agent : hyperkeratosis, headache, pyrexia, arthralgia, papilloma, alopecia, and palmar-plantar erythrodysesthesia syndrome; in combination with trametinib: pyrexia, chills, fatigue, rash, nausea, vomiting, diarrhea, abdominal pain, peripheral edema, cough, headache, arthralgia, night sweats, decreased appetite, constipation, and myalgia	New primary cutaneous malignancies; hemorrhagic events as single agent; CYP3A4, CYP2C8, CYP2C19, and CYP2B6 interactions
Encorafenib	<i>BRAFV600E</i> in melanoma (in combination with binimetinib)	Uveitis, hemorrhage, QTc prolongation, fatigue, nausea, vomiting	CYP3A4 interactions; avoid with hormonal contraceptives
Trametinib	<i>BRAFV600E</i> in melanoma (both as single agent and in combination with dabrafenib)	Rash, diarrhea, lymphedema; cardiomyopathy, ocular toxicity including retinal vein occlusion, interstitial lung disease, fever, hemorrhage, venous thromboembolism, hyperglycemia	In combination with dabrafenib: second neoplasms, hemorrhage, venous thrombosis, CHF, ocular, hyperglycemia; avoid CYP3A4, CYP2C8, CYP2C9, CYP2C19, or CYP2B6 interacting drugs
Vemurafenib	<i>BRAFV600E</i> in melanoma; alone and in combination with cobimetinib; may be useful in other tumors with <i>BRAFV600E</i>	Cutaneous squamous cell carcinoma, severe rash including Stevens-Johnson, allergic hypersensitivity, QTc prolongation, hepatic, ocular, photosensitivity	Usually combined with cobimetinib in melanoma; CYP3A4, CYP1A2, and CYP2D6 interactions
Apoptosis Modulation			
Venetoclax	Targets BCL2; indicated in CLL/SLL; AML: in combination with azacitidine or decitabine or low-dose cytarabine in treatment of newly diagnosed AML in adults who are age 75 years or older or who have comorbidities that preclude use of intensive induction chemotherapy	Neutropenia; infection: withhold for grade 3 and higher	Tumor lysis syndrome (TLS): anticipate TLS, assess risk in all patients. Premedicate with anti-hyperuricemics and ensure adequate hydration, with more intensive measures (intravenous hydration, frequent monitoring, hospitalization) as overall risk increases. Immunization: No live attenuated vaccines prior to, during, or after venetoclax treatment; CYP3A, Pgp interaction; take Pgp substrates at least 6 h before venetoclax
Multikinase Inhibitors			
Axitinib	Renal cell carcinoma, second line	HBP, hemorrhage/clotting; diarrhea, other GI including GI perforation, fatigue, hand-foot syndrome, hypothyroidism, reversible posterior leukoencephalopathy, proteinuria	Targets VEGFR, PDGFR, KIT; CYP3A4/5 interaction
Brigatinib	Advanced or metastatic ALK-positive NSCLC progressed on or intolerant to crizotinib	Interstitial lung disease, bradycardia, hypertension, visual disturbances, hyperglycemia, creatine phosphokinase elevations	Targets ALK and EGFR; CYP3A interaction; hormonal contraceptives may be ineffective due to decreased exposure as CYP3A4 substrates
Cabozantinib	Medullary thyroid cancer; renal cell cancer; hepatocellular carcinoma after sorafenib	Hypertension, thrombotic events, diarrhea, fistula/GI perforation/wound healing, reversible posterior leukoencephalopathy, hemorrhage, palmar-plantar erythrodysesthesia	Targets VEGFR2, MET, AXL, RET; modify dose with CYP3A4 interactors
Capmatinib	NSCLC with MET exon 14 skipping	Interstitial lung disease, hepatic, photosensitivity	Targets MET; avoid with CYP3A4 interactors
Entrectinib	NSCLC: advanced and/or ROS1 positive; any solid tumors with an <i>NTRK</i> gene fusion without a known acquired resistance mutation, with metastasis, or in which surgical resection is likely to result in severe morbidity, or in tumors with progression following treatment or no satisfactory alternative therapy	CHF, CNS effect, skeletal fractures; hepatotoxicity: monitor liver tests, including ALT and AST, every 2 weeks during the first month of treatment, then monthly thereafter; withhold or permanently discontinue based on severity; hyperuricemia: assess serum uric acid levels prior to initiation and periodically during treatment	Targets <i>NTRK</i> gene fusion proteins; QT prolongation: assess with electrolytes at baseline and during treatment; vision disorders: withhold for new visual changes and consider ophthalmologic evaluation; CYP3A4 interaction: patients with BSA >1.50 m ² , reduce the dose of entrectinib if co-administration of moderate or strong CYP3A inhibitors and if BSA ≤1.50 m ² ; avoid entrectinib; avoid with moderate and strong CYP3A inducers
Fedratinib	Intermediate-2 or high-risk primary or secondary (post-polycythemia vera or post-essential thrombocythemia) myelofibrosis	Anemia, thrombocytopenia, nausea, vomiting, diarrhea, hepatic, amylase/lipase, encephalopathy: check thiamine levels prior, replete if deficient	Targets Janus kinase 2, and FLT3, RET; CYP3A4, CYP2C19 interaction
Lenvatinib	Iodine-refractory differentiated thyroid cancer; with everolimus for renal cell carcinoma after one prior antiangiogenic; hepatocellular carcinoma; with pembrolizumab, for the treatment of advanced endometrial carcinoma that is not MSI-H or dMMR and disease progression following prior systemic therapy; candidates for curative surgery	Hypertension, cardiac dysfunction, arterial thromboembolism, hepatic, renal, proteinuria, diarrhea, fistula/GI perforation/wound healing, QTc prolongation, hypocalcemia, reversible posterior leukoencephalopathy, hemorrhage, altered thyroid	Targets VEGFR1/2/3, FGFR1/2/3/4, PDGF α , KIT, and RET

(Continued)

TABLE 73-6 Molecularly Targeted Agents^a (Continued)

DRUG	TARGET/INDICATION	ADVERSE EVENTS	NOTES
Midostaurin	Newly diagnosed <i>FLT3</i> -mutated AML during induction, consolidation, with daunorubicin/cytarabine-based chemotherapy; aggressive systemic mastocytosis, mast cell leukemia; systemic mastocytosis with associated hematologic malignancy	Interstitial lung disease; nausea; diarrhea	Targets mutant <i>FLT3</i> , protein kinase C, and many other protein kinases
Pazopanib	Renal cell carcinoma, soft tissue sarcoma (not GIST or adipocytic)	Fatigue, diarrhea/GI, hypertension; arterial and venous thrombosis with embolism, hemorrhage; hepatotoxicity: potentially severe/fatal; measure liver chemistries before and during treatment; GI perforation or fistula; proteinuria: monitor urine protein and interrupt treatment for 24-h urine protein ≥ 3 g and discontinue for repeat episodes despite dose reductions; infection: serious infections (with or without neutropenia); hypothyroidism	Targets VEGFRs, KIT, PDGFR, and FGFR; CHF ± prolonged QT intervals and torsades des pointes: monitor LVEF, ECG, and electrolytes at baseline and during treatment; reversible posterior leukoencephalopathy syndrome, interstitial lung disease/pneumonitis, thrombotic microangiopathy, including thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome (permanently discontinue); CYP3A4, CYP2D6, CYP2C8 interaction; use with simvastatin increases the risk of ALT elevations and should be undertaken with caution; avoid with drugs that raise gastric pH; consider short-acting antacids in place of PPIs and H ₂ receptor antagonists; separate antacid and pazopanib dosing by several hours
Pexidartinib	Indicated for tenosynovial giant cell tumor (TGCT) associated with severe morbidity or functional limitations and not amenable to improvement with surgery	Administer 1 h before or 2 h after food; can cause serious and potentially fatal liver injury; monitor liver tests prior to and during treatment and withhold, dose reduce, or permanently discontinue	Targets colony-stimulating factor-1 receptor, KIT, <i>FLT3</i> ; avoid with agents known to cause hepatotoxicity; CYP3A, UGT interaction; avoid with PPIs; use H ₂ receptor antagonists or antacids if needed
Regorafenib	Second-line colorectal cancer; GI stromal tumor	Hypertension, hemorrhage, hand-foot syndrome and other dermatologic toxicity, thromboses, GI perforations with fistula, wound healing delays	Targets VEGFR, cardiac ischemia with infarction, reversible posterior leukoencephalopathy syndrome; CYP3A4 interaction
Sorafenib	Renal cell, hepatocellular, differentiated thyroid carcinoma	Diarrhea, hemorrhage, hand-foot syndrome, other rash, hypertension, CHF, QTc prolongation, hepatic toxicity, GI perforation	Targets c-RAF more selectively than B-RAF; VEGFR; many other kinases; impaired TSH suppression in thyroid cancer; CYP3A4 interaction
Sunitinib	Renal cell carcinoma, advanced or adjuvant; pancreatic neuroendocrine tumor, GIST after imatinib	Hypertension, hemorrhagic events, GI perforation, proteinuria, leading to renal failure; interrupt treatment for 24-h urine protein ≥ 3 g; discontinue for repeat episodes despite dose reductions; thyroid dysfunction, hypoglycemia: check blood glucose levels and consider antidiabetic drug dose modifications; osteonecrosis of the jaw: consider preventive dentistry prior to treatment and avoid invasive dental procedures, particularly in patients receiving intravenous bisphosphonate therapy; impaired wound healing: temporary interruption prior to major surgical procedures; palmar-plantar erythrodysesthesia	Targets VEGFRs; PDGFR, RET, KIT; other protein kinases. Rare prolonged QT intervals and torsades des pointes: monitor at baseline and during treatment; maintain K, Mg levels; rare tumor lysis syndrome reported primarily in patients with RCC and GIST with high tumor burden; rare thrombotic microangiopathy, including thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome (discontinue); rare necrotizing fasciitis; severe cutaneous adverse events including erythema multiforme, Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN); discontinue if these events; CYP3A4 interaction
Vandetanib	Medullary thyroid cancer	Diarrhea, rash, hypertension, prolonged QTc, thromboses, fistulas, osteonecrosis, proteinuria	Targets VEGFR, RET, EGFR; CYP3A4 interaction
Cyclin-Dependent Kinase (CDK) Inhibitors			
Abemaciclib	Breast cancer: with an aromatase inhibitor as initial endocrine-based therapy for the treatment of postmenopausal HR+, HER2- advanced or metastatic breast cancer; or with fulvestrant for the treatment of women with HR+, HER2- advanced or metastatic breast cancer with disease progression following endocrine therapy; as monotherapy for the treatment of adult patients with HR+, HER2- advanced or metastatic breast cancer with disease progression following endocrine therapy and prior chemotherapy	Diarrhea, neutropenia, thrombocytopenia, hepatotoxicity, venous thromboembolism	Targets CDK4/6; avoid concomitant use of ketoconazole; CYP3A4 interaction

(Continued)

TABLE 73-6 Molecularly Targeted Agents^a (Continued)

DRUG	TARGET/INDICATION	ADVERSE EVENTS	NOTES
Palbociclib	Breast cancer: HR+, HER2– advanced or metastatic breast cancer in combination with an aromatase inhibitor as initial endocrine-based therapy in postmenopausal women; or fulvestrant in women with disease progression following endocrine therapy	Neutropenia, anemia, thrombocytopenia, stomatitis, diarrhea, fatigue	Targets CDK4/6; CYP3A interaction
Ribociclib	Breast cancer: with letrozole as initial endocrine-based therapy for the treatment of postmenopausal women with HR+, HER2– advanced or metastatic breast cancer	Hepatotoxicity; neutropenia	Targets CDK4/6; unusual QT interval prolongation; drugs known to prolong QT interval should be avoided such as antiarrhythmics; CYP3A interaction
Protein Homeostasis Modulators			
Bortezomib	Multiple myeloma, mantle cell lymphoma, second line	Neuropathy, thrombocytopenia, neutropenia, nausea, diarrhea, hypotension, tumor lysis syndrome with high tumor burden; hepatic: monitor hepatic enzymes during treatment, consider interruption	Proteasome inhibitor; infiltrative pulmonary disease, reversible posterior leukoencephalopathy syndrome: consider MRI for onset of visual or neurologic symptoms and discontinue if suspected; thrombotic microangiopathy; CYP3A4 interaction
Carfilzomib	Multiple myeloma: with dexamethasone or with lenalidomide plus dexamethasone in patients with relapsed or refractory multiple myeloma who have received one to three lines of therapy, or as a single agent for the treatment of patients with relapsed or refractory multiple myeloma who have received one or more lines of therapy	Infusion reaction: premedicate with dexamethasone; thrombocytopenia; tumor lysis syndrome, with need for hydration, monitoring of metabolic parameters	Proteasome inhibitor; cardiac toxicities: including failure or ischemia, withhold and evaluate; acute renal failure: monitor serum creatinine regularly; pulmonary toxicity, including pulmonary hypertension, acute respiratory distress/failure and diffuse infiltrative pulmonary disease: withhold and evaluate promptly; dose adjust with hepatic impairment; administer after a hemodialysis procedure
Ixazomib	Multiple myeloma: with lenalidomide and dexamethasone after at least one prior therapy	Thrombocytopenia, nausea, diarrhea, peripheral neuropathy, edema, hepatotoxicity	Proteasome inhibitor; avoid with strong CYP3A4 inducers; dose adjust with hepatic or renal impairment
Selinexor	Multiple myeloma (refractory): with dexamethasone after at least four prior therapies and refractory to at least two proteasome inhibitors, at least two immunomodulatory agents, and an anti-CD38 monoclonal antibody; DLBCL (relapsed or refractory) or arising from FL after at least two lines of systemic therapy	Thrombocytopenia, neutropenia, nausea, diarrhea, hyponatremia, neurotoxicity	Targets exportin 1 and therefore decreases efficient transport of proteins from nucleus to cytoplasm, leading to cell cycle arrest
Chromatin-Modifying Epigenetic Modulators			
DNA hypomethylating agents			
Azacitidine and decitabine	AML/myelodysplastic syndrome	Marrow, nausea, liver, neurologic, myalgia	“Suicide” inhibition of DNA; methyl transferase after incorporation into DNA
Histone deacetylase inhibitors			
Belinostat	Peripheral T-cell lymphoma, relapsed or refractory	Thrombocytopenia, neutropenia, lymphopenia, anemia, infection, hepatotoxicity	Tumor lysis syndrome monitoring
Panobinostat	Multiple myeloma in combination with bortezomib and dexamethasone, in patients with multiple myeloma who have received at least two prior regimens, including bortezomib and an immunomodulatory agent.	Diarrhea, potentially severe, requiring prophylaxis; cardiac ischemic events, arrhythmias, hemorrhage, hepatotoxicity, cytopenias	CYP3A4, CYP2D6 interactions; avoid concomitant antiarrhythmic drugs/ QT-prolonging drugs
Romidepsin	Cutaneous T-cell lymphoma, second line	QT prolongation, nausea, vomiting, cytopenias	Monitor QT at baseline and during treatment; monitor PT, INR with warfarin derivatives; CYP3A4 interaction
Vorinostat	Cutaneous T-cell lymphoma, second line	Fatigue, diarrhea, hyperglycemia, thrombocytopenia, embolism, GI bleeding, QT prolongation.	Monitor QT at baseline and during treatment; monitor PT, INR with warfarin derivatives

(Continued)

TABLE 73-6 Molecularly Targeted Agents^a (Continued)

DRUG	TARGET/INDICATION	ADVERSE EVENTS	NOTES
Histone methyltransferase inhibitors			
Tazemetostat	Epithelioid sarcoma: advanced or metastatic not eligible for surgical resection; FL with <i>EZH2</i> mutation after two prior therapies or any FL that has relapsed or is refractory to alternative therapies	Fatigue, nausea, constipation	Avoid CYP3A4 inducers/inhibitors; monitor for emergence of myelodysplastic syndrome, leukemia
Metabolism Modulators: mTOR Inhibitors/PI3 Kinase Inhibitors/IDH Inhibitors			
Alpelisib	Breast cancer: with fulvestrant for postmenopausal women, and men, with HR+, HER2-, <i>PIK3CA</i> -mutated, advanced or metastatic breast cancer following progression on or after an endocrine-based treatment	Hyperglycemia: safety not established in type 1 or uncontrolled type 2 diabetes; monitor glucose levels and hemoglobin A _{1c} ; optimize oral antihyperglycemics if warranted; interstitial pneumonitis: discontinue; diarrhea ≤ grade 2 frequent	Targets PI3K α isoform; severe hypersensitivity: permanently discontinue and initiate appropriate treatment; severe cutaneous reactions including SJS, erythema multiforme (EM), and TEN: consider consultation with a dermatologist; permanently discontinue if SJS, EM, or TEN confirmed; CYP3A4, CYP2C9; avoid with BCRP inhibitors
Copanlisib	Relapsed FL patients who have received at least two prior systemic therapies; pending confirmatory trial	Infection, hyperglycemia, HBP, noninfectious pneumonitis, neutropenia, cutaneous reactions	Targets PI3K α/δ isoforms; CYP3A4
Duvelisib	For relapsed or refractory CLL/SLL or FL; orphan drug designation for peripheral T-cell lymphoma	Neutropenia, hepatic toxicity, severe infections, diarrhea/colitis may require withholding; severe cutaneous reactions or pneumonitis in 5%	Targets PI3K γ/δ isoforms; CYP3A
Enasidenib	Relapsed or refractory AML with an <i>IDH2</i> mutation	Nausea, vomiting, diarrhea, elevated bilirubin, and anorexia	Targets <i>IDH2</i> mutant enzyme; unusual "differentiation syndrome" reflecting leukemia response to drug, but potentially fatal if not treated; use corticosteroid therapy, hemodynamic monitoring, consider hydroxyurea until symptom resolution
Everolimus	RCC, advanced; tuberous sclerosis-associated renal angiomyolipoma and/or subependymal giant cell astrocytoma; breast cancer, HR+, resistant to anastrozole or letrozole, in combination with exemestane; pancreatic, lung, or GI neuroendocrine, NOT functional carcinoid	Fatigue, noninfectious pneumonitis, infections, severe hypersensitivity reactions, renal impairment, impaired wound healing, hyperglycemia and hyperlipidemia, myelosuppression	Targets mTOR; angioedema with patients taking concomitant ACE inhibitors may be at increased risk; stomatitis: consider dexamethasone alcohol-free mouthwash when starting treatment; risk of reduced efficacy of vaccination: Pgp and strong CYP3A4 inhibitors: avoid concomitant use; Pgp and moderate CYP3A4 inhibitors: reduce dose; Pgp and strong CYP3A4 inducers: increase dose; geriatric patients: monitor and adjust dose for adverse reactions
Idelalisib	Relapsed CLL with rituximab; SLL, relapsed FL after two prior therapies	Hepatotoxicity, diarrhea or colitis, pneumonitis: monitor for pulmonary symptoms and bilateral interstitial infiltrates, then interrupt or discontinue; intestinal perforation: discontinue if suspected	Targets PI3K δ isoform; CYP3A4
Ivosidenib	AML: relapsed or refractory with an <i>IDH1</i> mutation; in the United States, newly diagnosed AML with a susceptible <i>IDH1</i> mutation, in patients who are at least 75 years old or who have comorbidities that preclude the use of intensive induction chemotherapy	Fatigue, leukocytosis, arthralgia, diarrhea, dyspnea, edema, nausea, mucositis, ECG QT prolonged, rash, pyrexia, cough, and constipation	Targets <i>IDH1</i> mutant; unusual QT prolongation (check electrolytes and hold or reduce dose); Guillain-Barré syndrome (permanently discontinue); CYP3A4; monitor/avoid with increased QTc-causing drugs
Tensirolimus	RCC, second line or poor prognosis	Hypersensitivity, hepatic (adjust dose in liver dysfunction), infection, interstitial lung disease, stomatitis, thrombocytopenia, nausea, anorexia, fatigue, hyperglycemia, hyperlipidemia, poor wound healing, GI perforation, renal impairment: check before treatment and periodically	Targets mTOR; CYP3A4/5 interactions; avoid live vaccines or exposure to subjects recently vaccinated with live vaccines
Poly-ADP Ribose Polymerase (PARP) Inhibitors			
Niraparib	Maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy	Cytopenias, nausea, diarrhea, fatigue	Myelodysplastic syndrome

(Continued)

TABLE 73-6 Molecularly Targeted Agents^a (Continued)

DRUG	TARGET/INDICATION	ADVERSE EVENTS	NOTES
Olaparib	Ovarian cancer: after two or more chemotherapies with deleterious <i>BRCA</i> mutation (germline and/or somatic); maintenance therapy when in complete or partial response to platinum-based chemotherapy Breast cancer: for the treatment of adult patients with deleterious or suspected deleterious gBRCAm, HER2– metastatic breast cancer who have been treated with chemotherapy in the neoadjuvant, adjuvant, or metastatic setting; if HR+, after prior endocrine therapy or inappropriate for endocrine therapy Pancreatic cancer: maintenance treatment of adult patients with deleterious or suspected deleterious gBRCAm metastatic pancreatic adenocarcinoma whose disease has not progressed on at least 16 weeks of a first-line platinum-based chemotherapy regimen	Nausea, fatigue, anemia, thrombocytopenia, neutropenia, stomatitis, liver function abnormalities	Myelodysplastic syndrome; rare interstitial pneumonitis
Rucaparib	Ovarian/fallopian tube/primary peritoneal cancer: as with olaparib	Nausea, fatigue, anemia, thrombocytopenia, neutropenia, stomatitis, liver function abnormalities	Severe heme toxicity with emergence of myelodysplastic syndrome
Talazoparib	Ovarian/fallopian tube/primary peritoneal cancer: as with olaparib	Nausea, fatigue (including asthenia), vomiting, abdominal pain, anemia, diarrhea, neutropenia, leukopenia, decreased appetite, constipation, stomatitis, dyspnea, and thrombocytopenia	Monitor for emergence of myelodysplasia; rare interstitial pneumonitis should lead to discontinuation; avoid with strong or moderate CYP3A inhibitors, but if concomitant use cannot be avoided, reduce dose; avoid with strong or moderate CYP3A inducers
Miscellaneous			
Arsenic trioxide	APL (target PML-RAR α and redox homeostasis)	↑ QT _c ; hypersensitivity with vasomotor symptoms	APL differentiation syndrome (see under tretinoin)
Glasdegib	AML: in combination with low-dose cytarabine, for the treatment of newly diagnosed AML in adult patients who are ≥ 75 years old or who have comorbidities that preclude use of intensive induction chemotherapy	Monitor ECG and electrolytes for QTc prolongation and interrupt treatment if it occurs	Targets smoothed receptor in hedgehog pathway; CYP3A4; avoid with QTc-prolonging drugs, but if co-administration is unavoidable monitor for increased QTc
Sonidegib	Metastatic basal cell carcinoma	Muscle spasm, fatigue, transmission through semen	Targets smoothed receptor in hedgehog pathway; CYP3A4
Tagraxofusp-erzs	Blastic plasmacytoid dendritic cell neoplasm	Hypersensitivity reactions (require premedication with steroids, antihistamines); hepatotoxicity, capillary leak syndrome	Targets CD123 (IL-3 receptor) to deliver a fragment of the diphtheria toxin
Tretinoin	APL, t(15;17) positive	Cutaneous including cheilitis, skin dryness; increased intracranial pressure; hyperlipidemia, abnormal liver function tests, usually resolve	Targets PML-RAR α ; APL differentiation syndrome: pulmonary dysfunction/infiltrate, pleural/pericardial effusion, fever
Vismodegib	Metastatic basal cell carcinoma	GI, hair loss, fatigue, muscle spasm, dysgeusia; no blood donation for 7 months after last dose	Targets smoothed receptor in hedgehog pathway
Ziv-aflibercept	Metastatic colorectal cancer in combination with 5-fluorouracil, leucovorin, irinotecan; resistant to or has progressed following an oxaliplatin-containing regimen	Fistula formation, GI perforation, hemorrhage, thrombosis, arterial thromboembolism, proteinuria, reversible posterior leukoencephalopathy	Targets VEGF by a solubilized receptor-trapping mechanism

^aAll agents in this category should be regarded as potentially fetotoxic, and use during pregnancy is either contraindicated or undertaken with clear understanding of risk of fetal harm; likewise not recommended for use during lactation.

Abbreviations: ACE, angiotensin-converting enzyme; ALL, acute lymphocytic leukemia; ALT, alanine aminotransferase; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; AST, aspartate aminotransferase; AV, atrioventricular; BCRP, breast cancer resistance protein drug transporter; BSA, body surface area; CHF, congestive heart failure; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CNS, central nervous system; CPK, creatine phosphokinase; CYP, cytochrome p450 interactions with drugs metabolized by the indicated isoform; DLBCL, diffuse large B-cell lymphoma; dMMR, deficient mismatch repair; EOG, electrocardiogram; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FDA, U.S. Food and Drug Administration; FL, follicular lymphoma; gBRCAm, germline mutated breast cancer associated protein; GI, gastrointestinal; GVHD, graft-versus-host disease; HBP, high blood pressure; MEK, mitogen activated protein kinase; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; IDH, isocitrate dehydrogenase; INR, international normalized ratio; LVEF, left ventricular ejection fraction; MSI-H, microsatellite instability-high; mTOR, mammalian target of rapamycin kinase; NSCLC, non-small-cell lung cancer; PDGFR, platelet-derived growth factor receptor; Pgp, P-glycoprotein; PT, prothrombin time; QTcF, QT corrected by Frederik formula; RCC, renal cell carcinoma; SLL, small lymphocytic lymphoma; TKI, tyrosine kinase inhibitor; TSH, thyroid-stimulating hormone; UGT, uridine diphosphate glucuronosyltransferase; VEGFR, vascular endothelial growth factor receptor.

tumors. Larotrectinib and entrectinib may be quite useful in managing these tumors; indeed, these agents are exemplary of “histology agnostic” agents, where the utility of the drug is not tied to a particular histologic diagnosis, but to the possession of a specific *NTRK* gene alteration. Neurotoxicity, a long half-life of the agents, and hepatotoxic adverse events are of concern. Also, assuring that solid tumors have been appropriately screened for the existence of such sensitizing mutations can be logistically and economically challenging.

RAS/ RAF/ MEK Antagonists The *BRAF* V600E mutation drives a substantial fraction of melanomas and certain NSCLCs and has been detected in certain thyroid tumors, colorectal tumors, hairy cell leukemias, and unusual gliomas. *BRAF* inhibitors such as dabrafenib, vemurafenib, and encorafenib have activity as single agents in many such tumors but are usually most active when co-administered as “doublets” with the MEK inhibitors trametinib, cobimetinib, and binimetinib, respectively, to promote “shut down” of RAF/MEK signaling at more than pathway member. Cutaneous adverse events including generally indolent cutaneous second neoplasms and thromboembolic, cardiac, and ocular toxicity can occur.

Sotorasib is a first-in-class inhibitor of *KRAS* G12C signaling that in early clinical reports has evidence of effecting stable disease in patients with a variety of neoplasm histologies bearing that mutation, with fewer actual responses. Its initial very favorable safety profile encourages further clinical investigations alone and in combination with other agents.

Multikinase Inhibitors Agents in this class also target specific macromolecules promoting the viability of tumor cells. They are “small-molecule” ATP site-directed antagonists that inhibit more than one protein kinase and may have value in the treatment of several solid tumors. Drugs of this type with prominent activity against the VEGFR tyrosine kinase have activity in renal cell carcinoma. Sorafenib is a VEGFR antagonist also with activity against the RAF serine-threonine protein kinase, and regorafenib is a closely related drug with value in relapsed advanced colon cancer. Pazopanib also prominently targets VEGFR and has activity in renal carcinoma and soft tissue sarcomas. Sunitinib has anti-VEGFR, anti-PDGFR, and anti-KIT activity. It causes prominent responses and stabilization of disease in renal cell cancers and GISTs. Side effects for agents with anti-VEGFR activity, similar to those of the anti-VEGF antibody bevacizumab, prominently include hypertension, proteinuria, and, more rarely, bleeding and clotting disorders, perforation of scarred gastrointestinal lesions, and posterior leukoencephalopathy, probably reflecting CNS vascular damage. Also encountered are fatigue, diarrhea, and hand-foot syndrome, with erythema and desquamation of the distal extremities, in some cases requiring dose modification, particularly with sorafenib.

Other agents in this class include agents such as brigatinib (clinical activity in ALK-dependent NSCLC, but also with anti-EGFR action), entrectinib (clinical activity in NTRK fusion protein diseases, but also in *ROS*-mutated NSCLC), and fedratinib (clinical activity in myeloproliferative neoplasms, but with RET activity in addition to JAK2 and *FLT3* antagonism).

Cyclin-Dependent Kinase Inhibitors Cyclin-dependent kinases (CDKs) are activated as the result of oncogene pathway activity, and CDK4 and CDK6 phosphorylate the retinoblastoma (RB) tumor-suppressor gene to allow entry into S-phase. Palbociclib, abemaciclib, and ribociclib, selective inhibitors of CDK4 and CDK6, have noteworthy activity in advanced breast cancers also expressing the estrogen receptor, usually in conjunction with continued efforts to suppress estrogen receptor signaling, and frequently in conjunction with mTOR inhibitors. Further clinical investigations in other RB intact tumors may broaden their role.

Protein Homeostasis Modulators The proteasome is a macromolecular complex that degrades misfolded proteins tagged for removal by ubiquitin ligases. Proteasome inhibitors were originally designed as potential anti-inflammatory agents owing to proteasome activity to produce inflammatory cytokines but had unexpected

antiproliferative activity in a variety of cell types. Proteasome inhibitors have clinical utility in myeloma and lymphoma, where unbalanced synthesis of immunoglobulin components can accumulate after proteasome inhibitor treatment and induce apoptosis or starve cells for amino acids, inducing autophagy. Boronic acid proteasome inhibitors, including bortezomib and ixazomib, cause thrombocytopenia, gastrointestinal dysfunction, and neuropathy. Carfilzomib is a distinct chemotype with attenuated neuropathy but increased incidence of infusion reactions and cytokine release, with attendant risk of cardiopulmonary adverse events.

Exportin 1 is a nuclear membrane transport protein that is responsible for normal exit and entry of a variety of nuclear proteins. Selinexor is an inhibitor of exportin action, resulting in abnormal nuclear accumulation of, e.g., tumor-suppressor gene products or needed export of other products, e.g., oncogene products. Useful clinical activity has been seen in myeloma and diffuse large B-cell lymphomas including those arising from previously treated indolent lymphomas. Cytopenias, gastrointestinal distress, and hyponatremia are features of its clinical use.

Chromatin-Modifying Agents Gene function is altered not only by mutation of DNA structure, but also by “epigenetic” mechanisms that alter the capacity of DNA to be transcribed or interact with regulatory proteins in the nucleus including transcription factors. Initial epigenetic approaches to modulate gene expression extended from the observation that low concentrations of certain nucleosides (5’azacytidine and decitabine) caused loss of methylated cytosine in DNA, associated with gene silencing, and had clinical activity in causing differentiation of AML cells with notably less toxicity than higher concentrations. 5’Azacytidine and decitabine are misincorporated into DNA and then scavenge DNA methyl transferase to disable DNA methylation of tumor-promoting genes and thus alter their transcription.

Histone deacetylase inhibitors alter the histone protein “packing” density of chromatin and induce global changes in expression of cell cycle regulatory proteins. Vorinostat, belinostat, and romidepsin are useful in cutaneous and peripheral T-cell lymphomas; panobinostat has activity in multiple myeloma. The agents are generally well tolerated but with the potential for cytopenias. The histone methyltransferase inhibitor tazemetostat is a first-in-class inhibitor of histone methyltransferase with unique activity in epithelioid sarcoma owing to its modulation of transcriptional mechanisms unique to that tumor and, recently, in certain follicular lymphomas.

Cancer Cell Metabolism Modulators Oncogenic transformation causes a “rewiring” of cellular metabolism away from oxidative phosphorylation to glycolysis (historically defined as the “Warburg effect” of aerobic glycolysis in animal and human tumors) with attendant tolerance of hypoxia and production of metabolites important for sustaining cell proliferation. Recent clinical studies have defined clinical value from inhibitors of the cell lipid membrane localized phosphoinositide-3 (PI3) kinase and mammalian target of rapamycin (mTOR) (the latter is a kinase whose inhibition was originally discovered as the mechanism by which the immunosuppressant rapamycin, isolated from a soil bacterium, decreased T-cell proliferation). PI3 kinase is activated by numerous oncogenic tyrosine kinases to ultimately cause a cascade of metabolic alterations including increased glucose uptake and activation of mTOR isoforms, which selectively increase translation efficiency of key regulators of cell cycle progression and protein synthetic capacity.

Temsirolimus and everolimus are mTOR inhibitors with activity in renal cancers. They produce stomatitis and fatigue; some hyperlipidemia (10%) and myelosuppression (10%); and rare lung toxicity and immunosuppression in regimens used clinically. Everolimus is also useful in patients with hormone receptor-positive breast cancers displaying resistance to hormonal inhibition and in certain neuroendocrine and brain tumors, the latter arising in patients with sporadic or inherited mutations in the pathway activating mTOR. Isoform-specific PI3 kinase inhibitors are of increasing importance in breast cancers with mutated *PI3Kα* (alpelisib; hyperglycemia and cutaneous

eruptions can occur) or owing to selective use of PI3Kδ by lymphoid tissues in lymphomas (idelalisib, copanlisib, and duvelisib).

Isocitrate dehydrogenase (IDH) inhibitors (ivosidenib specific for IDH1 and enasidenib specific for IDH2) have activity in tumors with IDH mutants (AML, cholangiocarcinomas) that generate the “oncometabolite” 2-hydroxyglutarate, which alters DNA and histone methyltransferase activity. The drugs thus function indirectly as epigenetic chromatin modulating agents through effects on cellular metabolism.

DNA Repair Pathway Modulators DNA repair systems act physiologically to lessen the impact of environmental genomic damaging agents and influence the susceptibility to certain chemotherapy agents. DNA repair enzyme mutations underlie inherited cancer susceptibility syndromes such as mutated *BRCA* tumor-suppressor gene-associated breast and ovarian cancers, among others.

Laboratory investigations revealed that poly-ADP ribose polymerase (PARP) acts as a synthetic lethal gene with mutations in the homologous recombination repair pathway, including the *BRCA* gene. PARP responds to detection of DNA lesions by creating chains of poly-ADP, which serve as scaffolds for the localization of DNA repair proteins still active even with mutated *BRCA* isoforms. However, without PARP activity, the scaffolds cannot form, and the DNA damage becomes lethal. This observation immediately suggested the potential utility of PARP inhibitors (e.g., olaparib) as treatments potentially useful for *BRCA*-induced tumors. Recently, PARP inhibitor utility has been extended to tumors that do not harbor *BRCA* mutations but have given evidence of responding to platinum drugs, as a way of extending the useful effect of the chemotherapy treatment. This finding underscores the likelihood that sensitivity to DNA-directed cytotoxic drugs on the part of a tumor is at least in part related to the drug's ability to take advantage of a sensitizing effect of a tumor's endogenous DNA repair capacity.

Miscellaneous Targeted Therapies The t(15;17) chromosomal translocation is diagnostic of acute promyelocytic leukemia (APL), a subset of AML. The translocation produces a chimeric fusion protein joining the retinoic acid receptor (RAR) α to the transcription factor PML. The abnormal protein, encoding PML-RARα, blocks differentiation of the cancer cells. All-trans-retinoic acid (ATRA) binds to the chimeric protein, releasing the block to differentiation inducing response in APL with fewer complications from cytopenias and disordered coagulation seen with cytotoxic agents. Its use can be attended by a “differentiation syndrome” characterized by cytokine release from and organ infiltration by the tumor cells. Pulmonary function can be severely compromised but is generally responsive to glucocorticoids. Increased intracranial pressure can occur from ATRA, and headache should occasion fundoscopic exam.

Arsenic trioxide was found empirically to also be of value in treating APL, and further study revealed that it also modulates PML-RARα levels, along with decreasing the tolerance of APL cells for free radical damage, inducing apoptosis. The combination of arsenic trioxide and ATRA is productive of very high rates of complete remission in APL. Arsenic trioxide can cause lengthening of the QT interval, and careful attention to concomitant medications, Mg²⁺, ionized Ca²⁺, and K⁺ is necessary during treatment.

The sonic hedgehog transcription factor pathway is regulated by the WNT ligands, which are active during embryonic and fetal life and in certain neoplasms. The sonic hedgehog inhibitors sonidegib and glassdegb are useful in non-surgically treatable cutaneous basal cell carcinomas and certain AMLs, respectively, where the pathway is active.

High-affinity binding to receptors on tumor cells can deliver toxins to tumor cells, exemplified by the IL-3-diphtheria toxin fusion protein tagraxofusp-erzs, targeting the IL-3 receptor (CD123) and useful in blastic plasmacytoid dendritic cell neoplasms. Capillary leak syndrome induced by the toxin component requires careful monitoring of fluid balance to avoid pulmonary dysfunction in particular. Specific receptors for cytokines and growth factors can also serve as “traps” to sequester needed growth factors. Ziv-aflibercept is not an antibody, but a solubilized VEGF receptor VEGF binding domain, and therefore

may have a distinct mechanism of action from bevacizumab, but with comparable side effects.

■ SYSTEMIC RADIATION THERAPY

Systemically administered isotopes of iodide have an important role in the treatment of thyroid neoplasms, owing to the selective upregulation of the iodide transporter in the tumor cell compartment. Likewise, isotopes of samarium and radium have been found useful in the palliation of bony metastases of prostate cancer owing to their selective deposition at the tumor–bone matrix interface. Antibody–radioisotope complexes such as Y⁹⁰–ibritumomab–tiuxetan target CD20, are useful in treating lymphoma, or an isotope can be complexed to a ligand for which the tumor has high affinity. The latter strategy is employed by Lu¹⁷⁷–dotatate, where an analog of somatostatin brings the lutetium isotope close to tumors such as somatostatin receptor–positive gastroenteropancreatic neuroendocrine tumors.

RESISTANCE TO CANCER TREATMENTS

Resistance mechanisms to the conventional cytotoxic agents were initially characterized in the late twentieth century as defects in drug uptake, metabolism, or export by tumor cells. The *multidrug resistance* (*MDR*) gene, encoding P-glycoprotein (Pgp), is prototypic of transport proteins that efficiently excrete many drugs from tumor cells; no clinically useful modulator of this process has yet emerged. Drug-metabolizing enzymes such as cytidine deaminase are upregulated in resistant tumor cells, and this is the basis for so-called ‘high-dose cytarabine’ regimens in the treatment of leukemia. Another resistance mechanism defined during this era involved increased expression of a drug's target, exemplified by amplification of the dihydrofolate reductase gene, in patients who had lost responsiveness to methotrexate, or mutation of topoisomerase II in tumors that relapsed after topoisomerase II modulator treatment.

A second class of resistance mechanisms involves loss of the cellular apoptotic mechanism activated after the engagement of a drug's target by the drug. This occurs in a way that is heavily influenced by the biology of the particular tumor type. For example, decreased alkylguanine alkyltransferase expression defines a subset of glioblastoma patients with the prospect of enhanced benefit from treatment with temozolamide but has no value in predicting benefit from temozolamide in epithelial neoplasms. Likewise, ovarian cancers resistant to platinating agents have decreased expression of the proapoptotic gene *BAX*.

A related class of resistance mechanisms emerged from sequencing of the targets of agents directed at oncogenic kinases, revealing mutated targets, as described previously. This relates to the phenomenon of tumor heterogeneity. Tumors harbor distinct populations of subclones that arise during the process of carcinogenesis, sharing to variable degrees mutations that may promote the growth of some subclones, but that are absent or are no longer relevant to the growth of other subclones. Really useful targeted therapies address a target present in all subclones and to which all tumor subclones require for tumor growth.

Finally, other mechanisms of resistance to targeted agents include the upregulation of alternate means of activating the pathway targeted by the agent. Thus, melanomas initially responsive to *BRAF* V600E antagonists such as vemurafenib may reactivate RAF signaling by employing variant isoforms that can bypass the drug. Likewise, inhibition of HER2/neu signaling in breast cancer cells can lead to the emergence of variants with distinct ways of activating downstream effectors such as PI3 kinase.

SUPPORTIVE CARE DURING CANCER TREATMENT

■ MYELOSUPPRESSION

Cytotoxic chemotherapeutic agents almost invariably affect bone marrow function. Titration of this effect determines the tolerated dose of the agent on a given schedule. Polymorphonuclear leukocytes (PMNs; $t_{1/2} = 6\text{--}8\text{ h}$), platelets ($t_{1/2} = 5\text{--}7\text{ days}$), and red blood cells (RBCs; $t_{1/2} = 120\text{ days}$) have most, less, and least susceptibility, respectively, to usually administered cytotoxic agents. The nadir count of each cell type

in response to classes of agents is characteristic. Maximal neutropenia occurs 6–14 days after conventional doses of anthracyclines, anti-folates, and antimetabolites. Alkylating agents differ from each other in the timing of cytopenias. Nitrosoureas, DTIC, and procarbazine can display delayed marrow toxicity, first appearing 6 weeks after dosing.

Complications of myelosuppression result from the predictable sequelae of the missing cells' function. *Febrile neutropenia* refers to the clinical presentation of fever and <1500 granulocytes/ μL . Management of febrile neutropenia is considered in Chap. 74. Transfusion of granulocytes has no role in the management of febrile neutropenia, owing to their exceedingly short half-life, mechanical fragility, and clinical syndromes of pulmonary compromise with leukostasis after their use. Instead, colony-stimulating factors (CSFs) are used to augment bone marrow production of PMNs. The American Society of Clinical Oncology has developed practice guidelines for the use of granulocyte CSF (G-CSF) and GM-CSF (Table 73-7).

TABLE 73-7 Indications for the Clinical Use of G-CSF or GM-CSF

Preventive Uses

With the first cycle of chemotherapy (so-called primary CSF administration)

- Not needed on a routine basis
- Use if the probability of febrile neutropenia is ≥20%
- Use if patient has preexisting neutropenia or active infection
- Age >65 years treated for lymphoma with curative intent or other tumors treated by similar regimens
- Poor performance status
- Extensive prior chemotherapy
- Dose-dense regimens in a clinical trial or with strong evidence of benefit

With subsequent cycles if febrile neutropenia has previously occurred (so-called secondary CSF administration)

- Not needed after short-duration neutropenia without fever
- Use if patient had febrile neutropenia in previous cycle
- Use if prolonged neutropenia (even without fever) delays therapy

Therapeutic Uses

Afebrile neutropenic patients

- No evidence of benefit

Febrile neutropenic patients

- No evidence of benefit

May feel compelled to use in the face of clinical deterioration from sepsis, pneumonia, or fungal infection, but benefit unclear

In bone marrow or peripheral blood stem cell transplantation

- Use to mobilize stem cells from marrow

- Use to hasten myeloid recovery

In acute myeloid leukemia

- G-CSF of minor or no benefit

- GM-CSF of no benefit and may be harmful

In myelodysplastic syndromes

- Not routinely beneficial

- Use intermittently in subset with neutropenia and recurrent infection

What Dose and Schedule Should Be Used?

G-CSF: 5 mg/kg per day subcutaneously

GM-CSF: 250 mg/ m^2 per day subcutaneously

Pegfilgrastim: one dose of 6 mg 24 h after chemotherapy

When Should Therapy Begin and End?

When indicated, start 24–72 h after chemotherapy

Continue until absolute neutrophil count is 10,000/ μL

Do not use concurrently with chemotherapy or radiation therapy

Abbreviations: CSF, colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

Source: From the American Society of Clinical Oncology: J Clin Oncol 24:3187, 2006.

Dangerous degrees of thrombocytopenia do not frequently complicate the management of patients with solid tumors receiving cytotoxic chemotherapy (with the possible exception of certain carboplatin-containing regimens), but they are frequent in patients with certain hematologic neoplasms where marrow is infiltrated with tumor. Severe bleeding related to thrombocytopenia occurs with increased frequency at platelet counts <20,000/ μL in patients with acute leukemia and <10,000/ μL in patients with solid tumors and is prevalent at counts <5000/ μL .

The precise "trigger" point at which to transfuse patients has been defined as a platelet count of 10,000/ μL or less in patients without medical comorbidities that may increase the risk of bleeding. This issue is important not only because of the costs of frequent transfusion but also because unnecessary platelet transfusions expose the patient to the risks of alloimmunization and loss of value from subsequent transfusion, as well as the infectious and hypersensitivity risks inherent in any transfusion. Prophylactic transfusions to keep platelets >20,000/ μL are reasonable in patients with leukemia who are stressed by fever or concomitant medical conditions. Careful review of medication lists to prevent exposure to nonsteroidal anti-inflammatory agents and maintenance of clotting factor levels adequate to support near-normal prothrombin and partial thromboplastin time tests are important in minimizing the risk of bleeding in the thrombocytopenic patient.

Anemia associated with chemotherapy can be managed by transfusion of packed RBCs. Transfusion is not undertaken until the hemoglobin falls to <80 g/L (8 g/dL), compromise of end-organ function occurs, or an underlying condition (e.g., coronary artery disease) calls for maintenance of hemoglobin >90 g/L (9 g/dL). Randomized trials in certain tumors have raised the possibility that erythropoietin (EPO) use may promote tumor cell survival.

■ NAUSEA AND VOMITING

The most common side effect of chemotherapy administration is nausea, with or without vomiting. Nausea may be acute (within 24 h of chemotherapy), delayed (>24 h), or anticipatory of the receipt of chemotherapy. Highly emetogenic drugs (risk of emesis >90%) include DTIC, cyclophosphamide at >1500 mg/ m^2 , and cisplatin; moderately emetogenic drugs (30–90% risk) include carboplatin, cytosine arabinoside (>1 g/ m^2), ifosfamide, conventional-dose cyclophosphamide, and anthracyclines; low-risk (10–30%) agents include 5-FU, taxanes, etoposide, and bortezomib, with minimal risk (<10%) afforded by treatment with antibodies, bleomycin, busulfan, fludarabine, and vinca alkaloids.

Serotonin antagonists (5-HT₃) and neurokinin 1 (NK1) receptor antagonists are useful in "high-risk" chemotherapy regimens. The combination acts at both peripheral gastrointestinal and CNS sites that control nausea and vomiting. For example, the 5-HT₃ blocker dolasetron, 100 mg intravenously or orally; dexamethasone, 12 mg; and the NK1 antagonist aprepitant, 125 mg orally, are combined on the day of administration of severely emetogenic regimens, with repetition of dexamethasone (8 mg) and aprepitant (80 mg) on days 2 and 3 for delayed nausea. Alternate 5-HT₃ antagonists include ondansetron, given as 0.15 mg/kg intravenously for three doses just before and at 4 and 8 h after chemotherapy; palonosetron at 0.25 mg over 30 s, 30 min before chemotherapy; and granisetron, given as a single dose of 0.01 mg/kg just before chemotherapy. Emesis from moderately emetic chemotherapy regimens may be prevented with a 5-HT₃ antagonist and dexamethasone alone for patients not receiving doxorubicin and cyclophosphamide combinations; the latter combination requires the 5-HT₃/dexamethasone/aprepitant on day 1, but aprepitant alone on days 2 and 3. Emesis from low-emetic-risk regimens may be prevented with 8 mg of dexamethasone alone or with non-5-HT₃, non-NK1 antagonist approaches including the following.

Antidopaminergic phenothiazines act directly at the chemoreceptor trigger zone (CTZ) in the brainstem medulla and include prochlorperazine (Compazine), 10 mg intramuscularly or intravenously, 10–25 mg orally, or 25 mg per rectum every 4–6 h for up to four doses;

and thiethylperazine, 10 mg by potentially all of the above routes every 6 h. Haloperidol is a butyrophenone dopamine antagonist given at 1 mg intramuscularly or orally every 8 h. Metoclopramide acts on peripheral dopamine receptors to augment gastric emptying and is used in high doses for highly emetogenic regimens (1–2 mg/kg intravenously 30 min before chemotherapy and every 2 h for up to three additional doses as needed); intravenous doses of 10–20 mg every 4–6 h as needed or 50 mg orally 4 h before and 8 and 12 h after chemotherapy are used for moderately emetogenic regimens. 5-9-Tetrahydrocannabinol (Marinol) is a rather weak antiemetic compared to other available agents, but it may be useful for persisting nausea and is used orally at 10 mg every 3–4 h as needed. Olanzapine, an “atypical antipsychotic” acting at multiple neurotransmitter receptors, may be of value, most clearly in cases refractory to the measures described above. Some practice guidelines have endorsed its earlier use in adults receiving highly emetogenic chemotherapy regimens in combination with an NK1 antagonist plus an HT3 antagonist plus dexamethasone.

■ DIARRHEA

Similar to the vomiting syndromes, chemotherapy-induced diarrhea may be immediate or can occur in a delayed fashion up to 48–72 h after the drugs. Careful attention to maintained hydration and electrolyte repletion, intravenously if necessary, along with antimotility treatments such as “high-dose” loperamide (4 mg at the first occurrence of diarrhea, with 2 mg repeated every 2 h until 12 h without loose stools, not to exceed a total daily dose of 16 mg), are appropriate. Octreotide (100–150 µg), a somatostatin analogue, or intraluminally acting opiate-based preparations may be considered for patients not responding to loperamide.

■ MUCOSITIS

Irritation and inflammation of the mucous membranes (mucositis) particularly afflicting the oral and anal mucosa, but potentially involving the entire gastrointestinal tract, may accompany cytotoxic chemotherapy. Topical therapies, including anesthetics and barrier-creating preparations, may provide symptomatic relief in mild cases. Palifermin, a keratinocyte growth factor and member of the fibroblast growth factor family, is effective in preventing severe mucositis in the setting of high-dose chemotherapy with stem cell transplantation for hematologic malignancies. It may also prevent or ameliorate mucositis from radiation.

■ ALOPECIA

Chemotherapeutic agents vary widely in causing alopecia, with anthracyclines, alkylating agents, and topoisomerase inhibitors reliably causing near-total alopecia when given at therapeutic doses. Antimetabolites are more variably associated with alopecia. Psychological support and the use of cosmetic resources are to be encouraged. “Chemo caps” that reduce scalp temperature to decrease the degree of alopecia are controversial during treatment with curative intent of neoplasms, such as leukemia or lymphoma, or in adjuvant breast cancer therapy. The richly vascularized scalp can certainly harbor micrometastatic or disseminated disease.

■ GONADAL DYSFUNCTION AND PREGNANCY

All cancer treatments described in this chapter should be regarded as potentially injurious to the developing fetus and to newborns via lactation. However, there are gradations to the degree of reproductive harm. All agents tend to have increased risk of adverse outcomes when administered during the first trimester, and strategies to delay chemotherapy, if possible, until after this milestone should be considered if the pregnancy is to continue to term. Patients in their second or third trimester can be treated with most regimens for the common

neoplasms afflicting women in their childbearing years, with the exception of antimetabolites, particularly antifolates, which have notable teratogenic or fetotoxic effects throughout pregnancy. The need for anticancer chemotherapy per se is infrequently a clear basis to recommend termination of a concurrent pregnancy, although each treatment strategy in this circumstance must be tailored to the individual needs of the patient.

Cessation of ovulation and azoospermia reliably result from regimens that contain alkylating agents and topoisomerase poison. The duration of these effects varies with age and sex. Sperm banking before treatment may be considered. Females experience amenorrhea with anovulation after alkylating agent therapy; egg preservation may be considered but may delay inception of urgent treatment. Recovery of normal menses is frequent if treatment is completed before age 30, but patients are unlikely to recover menses after age 35. Even those who regain menses usually experience premature menopause. Because the magnitude and extent of decreased fertility can be difficult to predict, patients should be counseled to maintain effective contraception, preferably by barrier means, during and after therapy. Resumption of efforts to conceive should be considered in the context of the patient’s likely prognosis. Hormone replacement therapy should be undertaken in women who do not have a hormonally responsive tumor. For patients who have had a hormone-sensitive tumor primarily treated by a local modality, conventional practice would counsel against hormone replacement, but this issue is under investigation.

■ PALLIATIVE AND SUPPORTIVE CARE

An important perspective the primary care provider may bring to patients and their families facing incurable cancer is that, given the limited value of chemotherapeutic approaches at some point in the natural history of most metastatic cancers, *palliative care* or *hospice-based* approaches, with meticulous and ongoing attention to symptom relief and with family, psychological, and spiritual support, should receive prominent attention as a valuable therapeutic plan (Chaps. 12 and 69). Optimizing the quality of life rather than attempting to extend it becomes a valued intervention. Patients facing the impending progression of disease in a life-threatening way frequently choose to undertake toxic treatments of little to no potential value, and support provided by the primary caregiver in accessing palliative and hospice-based options in contrast to receiving toxic and ineffective regimens can be critical in providing a basis for patients to make sensible choices.

Late effects of cancer and its treatment are reviewed in Chap. 95.

■ FURTHER READING

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Infections are a common cause of death and an even more common cause of morbidity in patients with a wide variety of neoplasms. Infections in cancer patients can result directly from tissue invasion by cancerous cells (either by replacement of healthy host marrow cells or by occlusion of an orifice) (Table 74-1) or as a result of treatment. In the era of cytotoxic chemotherapy, neutropenia as a result of chemotherapy was the major cause of infectious complications of cancer therapy. The routine use of granulocyte-stimulating cytokines has, in most cases, shortened the duration of neutropenia, and the increasing use of checkpoint inhibitors and chimeric antigen receptor (CAR) T cells has changed the field of oncology and led to better outcomes. Unfortunately, checkpoint inhibitors and immunomodulators are also associated with an increased risk of infections—particularly intracellular pathogens. An evolving approach to prevention and treatment of infectious complications of cancer has decreased infection-associated mortality rates and will probably continue to do so. This accomplishment has resulted from three major steps:

- 1. Early treatment:** The practice of using early empirical antibiotics reduced mortality rates among patients with leukemia and bacteremia from 84% in 1965 to 44% in 1972. The mortality rate due to infection in febrile neutropenic patients dropped to <10% by 2013. This dramatic improvement is attributed to early intervention with appropriate antimicrobial therapy.
- 2. Empirical treatment:** “Empirical” antifungal therapy has also lowered the incidence of disseminated fungal infection, with dramatic decreases in mortality rates. An antifungal agent is administered—on the basis of likely fungal infection—to neutropenic patients who, after 4–7 days of antibiotic therapy, remain febrile but have no positive cultures.
- 3. Prophylaxis:** Use of antibiotics for afebrile neutropenic patients as broad-spectrum prophylaxis against infections has decreased both mortality and morbidity even further. The current approach to treatment of severely neutropenic patients (e.g., those receiving high-dose chemotherapy for leukemia or high-grade lymphoma) is based on initial prophylactic therapy at the onset of neutropenia, subsequent “empirical” antibacterial therapy targeting the

organisms whose involvement is likely in light of physical findings (most often fever alone), and finally “empirical” antifungal therapy based on the known likelihood that fungal infection will become a serious issue after 4–7 days of broad-spectrum antibacterial therapy.

A physical predisposition to infection in patients with cancer (Table 74-1) can be a result of the neoplasm’s production of a break in the skin. For example, a squamous cell carcinoma may cause local invasion of the epidermis, which allows bacteria to gain access to subcutaneous tissue and permits the development of cellulitis. The artificial closing of a normally patent orifice can also predispose to infection; for example, obstruction of a ureter by a tumor can cause urinary tract infection, and obstruction of the bile duct can cause cholangitis. Part of the host’s normal defense against infection depends on the continuous emptying of a viscus; without emptying, a few bacteria that are present as a result of bacteremia or local transit can multiply and cause disease.

A similar problem can affect patients whose lymph node integrity has been disrupted by radical surgery, particularly patients who have had radical node dissections. A common clinical problem following radical mastectomy is the development of cellulitis (usually caused by streptococci or staphylococci) because of lymphedema and/or inadequate lymph drainage. In most cases, this problem can be addressed by local measures designed to prevent fluid accumulation and breaks in the skin, but antibiotic prophylaxis has been used in refractory cases.

A life-threatening problem common to many cancer patients is the loss of the reticuloendothelial capacity to clear microorganisms after splenectomy, which may be performed as part of the management of hairy cell leukemia, chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML) as well as Hodgkin’s disease. Even after curative therapy for the underlying disease, the lack of a spleen predisposes such patients to rapidly fatal infections. The loss of the spleen through trauma similarly predisposes the normal host to overwhelming infection throughout life. The splenectomized patient should be counseled about the risks of infection with certain organisms, such as the protozoan *Babesia* (Chap. 225) and *Capnocytophaga canimorsus*, a bacterium carried in the mouths of animals (Chaps. 141 and 158). Because encapsulated bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*) are the organisms most commonly associated with postsplenectomy sepsis, splenectomized persons should be vaccinated (and revaccinated; Table 74-2 and Chap. 123) against the capsular polysaccharides of these organisms. Many clinicians recommend giving splenectomized patients a small supply of antibiotics effective against *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* to avert rapid, overwhelming sepsis in the event that they cannot present for medical attention immediately after the onset of fever or other signs or

TABLE 74-1 Disruption of Normal Barriers in Patients with Cancer That May Predispose Them to Infections

Type of Defense	Specific Lesion or Deficiency	Cells Involved	Organism	Cancer Association	Disease
Physical barrier	Breaks in skin	Skin epithelial cells	Staphylococci, streptococci	Head and neck, squamous cell carcinoma	Cellulitis, extensive skin infection
Emptying of fluid collections	Occlusion of orifices: ureters, bile duct, colon	Luminal epithelial cells	Gram-negative bacilli	Renal, ovarian, biliary tree, metastatic diseases of many cancers	Rapid, overwhelming bacteremia; urinary tract infection
Lymphatic function	Node dissection	Lymph nodes	Staphylococci, streptococci	Breast cancer surgery	Cellulitis
Splenic clearance of microorganisms	Splenectomy	Splenic reticuloendothelial cells	<i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Neisseria meningitidis</i> , <i>Babesia</i> , <i>Capnocytophaga canimorsus</i>	Hodgkin’s disease, leukemia	Rapid, overwhelming sepsis
Phagocytosis	Lack of granulocytes	Granulocytes (neutrophils)	Staphylococci, streptococci, enteric organisms, fungi	Acute myeloid and acute lymphocytic leukemias, hairy cell leukemia	Bacteremia
Humoral immunity	Lack of antibodies	B cells	<i>S. pneumoniae</i> , <i>H. influenzae</i> , <i>N. meningitidis</i>	Chronic lymphocytic leukemia, multiple myeloma	Infections with encapsulated organisms, sinusitis, pneumonia
Cellular immunity	Lack of T cells	T cells and macrophages	<i>Mycobacterium tuberculosis</i> , <i>Listeria</i> , herpesviruses, fungi, intracellular parasites	Hodgkin’s disease, leukemia, T-cell lymphoma	Infections with intracellular bacteria, fungi, parasites; virus reactivation

Deceased.

TABLE 74-2 Vaccination of Cancer Patients Receiving Chemotherapy^a

VACCINE	USE IN INDICATED PATIENTS		
	INTENSIVE CHEMOTHERAPY	HODGKIN'S DISEASE	HEMATOPOIETIC STEM CELL TRANSPLANTATION
Diphtheria-tetanus-pertussis ^b	Primary series and boosters as necessary	No special recommendation	3 doses given 6–12 months after transplantation
Poliomyelitis ^c	Complete primary series and boosters	No special recommendation	3 doses given 6–12 months after transplantation
<i>Haemophilus influenzae</i> type b conjugate	Primary series and booster for children	Single dose for adults	3 doses given 6–12 months after transplantation (separated by 1 month)
Human papillomavirus (HPV)	HPV vaccine is approved for males and females 9–26 years of age. Check Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines) for updated recommendations.	HPV vaccine is approved for males and females 9–26 years of age. Check CDC website (www.cdc.gov/vaccines) for updated recommendations.	HPV vaccine is approved for males and females 9–26 years of age. Check CDC website (www.cdc.gov/vaccines) for updated recommendations.
Hepatitis A	As indicated for normal hosts on the basis of occupation and lifestyle	As indicated for normal hosts on the basis of occupation and lifestyle	As indicated for normal hosts on the basis of occupation and lifestyle
Hepatitis B	Same as for normal hosts	As indicated for normal hosts on the basis of occupation and lifestyle	3 doses given 6–12 months after transplantation
Pneumococcal conjugate vaccine (PCV13) Pneumococcal polysaccharide vaccine (PPSV23) ^d	Finish series prior to chemotherapy if possible.	Patients with splenectomy should receive both PCV13 and PPSV23.	Three doses of PCV13, beginning 3–6 months after transplantation, are followed by a dose of PPSV23 at least 8 weeks later. A second PPSV23 dose can be given 5 years later.
Quadrivalent meningococcal vaccine ^e	Should be administered to splenectomized patients and to patients living in endemic areas, including college students in dormitories	Should be administered to splenectomized patients and to patients living in endemic areas, including college students in dormitories. An additional dose can be given after 5 years.	Should be administered to splenectomized patients and to patients living in endemic areas, including college students in dormitories. An additional dose can be given after 5 years.
Meningococcal B vaccine	See above.	See above.	See above (see www.cdc.gov/vaccines for updated recommendations).
Influenza	Seasonal immunization	Seasonal immunization	Seasonal immunization (A seasonal dose is recommended and can be given as early as 4 months after transplantation; if given <6 months after transplantation, an additional dose is recommended.)
Measles/mumps/rubella	Contraindicated	Contraindicated during chemotherapy	After 24 months in patients without graft-versus-host disease
Varicella-zoster virus ^f	Zoster recombinant vaccine	Zoster recombinant vaccine	Two-dose zoster recombinant vaccine recommended

^aThe latest recommendations by the Advisory Committee on Immunization Practices and the CDC guidelines can be found at www.cdc.gov/vaccines. ^bA single dose of TDaP (tetanus–diphtheria–acellular pertussis), followed by a booster dose of Td (tetanus–diphtheria) every 10 years, is recommended for adults. ^cLive-virus vaccine is contraindicated; inactivated vaccine should be used. ^dTwo types of vaccines are used to prevent pneumococcal disease. A conjugate vaccine active against 13 serotypes (13-valent pneumococcal conjugate vaccine, or PCV13) is currently administered in three separate doses to all children. A polysaccharide vaccine active against 23 serotypes (23-valent pneumococcal polysaccharide vaccine, or PPSV23) elicits titers of antibody lower than those achieved with the conjugate vaccine, and immunity may wane more rapidly. Because the ablative chemotherapy given to recipients of hematopoietic stem cell transplants (HSCTs) eradicates immunologic memory, revaccination is recommended for all such patients. Vaccination is much more effective once immunologic reconstitution has occurred; however, because of the need to prevent serious disease, pneumococcal vaccine should be administered 6–12 months after transplantation in most cases. Because PPSV23 includes serotypes not present in PCV13, HSCT recipients should receive a dose of PPSV23 at least 8 weeks after the last dose of PCV13. Although antibody titers from PPSV23 clearly decay, experience with multiple doses of PPSV23 is limited, as are data on the safety, toxicity, or efficacy of such a regimen. For this reason, the CDC currently recommends the administration of one additional dose of PPSV23 at least 5 years after the last dose to immunocompromised patients, including transplant recipients, as well as patients with Hodgkin's disease, multiple myeloma, lymphoma, or generalized malignancies. Beyond this single additional dose, further doses are not recommended at this time. ^eMeningococcal conjugate vaccine (MenACWY) is recommended for adults ≤55 years old, and meningococcal polysaccharide vaccine (MPSV4) is recommended for those ≥56 years old. ^fVaricella vaccine is recommended for children and zoster recombinant vaccine for adults. ^gContact the manufacturer for more information on use in children with acute lymphocytic leukemia.

symptoms of bacterial infection. A few tablets of amoxicillin/clavulanic acid (or levofloxacin if resistant strains of *S. pneumoniae* are prevalent locally) are a reasonable choice for this purpose.

The level of suspicion of infections with certain organisms depends on the type of cancer diagnosed (Table 74-3). Diagnosis of multiple myeloma or CLL should alert the clinician to the possibility of hypogammaglobulinemia. While immunoglobulin replacement therapy can be effective, in most cases, prophylactic antibiotics are a cheaper, more convenient method of eliminating bacterial infections in CLL patients with hypogammaglobulinemia. Patients with acute lymphocytic leukemia (ALL), patients with non-Hodgkin's lymphoma, and all cancer patients treated with high-dose glucocorticoids (or glucocorticoid-containing chemotherapy regimens) should receive antibiotic prophylaxis for *Pneumocystis* infection (Table 74-3) for the duration of their chemotherapy. In addition to exhibiting susceptibility to certain infectious organisms, patients with cancer are likely to manifest their

infections in characteristic ways. For example, fever—generally a sign of infection in normal hosts—continues to be a reliable indicator in neutropenic patients. In contrast, patients receiving glucocorticoids and agents that impair T-cell function and cytokine secretion may have serious infections in the absence of fever. Similarly, neutropenic patients commonly present with cellulitis without purulence and with pneumonia without sputum or even x-ray findings (see below).

The use of monoclonal antibodies that target B and T cells as well as drugs that interfere with lymphocyte signal transduction events are associated with reactivation of latent infections. The use of infliximab and other anti-tumor necrosis factor (TNF) antibodies are associated with the development of reactivation tuberculosis. Similarly, the use of the anti-B cell antibody, rituximab, is associated with reactivation of hepatitis B and other latent viruses. Checkpoint inhibitors also predispose individuals to reactivation of intracellular pathogens, and clinicians must be aware of what viruses and other intracellular organisms

TABLE 74-3 Infections Associated with Specific Types of Cancer

CANCER	UNDERLYING IMMUNE ABNORMALITY	ORGANISM(S) CAUSING INFECTION
Multiple myeloma	Hypogammaglobulinemia	<i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Neisseria meningitidis</i>
Chronic lymphocytic leukemia	Hypogammaglobulinemia	<i>S. pneumoniae</i> , <i>H. influenzae</i> , <i>N. meningitidis</i>
Acute myeloid or lymphocytic leukemia	Granulocytopenia, skin and mucous membrane lesions	Extracellular gram-positive and gram-negative bacteria, fungi
Hodgkin's disease	Abnormal T-cell function	Intracellular pathogens (<i>Mycobacterium tuberculosis</i> , <i>Listeria</i> , <i>Salmonella</i> , <i>Cryptococcus</i> , <i>Mycobacterium avium</i>); herpesviruses
Non-Hodgkin's lymphoma and acute lymphocytic leukemia	Glucocorticoid chemotherapy, T- and B-cell dysfunction	<i>Pneumocystis</i>
Colon and rectal tumors	Local abnormalities ^a	<i>Streptococcus bovis</i> biotype 1 (bacteremia)
Hairy cell leukemia	Abnormal T-cell function	Intracellular pathogens (<i>M. tuberculosis</i> , <i>Listeria</i> , <i>Cryptococcus</i> , <i>M. avium</i>)

^aThe reason for this association is not well defined.

(mycobacteria, fungi, etc.) are likely to grow and pose a threat to an individual patient receiving these therapies. Like organ transplant recipients (Chap. 143), patients with latent bacterial disease (like tuberculosis) and latent viral disease (like herpes simplex or zoster) should be carefully monitored for reactivation disease.

SYSTEM SPECIFIC SYNDROMES

SKIN SPECIFIC SYNDROMES

Skin lesions are common in cancer patients, and the appearance of these lesions may permit the diagnosis of systemic bacterial or fungal infection. While cellulitis caused by skin organisms such as *Streptococcus* or *Staphylococcus* is common, neutropenic patients—that is, those with <500 functional polymorphonuclear leukocytes (PMNs)/µL—and patients with impaired blood or lymphatic drainage may develop infections with unusual organisms. Innocent-looking macules or papules may be the first sign of bacterial or fungal sepsis in immunocompromised patients (Fig. 74-1). In the neutropenic host, a macule progresses rapidly to ecthyma gangrenosum (see Fig. A1-34), a usually painless, round, necrotic lesion consisting of a central black or gray-black eschar with surrounding erythema. Ecthyma gangrenosum, which is located in nonpressure areas (as distinguished from necrotic lesions associated with lack of circulation), is often associated with *Pseudomonas aeruginosa* bacteremia (Chap. 164) but may be caused by other bacteria.

Candidemia (Chap. 216) is also associated with a variety of skin conditions (see Fig. A1-37) and commonly presents as a maculopapular rash. Punch biopsy of the skin may be the best method for diagnosis.

Cellulitis, an acute spreading inflammation of the skin, is most often caused by infection with group A *Streptococcus* or *Staphylococcus aureus*, virulent organisms normally found on the skin (Chap. 129). Although cellulitis tends to be circumscribed in normal hosts, it may spread rapidly in neutropenic patients. A tiny break in the skin may lead to spreading cellulitis, which is characterized by pain and erythema; in the affected patients, signs of infection (e.g., purulence) are often lacking. What might be a furuncle in a normal host may require amputation because of uncontrolled infection in a patient presenting with leukemia. A dramatic response to an infection that might be trivial in a normal host can mark the first sign of leukemia. Fortunately, granulocytopenic patients are likely to be infected with certain types of organisms (Table 74-4); thus, the selection of an antibiotic regimen is somewhat easier than it might otherwise be (see “Antibacterial Therapy,” below). It is essential to recognize cellulitis early and to treat it aggressively. Patients who are neutropenic or who have previously



A



B

FIGURE 74-1 A. Papules related to *Escherichia coli* bacteremia in a patient with acute lymphocytic leukemia. B. The same lesions on the following day.

TABLE 74-4 Organisms Likely to Cause Infections in Granulocytopenic Patients

Gram-Positive Cocci	
<i>Staphylococcus epidermidis</i> ^a	<i>Staphylococcus aureus</i>
<i>Viridans Streptococcus</i>	<i>Enterococcus faecalis</i>
<i>Streptococcus pneumoniae</i>	
Gram-Negative Bacilli	
<i>Escherichia coli</i>	<i>Serratia</i> spp.
<i>Klebsiella</i> spp.	<i>Acinetobacter</i> spp. ^a
<i>Pseudomonas aeruginosa</i>	<i>Stenotrophomonas</i> spp.
<i>Enterobacter</i> spp.	<i>Citrobacter</i> spp.
Non- <i>aeruginosa Pseudomonas</i> spp. ^a	
Gram-Positive Bacilli	
Diphtheroids	JK bacillus ^a
Fungi	
<i>Candida</i> spp.	<i>Mucor/Rhizopus</i>
<i>Aspergillus</i> spp.	

^aOften associated with intravenous catheters.

received antibiotics for other reasons may develop cellulitis with unusual organisms (e.g., *Escherichia coli*, *Pseudomonas*, or fungi). Early treatment, even of innocent-looking lesions, is essential to prevent necrosis and loss of tissue. Debridement to prevent spread may sometimes be necessary early in the course of disease, but it can often be performed after chemotherapy, when the PMN count increases.

Sweet syndrome, or *febrile neutrophilic dermatosis*, was originally described in women with elevated white blood cell (WBC) counts. The disease is characterized by the presence of leukocytes in the lower dermis, with edema of the papillary body. Ironically, this disease now is usually seen in neutropenic patients with cancer, most often in association with acute myeloid leukemia (AML) but also in association with a variety of other malignancies. Sweet syndrome usually presents as red or bluish-red papules or nodules that may coalesce and form sharply bordered plaques (see Fig. A1-40). The edema may suggest vesicles, but on palpation, the lesions are solid, and vesicles probably never arise in this disease. The lesions are most common on the face, neck, and arms. On the legs, they may be confused with erythema nodosum (see Fig. A1-39). The development of lesions is often accompanied by high fevers and an elevated erythrocyte sedimentation rate. Both the lesions and the temperature elevation respond dramatically to glucocorticoid administration. Treatment begins with high doses of glucocorticoids (prednisone, 60 mg/d) followed by tapered doses over the next 2–3 weeks.

Data indicate that *erythema multiforme* (see Fig. A1-24) with mucous membrane involvement is often associated with herpes simplex virus (HSV) infection and is distinct from Stevens-Johnson syndrome, which is associated with drugs and tends to have a more widespread distribution. Because cancer patients are both immunosuppressed (and therefore susceptible to herpes infections) and heavily treated with drugs (and therefore subject to Stevens-Johnson syndrome [see Fig. A3-4]), both of these conditions are common in this population.

Cytokines, which are used as adjuvants or primary treatments for cancer, can themselves cause characteristic rashes, further complicating the differential diagnosis. This phenomenon is a particular problem in bone marrow (stem cell) transplant recipients (Chap. 143), who, in addition to having the usual chemotherapy-, antibiotic-, and cytokine-induced rashes, are plagued by graft-versus-host disease.

CATHETER RELATED INFECTIONS

Because intravenous (IV) catheters are commonly used in cancer chemotherapy and are prone to cause infection (Chap. 142), they pose a major problem in the care of patients with cancer. Some catheter-associated infections can be treated with antibiotics, whereas in others, the catheter must be removed (Table 74-5). If the patient has a “tunneled” catheter (which consists of an entrance site, a subcutaneous

tunnel, and an exit site), a red streak over the subcutaneous part of the line (the tunnel) is grounds for immediate device removal. Failure to remove catheters under these circumstances may result in extensive cellulitis and tissue necrosis.

More common than tunnel infections are exit-site infections, often with erythema around the area where the line penetrates the skin. Most authorities (Chap. 147) recommend treatment (usually with vancomycin) for an exit-site infection caused by coagulase-negative *Staphylococcus*. Treatment of coagulase-positive staphylococcal infection is associated with a poorer outcome, and it is advisable to remove the catheter if possible. Similarly, most clinicians remove catheters associated with infections due to *P. aeruginosa* and *Candida* species, because such infections are difficult to treat and bloodstream infections with these organisms are likely to be deadly. Catheter infections caused by *Burkholderia cepacia*, *Stenotrophomonas* species, *Agrobacterium* species, *Acinetobacter baumannii*, *Pseudomonas* species other than *aeruginosa*, and carbapenem-resistant Enterobacteriaceae are likely to be very difficult to eradicate with antibiotics alone. Similarly, isolation of *Bacillus*, *Corynebacterium*, and *Mycobacterium* species should prompt removal of the catheter.

GASTROINTESTINAL TRACT SPECIFIC SYNDROMES

Upper Gastrointestinal Tract Disease • **INFECTIONS OF THE MOUTH** The oral cavity is rich in aerobic and anaerobic bacteria (Chap. 177) that normally live in a commensal relationship with the host. The antimetabolic effects of chemotherapy cause a breakdown of mucosal host defenses, leading to ulceration of the mouth and the potential for invasion by resident bacteria. Mouth ulcerations afflict most patients receiving cytotoxic chemotherapy and have been associated with viridans streptococcal bacteremia. *Candida* infections of the mouth are very common. Fluconazole is clearly effective in the treatment of both local infections (thrush) and systemic infections (esophagitis) due to *Candida albicans*. Other azoles (e.g., voriconazole) as well as echinocandins offer similar efficacy as well as activity against the fluconazole-resistant organisms that are associated with chronic fluconazole treatment (Chap. 216).

Noma (*cancrum oris*), commonly seen in malnourished children, is a penetrating disease of the soft and hard tissues of the mouth and adjacent sites, with resulting necrosis and gangrene. It has a counterpart in immunocompromised patients and is thought to be due to invasion of the tissues by *Bacteroides*, *Fusobacterium*, and other normal inhabitants of the mouth. Noma is associated with debility, poor oral hygiene, and immunosuppression.

TABLE 74-5 Approach to Catheter Infections in Immunocompromised Patients

CLINICAL PRESENTATION OR ISOLATED PATHOGEN	CATHETER REMOVAL	ANTIBIOTICS	COMMENTS
Evidence of Infection, Negative Blood Cultures			
Exit-site erythema	Not necessary if infection responds to treatment	Usually, begin treatment for gram-positive cocci.	Coagulase-negative staphylococci are most common.
Tunnel-site erythema	Required	Treat for gram-positive cocci pending culture results.	Failure to remove the catheter may lead to necrosis of the involved area requiring skin grafts in the future.
Blood Culture–Positive Infections			
Coagulase-negative staphylococci	Line removal optimal but may be unnecessary if patient is clinically stable and responds to antibiotics	Usually, start with vancomycin. Linezolid, quinupristin/dalfopristin, and daptomycin are alternative agents.	If there are no contraindications to line removal, this course of action is optimal. If the line is removed, antibiotics may not be necessary.
Other gram-positive cocci (e.g., <i>Staphylococcus aureus</i> , <i>Enterococcus</i>); gram-positive rods (<i>Bacillus</i> , <i>Corynebacterium</i> spp.)	Recommended	Treat with antibiotics to which the organism is sensitive, with duration based on the clinical setting.	The incidence of metastatic infections following <i>S. aureus</i> infection and the difficulty of treating enterococcal infection make line removal the recommended course of action. In addition, gram-positive rods do not respond readily to antibiotics alone.
Gram-negative bacteria	Recommended	Use an agent to which the organism is shown to be sensitive.	Organisms like <i>Stenotrophomonas</i> , <i>Pseudomonas</i> , and <i>Burkholderia</i> are notoriously hard to treat, as are carbapenem-resistant organisms.
Fungi	Recommended	—	Fungal infections of catheters are extremely difficult to treat.

Viruses, particularly HSV, are a prominent cause of morbidity in immunocompromised patients, in whom they are associated with severe mucositis. The use of acyclovir, either prophylactically or therapeutically, is of value.

ESOPHAGEAL INFECTIONS The differential diagnosis of esophagitis (usually presenting as substernal chest pain upon swallowing) includes herpes simplex and candidiasis, both of which are readily treatable.

Lower Gastrointestinal Tract Disease Hepatic candidiasis (*Chap. 216*) results from seeding of the liver (usually from a gastrointestinal source) in neutropenic patients. It is most common among patients being treated for AML and usually presents symptomatically around the time neutropenia resolves. The characteristic picture is that of persistent fever unresponsive to antibiotics, abdominal pain and tenderness or nausea, and elevated serum levels of alkaline phosphatase in a patient with hematologic malignancy who has recently recovered from neutropenia. The diagnosis of this disease (which may present in an indolent manner and persist for several months) is based on the finding of yeasts or pseudohyphae in granulomatous lesions. Hepatic ultrasound or CT may reveal bull's-eye lesions. MRI scans reveal small lesions not visible by other imaging modalities. The pathology (a granulomatous response) and the timing (with resolution of neutropenia and an elevation in granulocyte count) suggest that the host response to *Candida* is an important component of the manifestations of disease. In many cases, although organisms are visible, cultures of biopsied material may be negative. The designation *hepatosplenic candidiasis* or *hepatic candidiasis* is a misnomer because the disease often involves the kidneys and other tissues; the term *chronic disseminated candidiasis* may be more appropriate. Because of the risk of bleeding with liver biopsy, diagnosis is often based on imaging studies (MRI, CT). Treatment should be directed to the causative agent (usually *C. albicans* but sometimes *Candida tropicalis* or other less common *Candida* species).

Typhlitis *Typhlitis* (also referred to as necrotizing colitis, neutropenic colitis, necrotizing enteropathy, ileocecal syndrome, and cecitis) is a clinical syndrome of fever and right-lower-quadrant (or generalized abdominal) tenderness in an immunosuppressed host. This syndrome is classically seen in neutropenic patients after chemotherapy with cytotoxic drugs. It may be more common among children than among adults and appears to be much more common among patients with AML or ALL than among those with other types of cancer. Physical examination reveals right-lower-quadrant tenderness, with or without rebound tenderness. Associated diarrhea (often bloody) is common, and the diagnosis can be confirmed by the finding of a thickened cecal wall on CT, MRI, or ultrasonography. Plain films may reveal a right-lower-quadrant mass, but CT with contrast or MRI is a much more sensitive means of diagnosis. Although surgery is sometimes attempted to avoid perforation from ischemia, most cases resolve with medical therapy alone. The disease is sometimes associated with positive blood cultures (which usually yield aerobic gram-negative bacilli), and therapy is recommended for a broad spectrum of bacteria (particularly gram-negative bacilli, which are likely to be found in the bowel flora).

***Clostridioides difficile*-Induced Diarrhea** Patients with cancer are predisposed to the development of *C. difficile* diarrhea (*Chap. 134*) as a consequence of chemotherapy alone. Thus, they may test positive for *C. difficile* even without receiving antibiotics. Obviously, such patients are also subject to *C. difficile*-induced diarrhea as a result of antibiotic pressure. *C. difficile* should always be considered as a possible cause of diarrhea in cancer patients who have received either chemotherapy or antibiotics. New approaches to treat *C. difficile*-induced diarrhea and to prevent *C. difficile* expansion as part of the gut microbiota may make this disease less troublesome in the future.

CENTRAL NERVOUS SYSTEM SPECIFIC SYNDROMES

Meningitis The presentation of meningitis in patients with lymphoma or CLL and in patients receiving chemotherapy (particularly with glucocorticoids) for solid tumors suggests a diagnosis of

cryptococcal or listerial infection. As noted previously, splenectomized patients are susceptible to rapid, overwhelming infection with encapsulated bacteria (including *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*). Similarly, patients who are antibody-deficient (e.g., those with CLL, those who have received intensive chemotherapy, or those who have undergone bone marrow [stem cell] transplantation) are likely to have infections caused by these bacteria. Other cancer patients, however, because of their defective cellular immunity, are likely to be infected with other pathogens (Table 74-3). Central nervous system (CNS) tuberculosis should be considered, especially in patients from countries where tuberculosis is highly prevalent in the population.

Encephalitis The spectrum of disease resulting from viral encephalitis is expanded in immunocompromised patients. A predisposition to infections with intracellular organisms similar to those encountered in patients with AIDS (*Chap. 202*) is seen in cancer patients receiving (1) high-dose cytotoxic chemotherapy, (2) chemotherapy affecting T-cell function (e.g., fludarabine), or (3) antibodies that eliminate T cells (e.g., anti-CD3, alemtuzumab, anti-CD52) or cytokine activity (anti-tumor necrosis factor agents or interleukin 1 receptor antagonists). Infection with varicella-zoster virus (VZV) has been associated with encephalitis that may be caused by VZV-related vasculitis. Chronic viral infections may also be associated with dementia and encephalitic presentations. A diagnosis of progressive multifocal leukoencephalopathy (*Chap. 138*) should be considered when a patient who has received chemotherapy (rituximab in particular) presents with dementia (Table 74-6). Other abnormalities of the CNS that may be confused with infection include normal-pressure hydrocephalus and vasculitis resulting from CNS irradiation. It may be possible to differentiate these conditions by MRI.

Brain Masses Mass lesions of the brain most often present as headache with or without fever or neurologic abnormalities. Infections associated with mass lesions may be caused by bacteria (particularly *Nocardia*), fungi (particularly *Cryptococcus* or *Aspergillus*), or parasites (*Toxoplasma*). Epstein-Barr virus (EBV)-associated lymphoma may also present as single—or sometimes multiple—mass lesions of the brain. A biopsy may be required for a definitive diagnosis.

PULMONARY INFECTIONS

Pneumonia (*Chap. 126*) in immunocompromised patients may be difficult to diagnose because conventional methods of diagnosis depend on the presence of neutrophils. Bacterial pneumonia in neutropenic patients may present without purulent sputum—or, in fact, without any sputum at all—and may not produce physical findings suggestive of chest consolidation (rales or egophony).

In granulocytopenic patients with persistent or recurrent fever, the chest x-ray pattern may help to localize an infection and thus to determine which investigative tests and procedures should be undertaken and which therapeutic options should be considered (Table 74-7). In this setting, a simple chest x-ray is a screening tool; because the impaired host response results in less evidence of consolidation or infiltration, high-resolution CT is recommended for the diagnosis of pulmonary infections. The difficulties encountered in the management of pulmonary infiltrates relate in part to the difficulties of performing diagnostic

TABLE 74-6 Differential Diagnosis of Central Nervous System Infections in Patients with Cancer

FINDINGS ON CT OR MRI	UNDERLYING PREDISPOSITION	
	PROLONGED NEUTROPIENIA	DEFECTS IN CELLULAR IMMUNITY ^a
Mass lesions	<i>Aspergillus</i> , <i>Nocardia</i> , or <i>Cryptococcus</i> brain abscess	Toxoplasmosis, Epstein-Barr virus lymphoma (rare)
Diffuse encephalitis	Progressive multifocal leukoencephalopathy (JC virus)	Infection with varicella-zoster virus, cytomegalovirus, herpes simplex virus, human herpesvirus type 6, JC virus, <i>Listeria</i>

^aHigh-dose glucocorticoid therapy, cytotoxic chemotherapy.

TABLE 74-7 Differential Diagnosis of Chest Infiltrates in Immunocompromised Patients

INFILTRATE	CAUSE OF PNEUMONIA	
	INFECTIOUS	NONINFECTIOUS
Localized	Bacteria (including <i>Legionella</i> , mycobacteria)	Local hemorrhage or embolism, tumor
Nodular	Fungi (e.g., <i>Aspergillus</i> or <i>Mucor</i> , <i>Nocardia</i>)	Recurrent tumor
Diffuse	Viruses (especially cytomegalovirus), <i>Chlamydia</i> , <i>Pneumocystis</i> , <i>Toxoplasma gondii</i> , mycobacteria	Congestive heart failure, radiation pneumonitis, drug-induced lung injury, lymphangitic spread of cancer

procedures on the patients involved. When platelet counts can be increased to adequate levels by transfusion, microscopic and microbiologic evaluation of the fluid obtained by endoscopic bronchial lavage is often diagnostic. Lavage fluid should be cultured for *Mycoplasma*, *Chlamydia*, *Legionella*, *Nocardia*, more common bacterial pathogens, fungi, and viruses. In addition, the possibility of *Pneumocystis* pneumonia should be considered, especially in patients with ALL or lymphoma who have not received prophylactic trimethoprim-sulfamethoxazole (TMP-SMX). The characteristics of the infiltrate may be helpful in decisions about further diagnostic and therapeutic maneuvers. Nodular infiltrates suggest fungal pneumonia (e.g., that caused by *Aspergillus* or *Mucor*). Such lesions may best be approached by visualized biopsy procedures. It is worth noting that while bacterial pneumonias classically present as lobar infiltrates in normal hosts, bacterial pneumonias in granulocytopenic hosts present with a paucity of signs, symptoms, or radiographic abnormalities; thus, the diagnosis is difficult.

Aspergillus species (Chap. 217) can colonize the skin and respiratory tract or cause fatal systemic illness. Although this fungus may cause aspergillomas in a previously existing cavity or may produce allergic bronchopulmonary disease in some patients, the major problem posed by this genus in neutropenic patients is invasive disease, primarily due to *Aspergillus fumigatus* or *Aspergillus flavus*. The organisms enter the host following colonization of the respiratory tract, with subsequent invasion of blood vessels. The disease is likely to present as a thrombotic or embolic event because of this ability of the fungi to invade blood vessels. The risk of infection with *Aspergillus* correlates directly with the duration of neutropenia. In prolonged neutropenia, positive surveillance cultures for nasopharyngeal colonization with *Aspergillus* may predict the development of disease.

Patients with *Aspergillus* infection often present with pleuritic chest pain and fever, which are sometimes accompanied by cough. Hemoptysis may be an ominous sign. Chest x-rays may reveal new focal infiltrates or nodules. Chest CT may reveal a characteristic halo consisting of a mass-like infiltrate surrounded by an area of low attenuation. The presence of a "crescent sign" on chest x-ray or chest CT, in which the mass progresses to central cavitation, is characteristic of invasive *Aspergillus* infection but may develop as the lesions are resolving.

In addition to causing pulmonary disease, *Aspergillus* may invade through the nose or palate, with deep sinus penetration. The appearance of a discolored area in the nasal passages or on the hard palate should prompt a search for invasive *Aspergillus*. This situation is likely to require surgical debridement. Catheter infections with *Aspergillus* usually require both removal of the catheter and antifungal therapy. Antifungal prophylaxis has led to the emergence of non-fumigatus *Aspergillus* species as well as *Mucorales* and *Scedosporium/Lomentospora* spp. (Chaps. 217–219).

Diffuse interstitial infiltrates suggest viral, parasitic, or *Pneumocystis* pneumonia. If the patient has a diffuse interstitial pattern on chest x-ray, it may be reasonable, while considering invasive diagnostic procedures, to institute empirical treatment for *Pneumocystis* with TMP-SMX and for *Chlamydia*, *Mycoplasma*, and *Legionella* with a quinolone or azithromycin. Noninvasive procedures, such as staining of induced sputum smears for *Pneumocystis*, serum cryptococcal antigen tests, and urine testing for *Legionella* antigen, may be helpful. Serum galactomannan and β -D-glucan tests may be of value in diagnosing *Aspergillus*

infection, but their utility is limited by their lack of sensitivity and specificity. The presence of an elevated level of β -D-glucan in the serum of a patient being treated for cancer who is not receiving prophylaxis against *Pneumocystis* suggests the diagnosis of *Pneumocystis* pneumonia. Infections with viruses that cause only upper respiratory symptoms in immunocompetent hosts, such as respiratory syncytial virus (RSV), influenza viruses, and parainfluenza viruses, may be associated with fatal pneumonitis in immunocompromised hosts. CMV reactivation occurs in cancer patients receiving chemotherapy, but CMV pneumonia is most common among hematopoietic stem cell transplant (HSCT) recipients (Chap. 143). Polymerase chain reaction testing now allows rapid diagnosis of viral pneumonia, which can lead to treatment in some cases (e.g., influenza). Multiplex studies that can detect a wide array of viruses in the lung and upper respiratory tract are now available and will lead to specific diagnoses of viral pneumonias.

Bleomycin is the most common cause of chemotherapy-induced lung disease. Other causes include alkylating agents (such as cyclophosphamide, chlorambucil, and melphalan), nitrosoureas (carmustine [BCNU], lomustine [CCNU], and methyl-CCNU), busulfan, procarbazine, methotrexate, and hydroxyurea. Both infectious and noninfectious (drug- and/or radiation-induced) pneumonitis can cause fever and abnormalities on chest x-ray; thus, the differential diagnosis of an infiltrate in a patient receiving chemotherapy encompasses a broad range of conditions (Table 74-7). The treatment of radiation pneumonitis (which may respond dramatically to glucocorticoids) or drug-induced pneumonitis is different from that of infectious pneumonia, and a biopsy may be important in the diagnosis. Unfortunately, no definitive diagnosis can be made in ~30% of cases, even after bronchoscopy.

Open-lung biopsy is the gold standard of diagnostic techniques. Biopsy via a visualized thoracostomy can replace an open procedure in many cases. When a biopsy cannot be performed, empirical treatment can be undertaken: a quinolone or an erythromycin derivative (azithromycin) and TMP-SMX are used in the case of diffuse infiltrates, and an antifungal agent is administered in the case of nodular infiltrates. The risks should be weighed carefully in these cases. If inappropriate drugs are administered, empirical treatment may prove toxic or ineffective; either of these outcomes may be riskier than biopsy.

■ CARDIOVASCULAR INFECTIONS

Patients with Hodgkin's disease are prone to persistent infections by *Salmonella*, sometimes (and particularly often in elderly patients) affecting a vascular site. The use of IV catheters deliberately lodged in the right atrium is associated with a high incidence of bacterial endocarditis, presumably related to valve damage followed by bacteremia. Nonbacterial thrombotic endocarditis (marantic endocarditis) has been described in association with a variety of malignancies (most often solid tumors) and may follow bone marrow (stem cell) transplantation as well. The presentation of an embolic event with a new cardiac murmur suggests this diagnosis. Blood cultures are negative in this disease of unknown pathogenesis. Infective endocarditis can be a complication of cancer treatment because of the use of IV catheters that lead to bacterial infection. In addition, patients may present with infective endocarditis as an initial presentation of cancer, particularly in the case of gastrointestinal or genitourinary sources.

■ ENDOCRINE SYNDROMES

Infections of the endocrine system have been described in immunocompromised patients. *Candida* infection of the thyroid may be difficult to diagnose during the neutropenic period. It can be defined by indium-labeled WBC scans or gallium scans after neutrophil counts increase. CMV infection can cause adrenalitis with or without resulting adrenal insufficiency. The presentation of a sudden endocrine anomaly in an immunocompromised patient can be a sign of infection in the involved end organ.

■ MUSCULOSKELETAL INFECTIONS

Infection that is a consequence of vascular compromise, resulting in gangrene, can occur when a tumor restricts the blood supply to

muscles, bones, or joints. The process of diagnosis and treatment of such infection is similar to that in normal hosts, with the following caveats:

1. In terms of diagnosis, a lack of physical findings resulting from a lack of granulocytes in the granulocytopenic patient should make the clinician more aggressive in obtaining tissue rather than more willing to rely on physical signs.
2. In terms of therapy, aggressive debridement of infected tissues may be required. However, it is usually difficult to operate on patients who have recently received chemotherapy, both because of a lack of platelets (which results in bleeding complications) and because of a lack of WBCs (which may lead to secondary infection). A blood culture positive for *Clostridium perfringens*—an organism commonly associated with gas gangrene—can have a number of meanings (Chap. 154). *Clostridium septicum* bacteremia is associated with the presence of an underlying malignancy. Bloodstream infections with intestinal organisms such as *Streptococcus bovis* biotype 1 and *C. perfringens* may arise spontaneously from lower gastrointestinal lesions (tumor or polyps); alternatively, these lesions may be harbingers of invasive disease. The clinical setting must be considered in order to define the appropriate treatment for each case.

■ RENAL AND URETERAL INFECTIONS

Infections of the urinary tract are common among patients whose ureteral excretion is compromised (Table 74-1). *Candida*, which has a predilection for the kidney, can invade either from the bloodstream or in a retrograde manner (via the ureters or bladder) in immunocompromised patients. The presence of “fungus balls” or persistent candiduria suggests invasive disease. Persistent funguria (with *Aspergillus* as well as *Candida*) should prompt a search for a nidus of infection in the kidney.

Certain viruses are typically seen only in immunosuppressed patients. BK virus (polyomavirus hominis 1) has been documented in the urine of bone marrow transplant recipients and, like adenovirus, may be associated with hemorrhagic cystitis.

ABNORMALITIES THAT PREDISPOSE TO INFECTION

(Table 74-1)

■ THE LYMPHOID SYSTEM

It is beyond the scope of this chapter to detail how all the immunologic abnormalities that result from cancer or from chemotherapy for cancer lead to infections. Disorders of the immune system are discussed in other sections of this book. As has been noted, patients with antibody deficiency are predisposed to overwhelming infection with encapsulated bacteria (including *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*). Infections that result from the lack of a functional cellular immune system are described in Chap. 202. It is worth mentioning, however, that patients undergoing intensive chemotherapy for any form of cancer will have not only defects due to granulocytopenia but also lymphocyte dysfunction, which may be profound. Thus, these patients—especially those receiving glucocorticoid-containing regimens or drugs that inhibit either T-cell activation (calcineurin inhibitors or drugs like fludarabine, which affect lymphocyte function) or cytokine induction—should be given prophylaxis for *Pneumocystis* pneumonia.

Patients receiving treatment that eliminates B cells (e.g., with anti-CD20 antibodies or rituximab) are especially vulnerable to intercurrent viral infections. The incidence of progressive multifocal leukoencephalopathy (caused by JC virus) is elevated among these patients.

■ THE HEMATOPOIETIC SYSTEM

Initial studies in the 1960s revealed a dramatic increase in the incidence of infections (fatal and nonfatal) among cancer patients with a granulocyte count of $<500/\mu\text{L}$. The use of prophylactic antibacterial agents has reduced the number of bacterial infections, but 35–78% of febrile neutropenic patients being treated for hematologic malignancies develop infections at some time during chemotherapy. Aerobic pathogens (both

gram-positive and gram-negative) predominate in all series, but the exact organisms isolated vary from center to center. Infections with anaerobic organisms are uncommon. Geographic patterns affect the types of fungi isolated. Tuberculosis and malaria are common causes of fever in the developing world and may present in this setting as well.

Neutropenic patients are unusually susceptible to infection with a wide variety of bacteria; thus, antibiotic therapy should be initiated promptly to cover likely pathogens if infection is suspected. Indeed, early initiation of antibacterial agents is mandatory to prevent deaths. Like most immunocompromised patients, neutropenic patients are threatened by their own microbial flora, including gram-positive and gram-negative organisms found commonly on the skin and mucous membranes and in the bowel (Table 74-4). Because treatment with narrow-spectrum agents leads to infection with organisms not covered by the antibiotics used, the initial regimen should target all pathogens likely to be the initial causes of bacterial infection in neutropenic hosts. Studies performed in the 1970s suggested that administration of antimicrobial agents should be continued until neutropenia resolves—that is, the granulocyte count is sustained above $500/\mu\text{L}$ for at least 2 days. Recent studies have indicated that it is reasonable to stop antibiotics in patients who are afebrile and stable after 72 hours of treatment (Fig. 74-2). Fever may not resolve prior to granulocyte recovery. In some cases, patients remain febrile after resolution of neutropenia. In these instances, the risk of sudden death from overwhelming bacteremia is greatly reduced, and the following diagnoses should be seriously considered: (1) fungal infection, (2) bacterial abscesses or undrained foci of infection, and (3) drug fever (including reactions to antimicrobial agents as well as to chemotherapy or cytokines). In the proper setting, viral infection or graft-versus-host disease should be considered. In clinical practice, antibacterial therapy is usually discontinued when the patient is no longer neutropenic and all evidence of bacterial disease has been eliminated. Antifungal agents are then discontinued if there is no evidence of fungal disease. If the patient remains febrile, a search for viral diseases or unusual pathogens is conducted while unnecessary cytokines and other drugs are systematically eliminated from the regimen.

TREATMENT

Infections in Cancer Patients

ANTIBACTERIAL THERAPY

Hundreds of antibacterial regimens have been tested for use in patients with cancer. The major risk of infection is related to the degree of neutropenia seen as a consequence of either the disease or the therapy. Many of the relevant studies have involved small populations in which the outcomes have generally been good, and most have lacked the statistical power to detect differences among the regimens studied. Each febrile neutropenic patient should be approached as a unique problem, with particular attention given to previous infections and recent antibiotic exposures. Several general guidelines are useful in the initial treatment of neutropenic patients with fever (Fig. 74-2):

1. In the initial regimen, it is necessary to use antibiotics active against both gram-negative and gram-positive bacteria (Table 74-4).
2. Monotherapy with an aminoglycoside or an antibiotic lacking good activity against gram-positive organisms (e.g., ciprofloxacin or aztreonam) is not adequate in this setting.
3. The agents used should reflect both the epidemiology and the antibiotic resistance pattern of the hospital.
4. If the pattern of resistance justifies its use, a single third-generation cephalosporin constitutes an appropriate initial regimen in many hospitals.
5. Most standard regimens are designed for patients who have not previously received prophylactic antibiotics. The development of fever in a patient who has received antibiotics affects the choice of subsequent therapy, which should target resistant organisms and organisms known to cause infections in patients being treated with the antibiotics already administered.

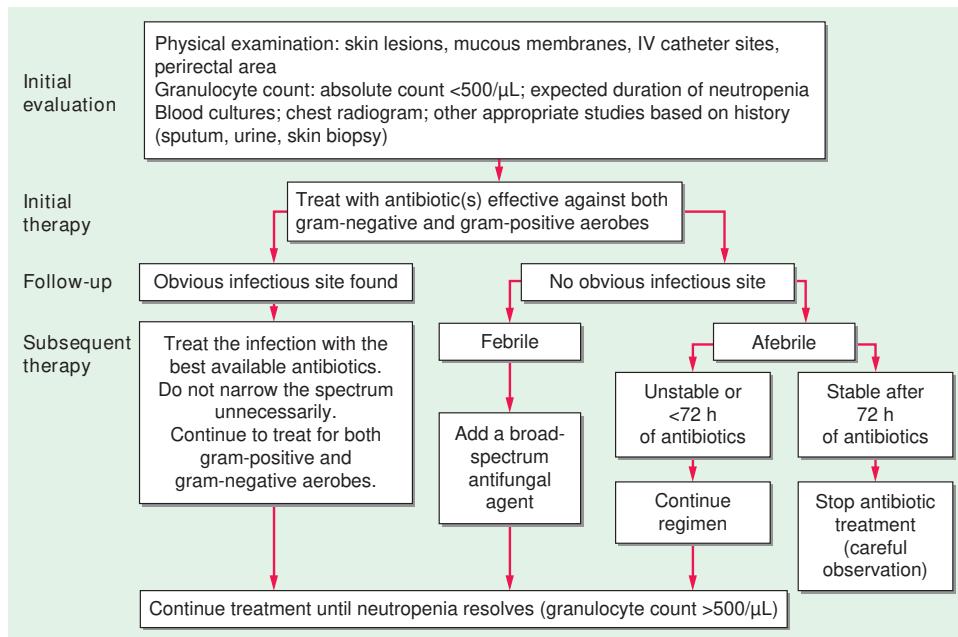


FIGURE 74-2 Algorithm for the diagnosis and treatment of fever and neutropenia.

- Randomized trials have indicated the safety of oral antibiotic regimens in the treatment of “low-risk” patients with fever and neutropenia. Outpatients who are expected to remain neutropenic for <10 days and who do not have concurrent medical problems (such as hypotension, pulmonary compromise, or abdominal pain) can be classified as low risk and treated with a broad-spectrum oral regimen.
- Several large-scale studies indicate that prophylaxis with a fluoroquinolone (ciprofloxacin or levofloxacin) decreases morbidity and mortality rates among afebrile patients who are anticipated to have neutropenia of long duration.

Commonly used antibiotic regimens for the treatment of febrile patients in whom prolonged neutropenia (>7 days) is anticipated include (1) ceftazidime or cefepime, (2) piperacillin/tazobactam, or (3) imipenem/cilastatin or meropenem. All three regimens have shown equal efficacy in large trials. All three are active against *P. aeruginosa* and a broad spectrum of aerobic gram-positive and gram-negative organisms. Imipenem/cilastatin has been associated with an elevated rate of *C. difficile* diarrhea, and many centers reserve carbapenem antibiotics for treatment of gram-negative bacteria that produce extended-spectrum β -lactamases; these limitations make carbapenems less attractive as an initial regimen. Despite the frequent involvement of coagulase-negative staphylococci, the initial use of vancomycin or its automatic addition to the initial regimen has not resulted in improved outcomes, and the antibiotic does exert toxic effects. For these reasons, only judicious use of vancomycin is recommended—for example, when there is good reason to suspect the involvement of coagulase-negative staphylococci (e.g., the appearance of erythema at the exit site of a catheter or a positive culture for methicillin-resistant *S. aureus* or coagulase-negative staphylococci). Because the sensitivities of bacteria vary from hospital to hospital, clinicians are advised to check their local sensitivities and to be aware that resistance patterns can change quickly, necessitating a change in the approach to patients with fever and neutropenia. Similarly, infection control services should monitor for basic antibiotic resistance and for fungal infections. The appearance of a large number of *Aspergillus* infections, in particular, suggests the possibility of an environmental source that requires further investigation and remediation.

The initial antibacterial regimen should be refined on the basis of culture results (Fig. 74-2). Blood cultures are the most relevant basis for selection of therapy; surface cultures of skin and mucous membranes may be misleading. In the case of gram-positive bacteraemia or another gram-positive infection, it is important that the antibiotic be optimal for the organism isolated. Once treatment with broad-spectrum antibiotics has begun, it is not desirable to discontinue all antibiotics because of the risk of failing to treat a potentially fatal bacterial infection; the addition of more and more antibacterial agents to the regimen is not appropriate unless there is a clinical or microbiologic reason to do so. Planned progressive therapy (the serial, empirical addition of one drug after another without culture data) is not efficacious in most settings and may have unfortunate consequences. Simply adding another antibiotic for fear that a gram-negative infection is present is a dubious practice. The synergy exhibited by β -lactams and aminoglycosides against certain gram-negative organisms (especially *P. aeruginosa*) provides the rationale for using two antibiotics in this setting, but recent analyses suggest that efficacy is not enhanced by the addition of aminoglycosides, while toxicity may be increased. Mere “double coverage,” with the addition of a quinolone or another antibiotic that is not likely to exhibit synergy, has not been shown to be beneficial and may cause additional toxicities and side effects. Cephalosporins can cause bone marrow suppression, and vancomycin is associated with neutropenia in some healthy individuals. Furthermore, the addition of multiple cephalosporins may induce β -lactamase production by some organisms; cephalosporins and double β -lactam combinations should probably be avoided altogether in *Enterobacter* infections.

ANTIFUNGAL THERAPY

Fungal infections in cancer patients are most often associated with neutropenia. Neutropenic patients are predisposed to the development of invasive fungal infections, most commonly those due to *Candida* and *Aspergillus* species and occasionally those caused by *Mucor*, *Rhizopus*, *Fusarium*, *Trichosporon*, *Bipolaris*, and others. Invasive candidal disease is usually caused by *C. albicans* or *C. tropicalis* but can be caused by *C. krusei*, *C. parapsilosis*, and *C. glabrata*. The worldwide spread of *C. auris*, a species that is typically resistant to fluconazole and often resistant to amphotericin B as

well, has further complicated the management of invasive *Candida* infections ([Chap. 216](#)).

For decades, it has been common clinical practice to add amphotericin B to antibacterial regimens if a neutropenic patient remains febrile despite 4–7 days of treatment with antibacterial agents. The rationale for this empirical addition is that it is difficult to culture fungi before they cause disseminated disease and that mortality rates from disseminated fungal infections in granulocytopenic patients are high. Before the introduction of newer azoles into clinical practice, amphotericin B was the mainstay of antifungal therapy. The insolubility of amphotericin B has resulted in the marketing of several lipid formulations that are less toxic than the amphotericin B deoxycholate complex. Echinocandins (e.g., caspofungin) are useful in the treatment of infections caused by azole-resistant *Candida* strains as well as in therapy for aspergillosis and have been shown to be equivalent to liposomal amphotericin B for the empirical treatment of patients with prolonged fever and neutropenia. Newer azoles have also been demonstrated to be effective in this setting. Although fluconazole is efficacious in the treatment of infections due to many *Candida* species, its use against serious fungal infections in immunocompromised patients is limited by its narrow spectrum: it has no activity against *Aspergillus* or against several non-albicans *Candida* species. The broad-spectrum azoles (e.g., voriconazole and posaconazole) provide another option for the treatment of *Aspergillus* infections ([Chap. 217](#)), including CNS infection. Clinicians should be aware that the spectrum of each azole is somewhat different and that no drug can be assumed to be efficacious against all fungi. *Aspergillus terreus* is resistant to amphotericin B. Although voriconazole is active against *Pseudallescheria boydii*, amphotericin B is not; however, voriconazole has no activity against *Mucor*. Posaconazole, which is administered orally, is useful as a prophylactic agent in patients with prolonged neutropenia. Studies in progress are assessing the use of these agents in combinations. For a full discussion of antifungal therapy, see [Chap. 211](#).

ANTIVIRAL THERAPY

The availability of a variety of agents active against herpes-group viruses, including some new agents with a broader spectrum of activity, has heightened focus on the treatment of viral infections, which pose a major problem in cancer patients. Viral diseases caused by the herpes group are prominent. Serious (and sometimes fatal) infections due to HSV and VZV are well documented in patients receiving chemotherapy. CMV may also cause serious disease, but fatalities from CMV infection are more common in hematopoietic stem cell transplant recipients. The roles of human herpesvirus (HHV)-6, HHV-7, and HHV-8 (Kaposi's sarcoma-associated herpesvirus) in cancer patients are still being defined ([Chap. 195](#)). EBV lymphoproliferative disease (LPD) can occur in patients receiving chemotherapy but is much more common among transplant recipients ([Chap. 143](#)). While clinical experience is most extensive with acyclovir, which can be used therapeutically or prophylactically, a number of derivative drugs offer advantages over this agent ([Chap. 191](#)).

In addition to the herpes group, several respiratory viruses (especially RSV) may cause serious disease in cancer patients. Although influenza vaccination is recommended (see below), it may be ineffective in this patient population. The availability of antiviral drugs with activity against influenza viruses gives the clinician additional options for the prophylaxis and treatment of these patients ([Chaps. 191 and 200](#)).

The COVID-19 pandemic has affected cancer patients disproportionately. Early analyses suggest that lung cancer patients in particular are more vulnerable to serious infection with SARS-CoV-2.

OTHER THERAPEUTIC MODALITIES

A variety of cytokines, including granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor, enhance granulocyte recovery after chemotherapy and consequently shorten the period of maximal vulnerability to fatal infections. Most authorities recommend their use only when neutropenia is both severe and prolonged, and they should be used only in the

appropriate setting (i.e., when stem cells are likely to be responsive) and not as an adjunct to antimicrobial agents. The cytokines themselves may have adverse effects, including fever, hypoxemia, and pleural effusions or serositis in other areas ([Chap. 349](#)).

Once neutropenia has resolved, the risk of infection decreases dramatically. However, depending on what drugs they receive, patients who continue on therapeutic protocols remain at high risk for certain diseases. Any patient receiving more than a maintenance dose of glucocorticoids (e.g., in many treatment regimens for diffuse lymphoma) should also receive prophylactic TMP-SMX because of the risk of *Pneumocystis* infection; those with ALL should receive such prophylaxis for the duration of chemotherapy.

PREVENTION OF INFECTION IN CANCER PATIENTS

EFFECT OF THE ENVIRONMENT

Outbreaks of fatal *Aspergillus* infection have been associated with construction projects and materials in several hospitals. The association between spore counts and risk of infection suggests the need for a high-efficiency air-handling system in hospitals that care for large numbers of neutropenic patients. The use of laminar-flow rooms and prophylactic antibiotics has decreased the number of infectious episodes in severely neutropenic patients. However, because of the expense of such a program and the failure to show that it dramatically affects mortality rates, most centers do not routinely use laminar flow to care for neutropenic patients. Some centers use “reverse isolation,” in which health care providers and visitors to a patient who is neutropenic wear gowns and gloves. Since most of the infections these patients develop are due to organisms that colonize the patients’ own skin and bowel, the validity of such schemes is dubious, and limited clinical data do not support their use. Hand washing by all staff caring for neutropenic patients should be required to prevent the spread of resistant organisms.

The presence of large numbers of bacteria (particularly *P. aeruginosa*) in certain foods, especially fresh vegetables, has led some authorities to recommend a special “low-bacteria” diet. A diet consisting of cooked and canned food is satisfactory to most neutropenic patients and does not involve elaborate disinfection or sterilization protocols. However, there are no studies to support even this type of dietary restriction. Counseling of patients to avoid leftovers, deli foods, undercooked meat, and unpasteurized dairy products is recommended since these foods have been associated with outbreaks of listerial infection.

PHYSICAL MEASURES

Although few studies address this issue, patients with cancer are predisposed to infections resulting from anatomic compromise (e.g., lymphedema resulting from node dissections after radical mastectomy). Surgeons who specialize in cancer surgery can provide specific guidelines for the care of such patients, and patients benefit from common-sense advice about how to prevent infections in vulnerable areas.

IMMUNOGLOBULIN REPLACEMENT

Many patients with multiple myeloma or CLL have immunoglobulin deficiencies as a result of their disease, and all allogeneic bone marrow transplant recipients are hypogammaglobulinemic for a period after transplantation. However, current recommendations reserve intravenous immunoglobulin replacement therapy for patients with severe, prolonged hypogammaglobulinemia (<400 mg of total IgG/dL) and a history of repeated infections. Antibiotic prophylaxis has been shown to be cheaper and is efficacious in preventing infections in most CLL patients with hypogammaglobulinemia. Routine use of immunoglobulin replacement is not recommended.

SEXUAL PRACTICES

The use of condoms is recommended for severely immunocompromised patients. Any sexual practice that results in oral exposure to feces is not recommended. Neutropenic patients should be advised to avoid any practice that results in trauma, as even microscopic cuts may result in bacterial invasion and fatal sepsis.



■ ANTIBIOTIC PROPHYLAXIS

Several studies indicate that the use of oral fluoroquinolones prevents infection and decreases mortality rates among severely neutropenic patients. Prophylaxis for *Pneumocystis* is mandatory for patients with ALL and for all cancer patients receiving glucocorticoid-containing chemotherapy regimens.

■ VACCINATION OF CANCER PATIENTS

In general, patients undergoing chemotherapy respond less well to vaccines than do normal hosts. Their greater need for vaccines thus leads to a dilemma in their management. Purified proteins and inactivated vaccines are almost never contraindicated and should be given to patients even during chemotherapy. For example, all adults should receive diphtheria–tetanus toxoid boosters at the indicated times as well as seasonal influenza vaccine. However, if possible, vaccination should not be undertaken concurrent with cytotoxic chemotherapy. If patients are expected to be receiving chemotherapy for several months and vaccination is indicated (e.g., influenza vaccination in the fall), the vaccine should be given midcycle—as far apart in time as possible from the antimetabolic agents that will prevent an immune response. The meningococcal and pneumococcal polysaccharide vaccines should be given to patients before splenectomy, if possible. The *H. influenzae* type b conjugate vaccine should be administered to all splenectomized patients.

In general, live virus (or live bacterial) vaccines should not be given to patients during intensive chemotherapy because of the risk of disseminated infection. Recommendations on vaccination are summarized in Table 74-2 (see <https://www.cdc.gov/vaccines/hcp/index.html> for updated recommendations).

■ IN MEMORIAM

Dr. Robert W. Finberg, Richard M. Haidack Distinguished Professor and Chair of Medicine, University of Massachusetts Chan Medical School (2000–2020), Professor of Medicine and Chair of Infectious Diseases, Dana Farber Cancer Institute (1996–1999), passed away on August 30, 2021. In addition to this chapter, he authored Chapter 143, “Infections in Transplant Recipients.” Dr. Finberg was an internationally renowned physician-scientist and an academic leader whose career spanned four decades. A brilliant talented researcher focused on viral pathogenesis he was also a consummate clinician who attended at the bedside throughout his career. Dr. Finberg played an important role in the COVID-19 pandemic by leading clinical trials for SARS-CoV-2 vaccines and therapeutics. Warm and generous with a keen wit, he was a beloved family man, colleague and friend. As an educator and mentor he truly cared about training the next generation, as evidenced by the legacy of a very large number of trainees he leaves behind. We are indebted to Dr. Finberg for his outstanding contributions to nine editions of Harrison’s Principles of Internal Medicine and to his considerable and significant contributions to the field of human health.

■ FURTHER READING

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- F DZP, S IS: Emerging fungal infections: New patients, new patterns, and new pathogens. *J Fungi* 5:67, 2019.
- M G et al: Infections associated with immunotherapeutic and molecular targeted agents in hematology and oncology. A position paper by the European Conference on Infections in Leukemia (ECIL). *Leukemia* 33:844, 2019.
- P PA: Management of patients with fever and neutropenia through the arc of time. *Ann Intern Med* 170:389, 2019.
- Z L et al: Clinical characteristics of COVID-19-infected cancer patients: A retrospective case study in three hospitals within Wuhan, China. *Ann Oncol* 31:894, 2020.

■ WEBSITE

Prevention and Treatment of Cancer-Related Infections; National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology Version 2.2020 (<https://www.nccn.org>).

Emergencies in patients with cancer may be classified into three groups: pressure or obstruction caused by a space-occupying lesion, metabolic or hormonal problems (paraneoplastic syndromes, Chap. 93), and treatment-related complications.

STRUCTURAL OBSTRUCTIVE ONCOLOGIC EMERGENCIES

■ SUPERIOR VENA CAVA SYNDROME

Superior vena cava syndrome (SVCS) is the clinical manifestation of superior vena cava (SVC) obstruction, with severe reduction in venous return from the head, neck, and upper extremities. Malignant tumors, such as lung cancer, lymphoma, and metastatic tumors, are responsible for the majority of SVCS cases. With the expanding use of intravascular devices (e.g., permanent central venous access catheters, pacemaker/defibrillator leads), the prevalence of benign causes of SVCS is now increasing, accounting for at least 40% of cases. Lung cancer, particularly of small-cell and squamous cell histologies, accounts for ~85% of all cases of malignant origin. In young adults, malignant lymphoma is a leading cause of SVCS. Hodgkin’s lymphoma involves the mediastinum more commonly than other lymphomas but rarely causes SVCS. When SVCS is noted in a young man with a mediastinal mass, the differential diagnosis is lymphoma versus primary mediastinal germ cell tumor. Metastatic cancers to the mediastinal lymph nodes, such as testicular and breast carcinomas, account for a small proportion of cases. Other causes include benign tumors, aortic aneurysm, thyromegaly, thrombosis, and fibrosing mediastinitis from prior irradiation, histoplasmosis, or Behcet’s syndrome. SVCS as the initial manifestation of Behcet’s syndrome may be due to inflammation of the SVC associated with thrombosis.

Patients with SVCS usually present with neck and facial swelling (especially around the eyes), dyspnea, and cough. Other symptoms include hoarseness, tongue swelling, headaches, nasal congestion, epistaxis, hemoptysis, dysphagia, pain, dizziness, syncope, and lethargy. Bending forward or lying down may aggravate the symptoms. The characteristic physical findings are dilated neck veins; an increased number of collateral veins covering the anterior chest wall; cyanosis; and edema of the face, arms, and chest. Facial swelling and plethora are typically exacerbated when the patient is supine. More severe cases include proptosis, glossal and laryngeal edema, and obtundation. The clinical picture is milder if the obstruction is located above the azygos vein. Symptoms are usually progressive, but in some cases, they may improve as collateral circulation develops.

Signs and symptoms of cerebral and/or laryngeal edema, though rare, are associated with a poorer prognosis and require urgent evaluation. Seizures are more likely related to brain metastases than to cerebral edema from venous occlusion. Patients with small-cell lung cancer and SVCS have a higher incidence of brain metastases than those without SVCS.

Cardiorespiratory symptoms at rest, particularly with positional changes, suggest significant airway and vascular obstruction and limited physiologic reserve. Cardiac arrest or respiratory failure can occur, particularly in patients receiving sedatives or undergoing general anesthesia.

Rarely, esophageal varices may develop, particularly in the setting of SVC syndrome due to hemodialysis catheter. These are “downhill” varices based on the direction of blood flow from cephalad to caudad (in contrast to “uphill” varices associated with caudad to cephalad flow from portal hypertension). If the obstruction to the SVC is proximal to the azygous vein, varices develop in the upper one-third of the esophagus. If the obstruction involves or is distal to the azygous vein, varices occur in the entire length of the esophagus. Variceal bleeding may be a late complication of chronic SVCS.

SVC obstruction may lead to bilateral breast edema with bilateral enlarged breasts. Unilateral breast dilation may be seen as a consequence of axillary or subclavian vein blockage.

The diagnosis of SVCS is a clinical one. The most significant chest radiographic finding is widening of the superior mediastinum, most commonly on the right side. Pleural effusion occurs in only 25% of patients, often on the right side. The majority of these effusions are exudative and occasionally chylous. However, a normal chest radiograph is still compatible with the diagnosis if other characteristic findings are present. Computed tomography (CT) provides the most reliable view of the mediastinal anatomy. The diagnosis of SVCS requires diminished or absent opacification of central venous structures with prominent collateral venous circulation. Magnetic resonance imaging (MRI) is increasingly being used to diagnose SVC obstruction with a 100% sensitivity and specificity, but dyspneic SVCS patients may have difficulty remaining supine for the entire imaging process. Invasive procedures, including bronchoscopy, percutaneous needle biopsy, mediastinoscopy, and even thoracotomy, can be performed by a skilled clinician without any major risk of bleeding. Endobronchial or esophageal ultrasound-guided needle aspiration may establish the diagnosis safely. For patients with a known cancer, a detailed workup usually is not necessary, and appropriate treatment may be started after obtaining a CT scan of the thorax. For those with no history of malignancy, a detailed evaluation is essential to rule out benign causes and determine a specific diagnosis to direct the appropriate therapy.

TREATMENT

Superior Vena Cava Syndrome

The one potentially life-threatening complication of a superior mediastinal mass is tracheal obstruction. Upper airway obstruction demands emergent therapy. Diuretics with a low-salt diet, head elevation, and oxygen may produce temporary symptomatic relief. Glucocorticoids have a limited role except in the setting of mediastinal lymphoma masses.

Radiation therapy is the primary treatment for SVCS caused by non-small-cell lung cancer and other metastatic solid tumors. Chemotherapy is effective when the underlying cancer is small-cell carcinoma of the lung, lymphoma, or germ cell tumor. SVCS recurs in 10–30% of patients; it may be palliated with the use of intravascular self-expanding stents (Fig. 75-1). Endovascular therapy is more frequently used first, to provide rapid relief of clinical symptoms with reduced complications. Early stenting may be necessary in patients with severe symptoms; however, the prompt increase in venous return after stenting may precipitate heart failure and pulmonary edema. Other complications of stent placement include hematoma at the insertion site, SVC perforation, stent migration in the right ventricle, stent fracture, and pulmonary embolism.

Clinical improvement occurs in most patients, although this improvement may be due to the development of adequate collateral circulation. The mortality associated with SVCS does not relate to caval obstruction but rather to the underlying cause.

SVCS AND CENTRAL VENOUS CATHETERS IN ADULTS

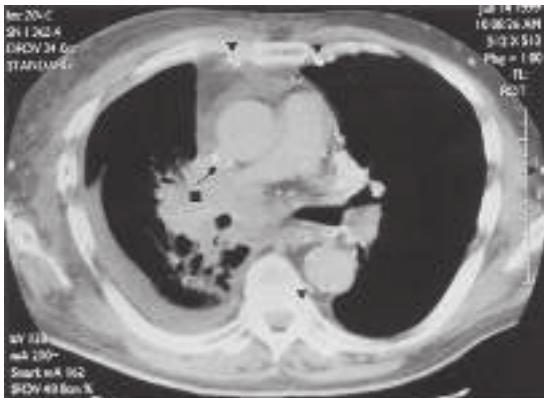
The use of long-term central venous catheters has become common practice in patients with cancer. Major vessel thrombosis may occur. In these cases, catheter removal should be combined with anticoagulation to prevent embolization. SVCS in this setting, if detected early, can be treated by fibrinolytic therapy without sacrificing the catheter. When managing patients with transvenous lead-related SVC syndrome, anticoagulation, local and systemic thrombolytic therapy, and surgical intervention can be effective therapy in select patients. Endovascular stenting has also been shown to be safe and promising, with minimal procedural or clinical complications. The role of anticoagulation after SVC stent placement is controversial.

PERICARDIAL EFFUSION/TAMPOONADE

Malignant pericardial disease is found at autopsy in 5–10% of patients with cancer, most frequently with lung cancer, breast cancer, leukemias,



A



B



C

FIGURE 75-1 Superior vena cava syndrome (SVCS). **A.** Chest radiographs of a 59-year-old man with recurrent SVCS caused by non-small-cell lung cancer showing right paratracheal mass with right pleural effusion. **B.** Computed tomography of same patient demonstrating obstruction of the superior vena cava with thrombosis (arrow) by the lung cancer (square) and collaterals (arrowheads). **C.** Balloon angioplasty (arrowhead) with Wallstent (arrow) in same patient.

and lymphomas. Cardiac tamponade as the initial presentation of extrathoracic malignancy is rare. The origin is not malignancy in ~50% of cancer patients with symptomatic pericardial disease, but it can be related to irradiation; drug-induced pericarditis, including chemotherapeutic agents such as all-trans retinoic acid, arsenic trioxide, imatinib, and other abl kinase inhibitors; hypothyroidism; idiopathic pericarditis; infection; or autoimmune diseases. Pericardial disease has been associated with immune checkpoint inhibitors specifically in patients with advanced non-small-cell lung cancer. Two types of radiation pericarditis occur: an acute inflammatory, effusive pericarditis occurring within months of irradiation, which usually resolves spontaneously, and a chronic effusive pericarditis that may appear up to 20 years after radiation therapy and is accompanied by a thickened pericardium.

Most patients with pericardial metastasis are asymptomatic. However, the common symptoms are dyspnea, cough, chest pain, orthopnea, and weakness. Pleural effusion, sinus tachycardia, jugular venous distention, hepatomegaly, peripheral edema, and cyanosis are the most frequent physical findings. Relatively specific diagnostic findings, such as paradoxical pulse, diminished heart sounds, pulsus alternans (pulse waves alternating between those of greater and lesser amplitude with successive beats), and friction rub are less common than with non-malignant pericardial disease. Chest radiographs and electrocardiogram (ECG) reveal abnormalities in 90% of patients, but half of these abnormalities are nonspecific. Echocardiography is the most helpful diagnostic test. Pericardial fluid may be serous, serosanguineous, or hemorrhagic, and cytologic examination of pericardial fluid is diagnostic in most patients. Measurements of tumor markers in the pericardial fluid are not helpful in the diagnosis of malignant pericardial fluid. Pericardioscopy with targeted pericardial and epicardial biopsy may differentiate neoplastic and benign pericardial disease. A combination of cytology, pericardial and epicardial biopsy, and guided pericardioscopy gives the best diagnostic yield. CT scan of chest may also reveal the presence of a concomitant thoracic neoplasm. Cancer patients with pericardial effusion containing malignant cells on cytology have a very poor survival, ~7 weeks.

TREATMENT

Pericardial Effusion/Tamponade

Pericardiocentesis with or without the introduction of sclerosing agents, the creation of a pericardial window, complete pericardial stripping, cardiac irradiation, or systemic chemotherapy are effective treatments. Acute pericardial tamponade with life-threatening hemodynamic instability requires immediate drainage of fluid. This can be quickly achieved by pericardiocentesis. The recurrence rate after percutaneous catheter drainage is ~20%. Sclerotherapy (pericardial instillation of bleomycin, mitomycin C, or tetracycline) may decrease recurrences. Alternatively, subxiphoid pericardiotomy can be performed in 45 min under local anesthesia. Thoracoscopic pericardial fenestration can be employed for benign causes; however, 60% of malignant pericardial effusions recur after this procedure. In a subset of patients, drainage of the pericardial effusion is paradoxically followed by worsening hemodynamic instability. This so-called “postoperative low cardiac output syndrome” occurs in up to 10% of patients undergoing surgical drainage and carries poor short-term survival.

■ INTESTINAL OBSTRUCTION

Intestinal obstruction and reobstruction are common problems in patients with advanced cancer, particularly colorectal or ovarian carcinoma. However, other cancers, such as lung or breast cancer and melanoma, can metastasize within the abdomen, leading to intestinal obstruction. Metastatic disease from colorectal, ovarian, pancreatic, gastric, and occasionally breast cancer can lead to peritoneal carcinomatosis, with infiltration of the omentum and peritoneal surface, thus limiting bowel motility. Typically, obstruction occurs at multiple sites in peritoneal carcinomatosis. Melanoma has a predilection to involve the small bowel; this involvement may be isolated, and resection may

result in prolonged survival. Intestinal pseudoobstruction is caused by infiltration of the mesentery or bowel muscle by tumor, involvement of the celiac plexus, or paraneoplastic neuropathy in patients with small-cell lung cancer. Paraneoplastic neuropathy is associated with IgG antibodies reactive to neurons of the myenteric and submucosal plexuses of the jejunum and stomach. Ovarian cancer can lead to authentic luminal obstruction or to pseudoobstruction that results when circumferential invasion of a bowel segment arrests the forward progression of peristaltic contractions.

The onset of obstruction is usually insidious. Pain is the most common symptom and is usually colicky in nature. Pain can also be due to abdominal distention, tumor masses, or hepatomegaly. Vomiting can be intermittent or continuous. Patients with complete obstruction usually have constipation. Physical examination may reveal abdominal distention with tympany, ascites, visible peristalsis, high-pitched bowel sounds, and tumor masses. Erect plain abdominal films may reveal multiple air-fluid levels and dilation of the small or large bowel. Acute cecal dilation to >12–14 cm is considered a surgical emergency because of the high likelihood of rupture. CT scan is useful in defining the extent of disease and the exact nature of the obstruction and differentiating benign from malignant causes of obstruction in patients who have undergone surgery for malignancy. Malignant obstruction is suggested by a mass at the site of obstruction or prior surgery, adenopathy, or an abrupt transition zone and irregular bowel thickening at the obstruction site. Benign obstruction is more likely when CT shows mesenteric vascular changes, a large volume of ascites, or a smooth transition zone and smooth bowel thickening at the obstruction site. In challenging patients with obstructive symptoms, particularly low-grade small-bowel obstruction (SBO), CT enteroclysis often can help establish the diagnosis by providing distention of small-bowel loops. In this technique, water-soluble contrast is infused through a nasoenteric tube into the duodenum or proximal small bowel followed by CT images. The prognosis for the patient with cancer who develops intestinal obstruction is poor; median survival is 3–4 months. About 25–30% of patients are found to have intestinal obstruction due to causes other than cancer. Adhesions from previous operations are a common benign cause. Ileus induced by vinca alkaloids, narcotics, or other drugs is another reversible cause.

TREATMENT

Intestinal Obstruction

The management of intestinal obstruction in patients with advanced malignancy depends on the extent of the underlying malignancy, options for further antineoplastic therapy, estimated life expectancy, the functional status of the major organs, and the extent of the obstruction. The initial management should include surgical evaluation. Operation is not always successful and may lead to further complications with a substantial mortality rate (10–20%). Laparoscopy can diagnose and treat malignant bowel obstruction in some cases. Self-expanding metal stents placed in the gastric outlet, duodenum, proximal jejunum, colon, or rectum may palliate obstructive symptoms at those sites without major surgery. Patients known to have advanced intraabdominal malignancy should receive a prolonged course of conservative management, including nasogastric decompression. Percutaneous endoscopic or surgical gastrostomy tube placement is an option for palliation of nausea and vomiting, the so-called “venting gastrostomy.” Treatment with antiemetics, antispasmodics, and analgesics may allow patients to remain outside the hospital. Octreotide may relieve obstructive symptoms through its inhibitory effect on gastrointestinal secretion. Glucocorticoids have anti-inflammatory effects and may help the resolution of bowel obstruction. They also have antiemetic effects.

■ URINARY OBSTRUCTION

Urinary obstruction may occur in patients with prostatic or gynecologic malignancies, particularly cervical carcinoma; metastatic disease

from other primary sites such as carcinomas of the breast, stomach, lung, colon, and pancreas; or lymphomas. Radiation therapy to pelvic tumors may cause fibrosis and subsequent ureteral obstruction. Bladder outlet obstruction is usually due to prostate and cervical cancers and may lead to bilateral hydronephrosis and renal failure.

Flank pain is the most common symptom. Persistent urinary tract infection, persistent proteinuria, or hematuria in patients with cancer should raise suspicion of ureteral obstruction. Total anuria and/or anuria alternating with polyuria may occur. A slow, continuous rise in the serum creatinine level necessitates immediate evaluation. Renal ultrasound is the safest and cheapest way to identify hydronephrosis. The function of an obstructed kidney can be evaluated by a nuclear scan. CT scan can reveal the point of obstruction and identify a retroperitoneal mass or adenopathy.

TREATMENT

Urinary Obstruction

Obstruction associated with flank pain, sepsis, or fistula formation is an indication for immediate palliative urinary diversion. Internal ureteral stents can be placed under local anesthesia. Percutaneous nephrostomy offers an alternative approach for drainage. The placement of a nephrostomy is associated with a significant rate of pyelonephritis. In the case of bladder outlet obstruction due to malignancy, a suprapubic cystostomy can be used for urinary drainage. An aggressive intervention with invasive approaches to improve the obstruction should be weighed against the likelihood of antitumor response, and the ability to reverse renal insufficiency should be evaluated.

MALIGNANT BILIARY OBSTRUCTION

This common clinical problem can be caused by a primary carcinoma arising in the pancreas, ampulla of Vater, bile duct, or liver or by metastatic disease to the periductal lymph nodes or liver parenchyma. The most common metastatic tumors causing biliary obstruction are gastric, colon, breast, and lung cancers. Jaundice, light-colored stools, dark urine, pruritus, and weight loss due to malabsorption are usual symptoms. Pain and secondary infection are uncommon in malignant biliary obstruction. Ultrasound, CT scan, or percutaneous transhepatic or endoscopic retrograde cholangiography will identify the site and nature of the biliary obstruction.

TREATMENT

Malignant Biliary Obstruction

Palliative intervention is indicated only in patients with disabling pruritus resistant to medical treatment, severe malabsorption, or infection. Stenting under radiographic control, surgical bypass, or radiation therapy with or without chemotherapy may alleviate the obstruction. The choice of therapy should be based on the site of obstruction (proximal vs distal), the type of tumor (sensitive to radiotherapy, chemotherapy, or neither), and the general condition of the patient. Stenting under radiographic or endoscopic control, surgical bypass, or radiation therapy with or without chemotherapy may alleviate the obstruction. Photodynamic therapy and radiofrequency ablation are promising endoscopic therapies for malignant biliary obstruction.

Endoscopic ultrasonography-guided biliary drainage is an evolving method of biliary drainage in patients with malignant biliary obstruction, particularly in patients whom standard endoscopic retrograde cholangiopancreatography failed.

SPINAL CORD COMPRESSION

Malignant spinal cord compression (MSCC) is defined as compression of the spinal cord and/or cauda equina by an extradural tumor mass. The minimum radiologic evidence for cord compression is indentation of the theca at the level of clinical features. Spinal cord compression

(SCC) occurs in 5–10% of patients with cancer. Epidural tumor is the first manifestation of malignancy in ~10% of patients. The underlying cancer is usually identified during the initial evaluation; lung cancer is the most common cause of MSCC.

Metastatic tumor involves the vertebral column more often than any other part of the bony skeleton. Lung, breast, and prostate cancers are the most frequent offenders. Multiple myeloma also has a high incidence of spine involvement. Lymphomas, melanoma, renal cell cancer, and genitourinary cancers also cause cord compression. The thoracic spine is the most common site (70%), followed by the lumbosacral spine (20%) and the cervical spine (10%). Involvement of multiple sites is most frequent in patients with breast and prostate carcinoma. Cord injury develops when metastases to the vertebral body or pedicle enlarge and compress the underlying dura. Another cause of cord compression is direct extension of a paravertebral lesion through the intervertebral foramen. These cases usually involve a lymphoma, myeloma, or pediatric neoplasm. Parenchymal spinal cord metastasis due to hematogenous spread is rare. Intramedullary metastases can be seen in lung cancer, breast cancer, renal cancer, melanoma, and lymphoma, and are frequently associated with brain metastases and leptomeningeal disease.

Expanding extradural tumors induce injury through several mechanisms. Expanding extradural tumors induce mechanical injury to axons and myelin. Compression compromises blood flow, leading to ischemia and/or infarction.

The most common initial symptom in patients with SCC is localized back pain and tenderness due to involvement of vertebrae by tumor. Pain is usually present for days or months before other neurologic findings appear. It is exacerbated by movement and by coughing or sneezing. It can be differentiated from the pain of disk disease by the fact that it worsens when the patient is supine. Radicular pain is less common than localized back pain and usually develops later. Radicular pain in the cervical or lumbosacral areas may be unilateral or bilateral. Radicular pain from the thoracic roots is often bilateral and is described by patients as a feeling of tight, band-like constriction around the thorax and abdomen. Typical cervical radicular pain radiates down the arm; in the lumbar region, the radiation is down the legs. *Lhermitte's sign*, a tingling or electric sensation down the back and upper and lower limbs upon flexing or extending the neck, may be an early sign of cord compression. Loss of bowel or bladder control may be the presenting symptom but usually occurs late in the course. Occasionally, patients present with ataxia of gait without motor and sensory involvement due to involvement of the spinocerebellar tract.

On physical examination, pain induced by straight leg raising, neck flexion, or vertebral percussion may help to determine the level of cord compression. Patients develop numbness and paresthesias in the extremities or trunk. Loss of sensibility to pinprick is as common as loss of sensibility to vibration or position. The upper limit of the zone of sensory loss is often one or two vertebrae below the site of compression. Motor findings include weakness, spasticity, and abnormal muscle stretching. An extensor plantar reflex reflects significant compression. Deep tendon reflexes may be brisk. Motor and sensory loss usually precedes sphincter disturbance. Patients with autonomic dysfunction may present with decreased anal tonus, decreased perineal sensibility, and a distended bladder. The absence of the anal wink reflex or the bulbocavernosus reflex confirms cord involvement. In doubtful cases, evaluation of postvoiding urinary residual volume can be helpful. A residual volume of >150 mL suggests bladder dysfunction. Autonomic dysfunction is an unfavorable prognostic factor. Patients with progressive neurologic symptoms should have frequent neurologic examinations and rapid therapeutic intervention. Other illnesses that may mimic cord compression include osteoporotic vertebral collapse, disk disease, pyogenic abscess or vertebral tuberculosis, radiation myelopathy, neoplastic leptomeningitis, benign tumors, epidural hematoma, and spinal lipomatosis.

Cauda equina syndrome is characterized by low back pain; diminished sensation over the buttocks, posterior-superior thighs, and perineal area in a saddle distribution; rectal and bladder dysfunction; sexual impotence; absent bulbocavernous, patellar, and Achilles'

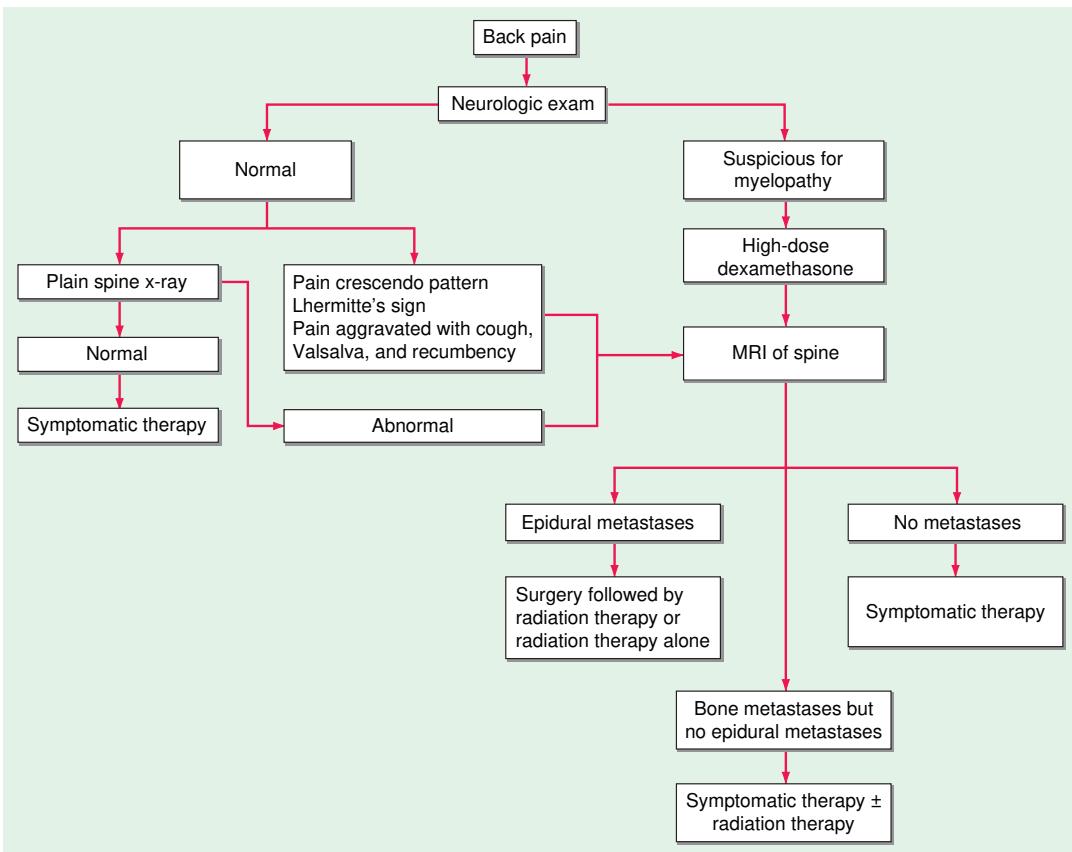


FIGURE 75-2 Management of cancer patients with back pain.

reflexes; and variable amount of lower-extremity weakness. This reflects compression of nerve roots as they form the cauda equina after leaving the spinal cord. The majority of cauda equina tumors are primary tumors of glial or nerve sheath origin; metastases are very rare.

Patients with cancer who develop back pain should be evaluated for SCC as quickly as possible (Fig. 75-2). Treatment is more often successful in patients who are ambulatory and still have sphincter control at the time treatment is initiated. Patients should have a neurologic examination and plain films of the spine. Those whose physical examination suggests cord compression should receive dexamethasone starting immediately and undergo MRI imaging.

Erosion of the pedicles (the “winking owl” sign) is the earliest radiologic finding of vertebral tumor in plain films; however, plain films are insensitive. Other radiographic changes include increased intrapedicular distance, vertebral destruction, lytic or sclerotic lesions, scalloped vertebral bodies, and vertebral body collapse. Vertebral collapse is not a reliable indicator of the presence of tumor; ~20% of cases of vertebral collapse, particularly those in older patients and postmenopausal women, are due not to cancer but to osteoporosis. Also, a normal appearance on plain films of the spine does not exclude the diagnosis of cancer. The role of bone scans in the detection of cord compression is not clear; this method is sensitive but less specific than spinal radiography.

The full-length image of the cord provided by MRI is the imaging procedure of choice. Multiple epidural metastases are noted in 25% of patients with cord compression, and their presence influences treatment plans. On T1-weighted images, good contrast is noted between the cord, cerebrospinal fluid (CSF), and extradural lesions. Owing to its sensitivity in demonstrating the replacement of bone marrow by tumor, MRI can show which parts of a vertebra are involved by tumor. MRI also visualizes intraspinal extradural masses compressing

the cord. T2-weighted images are most useful for the demonstration of intramedullary pathology. Gadolinium-enhanced MRI can help to delineate intramedullary disease. MRI is as good as or better than myelography plus postmyelogram CT scan in detecting metastatic epidural disease with cord compression. Myelography should be reserved for patients who have poor MRIs or who cannot undergo MRI promptly. CT scan in conjunction with myelography enhances the detection of small areas of spinal destruction.

In patients with cord compression and an unknown primary tumor, a simple workup including chest radiography, mammography, measurement of prostate-specific antigen, and abdominal CT usually reveals the underlying malignancy.

TREATMENT

Spinal Cord Compression

The treatment of patients with SCC is aimed at relief of pain and restoration/preservation of neurologic function (Fig. 75-2). Management of MSCC requires a multidisciplinary approach.

Radiation therapy plus glucocorticoids is generally the initial treatment of choice for most patients with SCC. The management decision of SCC involves assessment of neurologic (N), oncologic (O), mechanical (M), and systemic factors (S). NOMS was developed by Memorial Sloan Kettering Cancer Center (MSKCC) researchers to provide an algorithm for management of SCC. The neurologic assessment is based on the degree of epidural SCC, myelopathy, and/or functional radiculopathy. Oncologic assessment involves the radiosensitivity of the tumor type. In patients with radioresistant tumors, stereotactic body radiotherapy (SBRT) is the preferred approach if radiation is appropriate. Safe delivery

of SBRT requires a 2- to 3-mm margin away from the spinal cord. Separation surgery followed by SBRT is necessary in patients with high-grade SCC due to radioresistant tumors. Separation surgery is the circumferential excision of epidural tumor to reconstitute the thecal sac and provide a 2-mm margin for safe delivery of an ablative radiation dose. In patients with mechanical instability or retropulsion of bone fragments into the spinal canal or cord, a surgical approach is the treatment of choice. Systemic factors that need to be considered are the extent of disease and medical comorbidities that determine the patient's ability to tolerate planned therapy. Chemotherapy may have a role in patients with chemosensitive tumors who have had prior radiotherapy to the same region and who are not candidates for surgery. Patients who previously received radiotherapy for MSCC with an in-field tumor progression can be treated with reirradiation with spine stereotactic radiosurgery (SRS) if they are not surgical candidates.

Patients with painful pathologic compression fractures without spinal instability may benefit from percutaneous vertebroplasty or kyphoplasty, the injection of acrylic cement into a collapsed vertebra to stabilize the fracture. Pain palliation is common, and local antitumor effects have been noted. Cement leakage may cause symptoms in ~10% of patients. Bisphosphonates and/or denosumab may be helpful in prevention of SCC in patients with bony involvement.

The histology of the tumor is an important determinant of both recovery and survival. Rapid onset and progression of signs and symptoms are poor prognostic features.

■ INCREASED INTRACRANIAL PRESSURE

About 25% of patients with cancer die with intracranial metastases. The cancers that most often metastasize to the brain are lung and breast cancers and melanoma. Brain metastases often occur in the presence of systemic disease, and they frequently cause major symptoms, disability, and early death. The initial presentation of brain metastases from a previously unknown primary cancer is common. Lung cancer is most commonly the primary malignancy. CT scans of the chest/abdomen and MRI of the brain as the initial diagnostic studies can identify a biopsy site in most patients.

The signs and symptoms of a metastatic brain tumor are similar to those of other intracranial expanding lesions: headache, nausea, vomiting, behavioral changes, seizures, and focal, progressive neurologic changes. Occasionally the onset is abrupt, resembling a stroke, with the sudden appearance of headache, nausea, vomiting, and neurologic deficits. This picture is usually due to hemorrhage into the metastasis. Melanoma, germ cell tumors, and renal cell cancers have a particularly high incidence of intracranial bleeding. The tumor mass and surrounding edema may cause obstruction of the circulation of CSF, with resulting hydrocephalus. Patients with increased intracranial pressure may have papilledema with visual disturbances and neck stiffness. As the mass enlarges, brain tissue may be displaced through the fixed cranial openings, producing various herniation syndromes.

MRI is superior to CT scan. Gadolinium-enhanced MRI is more sensitive than CT at revealing meningeal involvement and small lesions, particularly in the brainstem or cerebellum. The MRI of the brain shows brain metastases as multiple enhancing lesions of various sizes with surrounding areas of low-density edema.

Intracranial hypertension ("pseudotumor cerebri") secondary to tretinoin therapy for acute promyelocytic leukemia has been reported as another cause of intracranial pressure in the setting of a malignancy.

TREATMENT

Increased Intracranial Pressure

Dexamethasone is the best initial treatment for all symptomatic patients with brain metastases. The current success of immunotherapy approaches for primary and metastatic brain tumors may preclude or limit glucocorticoid use since it may decrease antitumor response. Bevacizumab should be considered in patients who are unable to wean completely off of steroids as well as those who have

symptomatic brain edema and are on immunotherapy. Patients with multiple lesions should usually receive whole-brain radiation. Patients with a single brain metastasis and with controlled extracranial disease may be treated with surgical excision followed by whole-brain radiation therapy, especially if they are aged <60 years. Radioresistant tumors should be resected if possible. Stereotactic radiosurgery (SRS) is recommended in patients with a limited number of brain metastases (one to four) who have stable, systemic disease or reasonable systemic treatment options and in patients who have a small number of metastatic lesions in whom whole-brain radiation therapy has failed. With a gamma knife or linear accelerator, multiple small, well-collimated beams of ionizing radiation destroy lesions seen on MRI. Some patients with increased intracranial pressure associated with hydrocephalus may benefit from shunt placement. If neurologic deterioration is not reversed with medical therapy, ventriculotomy to remove CSF or craniotomy to remove tumors or hematomas may be necessary.

Targeted agents and checkpoint inhibitors have significant activity in brain metastases from non-small-cell lung cancer, breast cancer, renal cancer, and melanoma.

■ NEOPLASTIC MENINGITIS

Tumor involving the leptomeninges is a complication of both primary central nervous system (CNS) tumors and tumors that metastasize to the CNS. The incidence is estimated at 3–8% of patients with cancer. Melanoma, breast and lung cancer, lymphoma (including AIDS-associated), and acute leukemia are the most common causes. The lobular or triple-negative subtypes of breast cancer, as well as tumors with expression of the mutant epidermal growth factor receptor (EGFR) or the anaplastic lymphoma kinase (ALK) rearrangement in non-small-cell lung cancer (NSCLC), are more likely to have CNS involvement including neoplastic meningitis and brain metastases. Synchronous intraparenchymal brain metastases are evident in 11–31% of patients with neoplastic meningitis. Leptomeningeal seeding is frequent in patients undergoing resection of brain metastases or receiving stereotactic radiotherapy for brain metastases.

Patients typically present with multifocal neurologic signs and symptoms, including headache, gait abnormality, mental changes, nausea, vomiting, seizures, back or radicular pain, and limb weakness. Signs include cranial nerve palsies, extremity weakness, paresthesia, and decreased deep tendon reflexes.

Diagnosis is made by demonstrating malignant cells in the CSF; however, up to 40% of patients may have false-negative CSF cytology. An elevated CSF protein level is nearly always present (except in HTLV-1-associated adult T-cell leukemia). Patients with neurologic signs and symptoms consistent with neoplastic meningitis who have a negative CSF cytology should have the spinal tap repeated at least one more time for cytologic examination. MRI findings suggestive of neoplastic meningitis include leptomeningeal, subependymal, dural, or cranial nerve enhancement; superficial cerebral lesions; intradural nodules; and communicating hydrocephalus. Spinal cord imaging by MRI is a necessary component of the evaluation of nonleukemia neoplastic meningitis because ~20% of patients have cord abnormalities, including intradural enhancing nodules that are diagnostic for leptomeningeal involvement. Cauda equina lesions are common, but lesions may be seen anywhere in the spinal canal. The value of MRI for the diagnosis of leptomeningeal disease is limited in patients with hematopoietic malignancy. Radiolabeled CSF flow studies are abnormal in up to 70% of patients with neoplastic meningitis; ventricular outlet obstruction, abnormal flow in the spinal canal, or impaired flow over the cerebral convexities may affect distribution of intrathecal chemotherapy, resulting in decreased efficacy or increased toxicity. Radiation therapy may correct CSF flow abnormalities before use of intrathecal chemotherapy. Neoplastic meningitis can also lead to intracranial hypertension and hydrocephalus. Placement of a ventriculoperitoneal shunt may effectively palliate symptoms in these patients.

The development of neoplastic meningitis usually occurs in the setting of uncontrolled cancer outside the CNS; thus, prognosis is poor

(median survival 10–12 weeks). However, treatment of the neoplastic meningitis may successfully alleviate symptoms and control the CNS spread.

TREATMENT

Neoplastic Meningitis

Chemotherapy provided by either intrathecal injection or systemic routes is used to control leptomeningeal disease throughout the entire neuroaxis. Intrathecal chemotherapy, usually methotrexate, cytarabine, or thiotapec, is delivered by lumbar puncture or by an intraventricular reservoir (Ommaya). Among solid tumors, breast cancer responds best to therapy. Focal radiotherapy may have a role in bulky disease and in symptomatic or obstructive lesions. Targeted therapy such as systemically administered EGFR tyrosine kinase inhibitors (TKIs) in non-small-cell lung cancer may lead to improvement in some patients with leptomeningeal spread. Patients with neoplastic meningitis from either acute leukemia or lymphoma may be cured of their CNS disease if the systemic disease can be eliminated.

■ SEIZURES

Seizures occurring in a patient with cancer can be caused by the tumor itself, by metabolic disturbances, by radiation injury, by cerebral infarctions, by chemotherapy-related encephalopathies, or by CNS infections. Metastatic disease to the CNS is the most common cause of seizures in patients with cancer. However, seizures occur more frequently in primary brain tumors than in metastatic brain lesions. Seizures are a presenting symptom of CNS metastasis in 6–29% of cases. Approximately 10% of patients with CNS metastasis eventually develop seizures. Tumors that affect the frontal, temporal, and parietal lobes are more commonly associated with seizures than are occipital lesions. Both early and late seizures are uncommon in patients with posterior fossa and sellar lesions. Seizures are common in patients with CNS metastases from melanoma and low-grade primary brain tumors. Very rarely, cytotoxic drugs such as etoposide, busulfan, ifosfamide, and chlorambucil cause seizures. Treatment with bispecific antibodies and chimeric antigen receptor (CAR) T cells may also cause CNS toxicity including seizures and encephalopathy. Another cause of seizures related to drug therapy is reversible posterior leukoencephalopathy syndrome (RPLS). Chemotherapy, targeted therapy, and immunotherapies have been associated with the development of RPLS. RPLS occurs in patients undergoing allogeneic bone marrow or solid-organ transplantation. RPLS is characterized by headache, altered consciousness, generalized seizures, visual disturbances, hypertension, and symmetric posterior cerebral white matter vasogenic edema on CT/MRI. Seizures may begin focally but are typically generalized.

TREATMENT

Seizures

Patients in whom seizures due to CNS metastases have been demonstrated should receive anticonvulsive treatment with phenytoin or levetiracetam. If this is not effective, valproic acid can be added. Prophylactic anticonvulsant therapy is not recommended. In postcraniotomy patients, prophylactic antiepileptic drugs should be withdrawn during the first week after surgery. Most antiseizure medications including phenytoin induce cytochrome P450 (CYP450), which alters the metabolism of many antitumor agents, including irinotecan, taxanes, and etoposide as well as molecular targeted agents, including imatinib, gefitinib, erlotinib, tipifarnib, sorafenib, sunitinib, temsirolimus, everolimus, and vemurafenib. Levetiracetam and topiramate are anticonvulsant agents not metabolized by the hepatic CYP450 system and do not alter the metabolism of antitumor agents. They have become the preferred drugs. Surgical resection and other antitumor treatments such as radiotherapy and chemotherapy may improve seizure control.

■ PULMONARY AND INTRACEREBRAL LEUKOSTASIS

Hyperleukocytosis and the leukostasis syndrome associated with it are potentially fatal complications of acute leukemia (particularly myeloid leukemia) that can occur when the peripheral blast cell count is $>100,000/\text{mL}$. The frequency of hyperleukocytosis is 5–13% in acute myeloid leukemia (AML) and 10–30% in acute lymphoid leukemia; however, leukostasis is rare in lymphoid leukemia. At such high blast cell counts, blood viscosity is increased, blood flow is slowed by aggregates of tumor cells, and the primitive myeloid leukemic cells are capable of invading through the endothelium and causing hemorrhage. Brain and lung are most commonly affected. Patients with brain leukostasis may experience stupor, headache, dizziness, tinnitus, visual disturbances, ataxia, confusion, coma, or sudden death. On examination, papilledema, retinal vein distension, retinal hemorrhages, and focal deficit may be present. Pulmonary leukostasis may present as respiratory distress and hypoxemia and progress to respiratory failure. Chest radiographs may be normal but usually show interstitial or alveolar infiltrates. Hyperleukocytosis rarely may cause acute leg ischemia, renal vein thrombosis, myocardial ischemia, bowel infarction, and priapism. Arterial blood gas results should be interpreted cautiously. Rapid consumption of plasma oxygen by the markedly increased number of white blood cells can cause spuriously low arterial oxygen tension. Pulse oximetry is the most accurate way of assessing oxygenation in patients with hyperleukocytosis. Hydroxyurea can rapidly reduce a high blast cell count while the diagnostic workup is in progress. After the diagnosis is established, the patient should start quickly with effective induction chemotherapy. Leukapheresis should be used in patients with symptoms of hyperleukocytosis. Patients with hyperleukocytosis are also at the risk for disseminated intravascular coagulation and tumor lysis syndrome. The clinician should monitor the patient for these complications and take preventive and therapeutic actions during induction therapy. Intravascular volume depletion and unnecessary blood transfusions may increase blood viscosity and worsen the leukostasis syndrome. Leukostasis is very rarely a feature of the high white cell counts associated with chronic lymphoid or chronic myeloid leukemia.

When acute promyelocytic leukemia is treated with differentiating agents like tretinoin and arsenic trioxide, cerebral or pulmonary leukostasis may occur as tumor cells differentiate into mature neutrophils. This complication can be largely avoided by using cytotoxic chemotherapy together with the differentiating agents.

■ HEMOPTYSIS

Hemoptysis may be caused by nonmalignant conditions, but lung cancer accounts for a large proportion of cases. Up to 20% of patients with lung cancer have hemoptysis some time in their course. Endobronchial metastases from carcinoid tumors, breast cancer, colon cancer, kidney cancer, and melanoma may also cause hemoptysis. The volume of bleeding is often difficult to gauge. Massive hemoptysis is defined as $>200\text{--}600 \text{ mL}$ of blood produced in 24 h. However, any hemoptysis should be considered massive if it threatens life. When respiratory difficulty occurs, hemoptysis should be treated emergently. The first priorities are to maintain the airway, optimize oxygenation, and stabilize the hemodynamic status. If the bleeding side is known, the patient should be placed in a lateral decubitus position, with the bleeding side down to prevent aspiration into the unaffected lung, and given supplemental oxygen. If large-volume bleeding continues or the airway is compromised, the patient should be intubated and undergo emergency bronchoscopy. If the site of bleeding is detected, either the patient undergoes a definitive surgical procedure or the lesion is treated with a neodymium:yttrium-aluminum-garnet (Nd:YAG) laser, argon plasma coagulation, or electrocautery. In stable patients, multidetector CT angiography delineates bronchial and nonbronchial systemic arteries and identifies the source of bleeding and underlying pathology with high sensitivity. Massive hemoptysis usually originates from the high-pressure bronchial circulation. Bronchial artery embolization is considered a first-line definitive procedure for managing hemoptysis. Bronchial artery embolization may control brisk bleeding in 75–90% of

patients, permitting the definitive surgical procedure to be done more safely if it is appropriate.

Embolization without definitive surgery is associated with rebleeding in 20–50% of patients. Recurrent hemoptysis usually responds to a second embolization procedure. A postembolization syndrome characterized by pleuritic pain, fever, dysphagia, and leukocytosis may occur; it lasts 5–7 days and resolves with symptomatic treatment. Bronchial or esophageal wall necrosis, myocardial infarction, and spinal cord infarction are rare complications. Surgery, as a salvage strategy, is indicated after failure of embolization and is associated with better survival when performed in a nonurgent setting.

Pulmonary hemorrhage with or without hemoptysis in hematologic malignancies is often associated with fungal infections, particularly *Aspergillus* spp. After granulocytopenia resolves, the lung infiltrates in aspergillosis may cavitate and cause massive hemoptysis. Thrombocytopenia and coagulation defects should be corrected, if possible. Surgical evaluation is recommended in patients with aspergillosis-related cavitary lesions.

Bevacizumab, an antibody to vascular endothelial growth factor (VEGF) that inhibits angiogenesis, has been associated with life-threatening hemoptysis in patients with non-small-cell lung cancer, particularly of squamous cell histology. Non-small-cell lung cancer patients with cavitary lesions or previous hemoptysis (≥ 2.5 mL) within the past 3 months have higher risk for pulmonary hemorrhage.

AIRWAY OBSTRUCTION

Airway obstruction refers to a blockage at the level of the mainstem bronchi or above. It may result either from intraluminal tumor growth or from extrinsic compression of the airway. The most common cause of malignant upper airway obstruction is invasion from an adjacent primary tumor, most commonly lung cancer, followed by esophageal, thyroid, and mediastinal malignancies including lymphomas. Extrathoracic primary tumors such as renal, colon, or breast cancer can cause airway obstruction through endobronchial and/or mediastinal lymph node metastases. Patients may present with dyspnea, hemoptysis, stridor, wheezing, intractable cough, postobstructive pneumonia, or hoarseness. Chest radiographs usually demonstrate obstructing lesions. CT scans reveal the extent of tumor. Cool, humidified oxygen, glucocorticoids, and ventilation with a mixture of helium and oxygen (Heliox) may provide temporary relief. If the obstruction is proximal to the larynx, a tracheostomy may be lifesaving. For more distal obstructions, particularly intrinsic lesions incompletely obstructing the airway, bronchoscopy with mechanical debulking and dilation or ablational treatments including laser treatment, photodynamic therapy, argon plasma coagulation, electrocautery, or stenting can produce immediate relief in most patients (Fig. 75-3). However, radiation therapy (either external-beam irradiation or brachytherapy) given together with glucocorticoids may also open the airway. Symptomatic extrinsic compression may be palliated by stenting. Patients with primary airway tumors such as squamous cell carcinoma, carcinoid tumor, adenocystic carcinoma, or non-small-cell lung cancer, if resectable, should have surgery.

METABOLIC EMERGENCIES

HYPERCALCEMIA

Hypercalcemia is the most common paraneoplastic syndrome. Its pathogenesis and management are discussed fully in Chaps. 93 and 410.

SYNDROME OF INAPPROPRIATE SECRETION OF ANTIDIURETIC HORMONE

Hyponatremia is a common electrolyte abnormality in cancer patients, and syndrome of inappropriate secretion of antidiuretic hormone (SIADH) is the most common cause among patients with cancer. SIADH is discussed fully in Chaps. 93 and 381.

LACTIC ACIDOSIS

Lactic acidosis is a rare and potentially fatal metabolic complication of cancer. Lactic acidosis associated with sepsis and circulatory failure is a common preterminal event in many malignancies. Lactic acidosis in the



A



B

FIGURE 75-3 Airway obstruction. A. Computed tomography scan of a 62-year-old man with tracheal obstruction caused by renal carcinoma showing paratracheal mass with tracheal invasion/obstruction (arrow). B. Chest x-ray of same patient after stent (arrows) placement.

absence of hypoxemia may occur in patients with leukemia, lymphoma, or solid tumors. In some cases, hypoglycemia also is present. Extensive involvement of the liver by tumor is often present. In most cases, decreased metabolism and increased production by the tumor both contribute to lactate accumulation. Tumor cell overexpression of certain glycolytic enzymes and mitochondrial dysfunction can contribute to its increased lactate production. HIV-infected patients have an increased risk of aggressive lymphoma; lactic acidosis that occurs in such patients may be related either to the rapid growth of the tumor or from toxicity of nucleoside reverse transcriptase inhibitors. Symptoms of lactic acidosis include tachypnea, tachycardia, change of mental status, and hepatomegaly. The serum level of lactic acid may reach 10–20 mmol/L (90–180 mg/dL). Treatment is aimed at the underlying disease. *The danger from lactic acidosis is from the acidosis, not the lactate.* Sodium bicarbonate should be added if acidosis is very severe or if hydrogen ion production is very rapid and uncontrolled. Other treatment options include renal replacement therapy, such as hemodialysis, and thiamine replacement. The prognosis is poor regardless of the treatment offered.

HYPOGLYCEMIA

Persistent hypoglycemia is occasionally associated with tumors other than pancreatic islet cell tumors. Usually these tumors are large; tumors of mesenchymal origin, hepatomas, or adrenocortical tumors may cause hypoglycemia. Mesenchymal tumors are usually located in the retroperitoneum or thorax. Obtundation, confusion, and behavioral aberrations occur in the postabsorptive period and may precede the diagnosis of the tumor. These tumors often secrete incompletely processed insulin-like growth factor II (IGF-II), a hormone capable

of activating insulin receptors and causing hypoglycemia. Tumors secreting incompletely processed big IGF-II are characterized by an increased IGF-II to IGF-I ratio, suppressed insulin and C-peptide level, and inappropriately low growth hormone and β -hydroxybutyrate concentrations. Rarely, hypoglycemia is due to insulin secretion by a non-islet cell carcinoma. The development of hepatic dysfunction from liver metastases and increased glucose consumption by the tumor can contribute to hypoglycemia. If the tumor cannot be resected, hypoglycemia symptoms may be relieved by the administration of glucose, glucocorticoids, recombinant growth hormone, or glucagon.

Hypoglycemia can be artificial; hyperleukocytosis from leukemia, myeloproliferative diseases, leukemoid reactions, or colony-stimulating factor treatment can increase glucose consumption in the test tube after blood is drawn, leading to pseudohypoglycemia.

■ ADRENAL INSUFFICIENCY

In patients with cancer, adrenal insufficiency may go unrecognized because the symptoms, such as nausea, vomiting, anorexia, and orthostatic hypotension, are nonspecific and may be mistakenly attributed to progressive cancer or to therapy. Primary adrenal insufficiency may develop owing to replacement of both glands by metastases (lung, breast, colon, or kidney cancer; lymphoma), to removal of both glands, or to hemorrhagic necrosis in association with sepsis or anticoagulation. Impaired adrenal steroid synthesis occurs in patients being treated for cancer with mitotane, ketoconazole, or aminoglutethimide or undergoing rapid reduction in glucocorticoid therapy. Megestrol acetate, used to manage cancer and HIV-related cachexia, may suppress plasma levels of cortisol and adrenocorticotrophic hormone (ACTH). Patients taking megestrol may develop adrenal insufficiency, and even those whose adrenal dysfunction is not symptomatic may have inadequate adrenal reserve if they become seriously ill. Paradoxically, some patients may develop Cushing's syndrome and/or hyperglycemia because of the glucocorticoid-like activity of megestrol acetate. Ipilimumab, an anti-CTLA-4 antibody used for treatment of malignant melanoma, may cause autoimmunity including autoimmune-like enterocolitis, hypophysitis (leading to secondary adrenal insufficiency), hepatitis, and, rarely, primary adrenal insufficiency. Autoimmune hypophysitis may present with headache, visual field defects, and pituitary hormone deficiencies manifesting as hypopituitarism, adrenal insufficiency (including adrenal crisis), or hypothyroidism. Ipilimumab-associated hypophysitis symptoms occur at an average of 6–12 weeks after initiation of therapy. An MRI usually shows homogenous enhancement of pituitary gland. Early glucocorticoid treatment and hormone replacement are the initial treatment. The role of high-dose glucocorticoids in the treatment of hypophysitis is not clear. High-dose glucocorticoids may not improve the frequency of pituitary function recovery. Autoimmune adrenalitis can also be observed with anti-CTLA-4 antibody. Pituitary dysfunction is usually permanent, requiring long-term hormone replacement therapy. Other checkpoint inhibitors, such as monoclonal antibodies targeting programmed cell death-1 (PD-1), an inhibitory receptor expressed by T cells or one of its ligands (PD-L1), may cause hypophysitis infrequently (~1%). Autoimmune adrenalitis is more frequent with use of PD-1/PD-L1 than with CTLA-4 inhibitors, but incidence is low. Cranial irradiation for childhood brain tumors may affect the hypothalamus-pituitary-adrenal axis, resulting in secondary adrenal insufficiency. Rarely, metastatic replacement causes primary adrenal insufficiency as the first manifestation of an occult malignancy. Metastasis to the pituitary or hypothalamus is found at autopsy in up to 5% of patients with cancer, but associated secondary adrenal insufficiency is rare.

Acute adrenal insufficiency is potentially lethal. Treatment of suspected adrenal crisis is initiated after the sampling of serum cortisol and ACTH levels ([Chap. 386](#)).

TREATMENT RELATED EMERGENCIES

■ TUMOR LYSIS SYNDROME

Tumor lysis syndrome (TLS) is characterized by hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia and is caused by the

destruction of a large number of rapidly proliferating neoplastic cells. Acidosis may also develop. Acute renal failure occurs frequently.

TLS is most often associated with the treatment of Burkitt's lymphoma, acute lymphoblastic leukemia, and other rapidly proliferating lymphomas, but it also may be seen with chronic leukemias and, rarely, with solid tumors. This syndrome has been seen in patients with chronic lymphocytic leukemia after treatment with nucleosides like fludarabine and is increased in frequency in lymphoid neoplasms treated with venetoclax, a bcl-2 antagonist. TLS has been observed with administration of glucocorticoids, hormonal agents such as letrozole and tamoxifen, and monoclonal antibodies such as rituximab, obinutuzumab, ofatumumab, and gemtuzumab. TLS usually occurs during or shortly (1–5 days) after chemotherapy. Rarely, spontaneous necrosis of malignancies causes TLS.

Hyperuricemia may be present at the time of chemotherapy. Effective treatment kills malignant cells and leads to increased serum uric acid levels from the turnover of nucleic acids. Owing to the acidic local environment, uric acid can precipitate in the tubules, medulla, and collecting ducts of the kidney, leading to renal failure. Lactic acidosis and dehydration may contribute to the precipitation of uric acid in the renal tubules. The finding of uric acid crystals in the urine is strong evidence for uric acid nephropathy. The ratio of urinary uric acid to urinary creatinine is >1 in patients with acute hyperuricemic nephropathy and <1 in patients with renal failure due to other causes. Other events may lead to renal failure in TLS. Calcium phosphate also precipitates in the interstitium and renal microvasculature, leading to nephrocalcinosis. Both types of crystals are toxic to the tubular epithelium, inducing local active inflammatory and pro-oxidative responses. Soluble uric acid may induce hemodynamic changes, with decreased renal blood flow due to vasoconstriction and impaired autoregulation (crystal-independent pathway).

Hyperphosphatemia, which can be caused by the release of intracellular phosphate pools by tumor lysis, produces a reciprocal depression in serum calcium, which causes severe neuromuscular irritability and tetany. Deposition of calcium phosphate in the kidney and hyperphosphatemia may cause renal failure. Potassium is the principal intracellular cation, and massive destruction of malignant cells may lead to hyperkalemia. Hyperkalemia in patients with renal failure may rapidly become life threatening by causing ventricular arrhythmias and sudden death.

The likelihood that TLS will occur in patients with Burkitt's lymphoma is related to the tumor burden and renal function. Hyperuricemia and high serum levels of lactate dehydrogenase (LDH >1500 U/L), both of which correlate with total tumor burden, also correlate with the risk of TLS. In patients at risk for TLS, pretreatment evaluations should include a complete blood count, serum chemistry evaluation, and urine analysis. High leukocyte and platelet counts may artificially elevate potassium levels ("pseudohyperkalemia") due to lysis of these cells after the blood is drawn. In these cases, plasma potassium instead of serum potassium should be followed. In pseudohyperkalemia, no electrocardiographic abnormalities are present. In patients with abnormal baseline renal function, the kidneys and retroperitoneal area should be evaluated by sonography and/or CT to rule out obstructive uropathy. Urine output should be watched closely.

TREATMENT

Tumor Lysis Syndrome

Recognition of risk and prevention are the most important steps in the management of this syndrome ([Fig. 75-4](#)). The standard preventive approach consists of allopurinol and aggressive hydration. Urinary alkalization with sodium bicarbonate is no longer recommended. It increases uric acid solubility, but a high pH decreases the solubility of xanthine, hypoxanthine, and calcium phosphate, potentially increasing the likelihood of intratubular crystallization. Intravenous allopurinol may be given in patients who cannot tolerate oral therapy. Febuxostat, a potent nonpurine selective xanthine oxidase inhibitor, is indicated for treatment of hyperuricemia. It

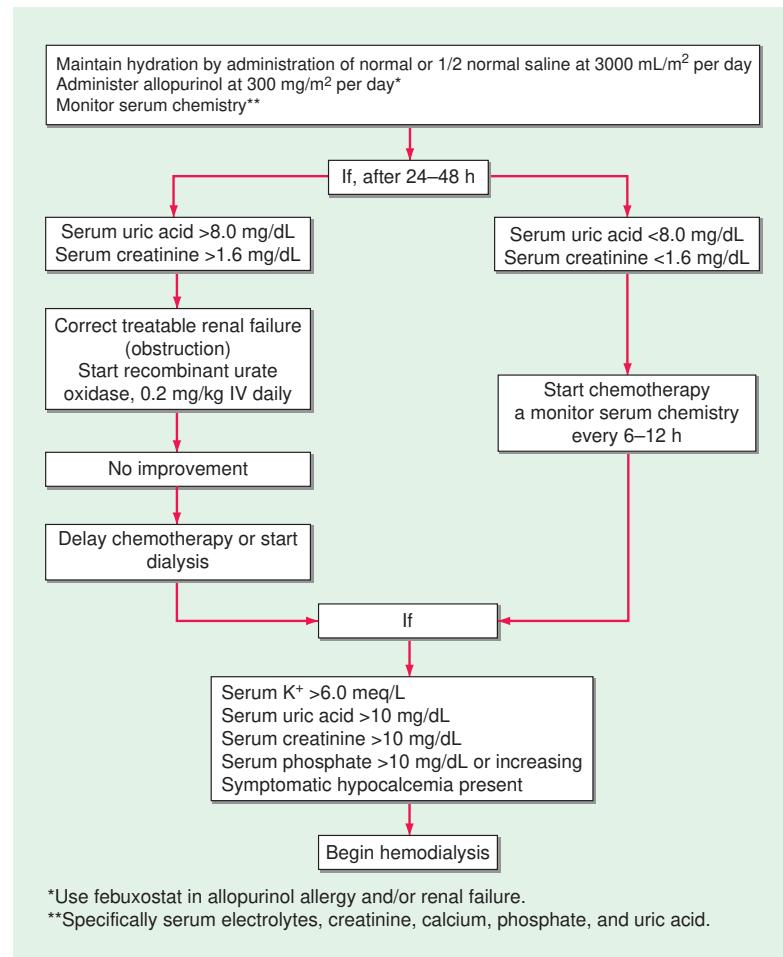


FIGURE 75-4 Management of patients at high risk for the tumor lysis syndrome.

results in fewer hypersensitivity reactions than allopurinol. Febuxostat does not require dosage adjustment in patients with mild to moderate renal impairment. Febuxostat achieved significantly superior serum uric acid control in comparison to allopurinol in patients with hematologic malignancies at intermediate to high TLS risk. In some cases, uric acid levels cannot be lowered sufficiently with the standard preventive approach. Rasburicase (recombinant urate oxidase) can be effective in these instances, particularly when renal failure is present. Urate oxidase is missing from primates and catalyzes the conversion of poorly soluble uric acid to readily soluble allantoic acid. Rasburicase acts rapidly, decreasing uric acid levels within hours; however, it may cause hypersensitivity reactions such as bronchospasm, hypoxemia, and hypotension. Rasburicase should also be administered to high-risk patients for TLS prophylaxis. Rasburicase is contraindicated in patients with glucose-6-phosphate dehydrogenase deficiency who are unable to break down hydrogen peroxide, an end product of the urate oxidase reaction. Rasburicase is known to cause ex vivo enzymatic degradation of uric acid in test tube at room temperature. This leads to spuriously low uric acid levels during laboratory monitoring of the patient with TLS. Samples must be cooled immediately to deactivate the urate oxidase. Despite aggressive prophylaxis, TLS and/or oliguric or anuric renal failure may occur. Renal replacement therapy is often necessary and should be considered early in the course. Hemodialysis is preferred. Hemofiltration offers a gradual, continuous method of removing cellular by-products and fluid.

HUMAN ANTIBODY INFUSION REACTIONS

The initial infusion of human or humanized antibodies (e.g., rituximab, gemtuzumab, trastuzumab, alemtuzumab, panitumumab, brentuximab vedotin, blinatumomab) is associated with fever, chills, nausea, asthenia, and headache in up to half of treated patients. Bronchospasm and hypotension occur in 1% of patients. Severe manifestations including pulmonary infiltrates, acute respiratory distress syndrome (ARDS), and cardiogenic shock occur rarely. Laboratory manifestations include elevated hepatic aminotransferase levels, thrombocytopenia, and prolongation of prothrombin time. The pathogenesis is thought to be activation of immune effector processes (cells and complement) and release of inflammatory cytokines, such as tumor necrosis factor α , interferon γ , interleukin (IL) 6, and IL-10 (cytokine release syndrome [CRS]). Although its origins are not completely understood, CRS is believed to be due to activation of a variety of cell types including monocytes/macrophages and T and B lymphocytes. Hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome (MAS) can develop as part of CRS and usually is a manifestation of severe CRS.

Severe CRS may require intensive support for ARDS and resistant hypotension. Emerging clinical experience at several institutions has concluded that tocilizumab is an effective treatment for severe or life-threatening CRS. Tocilizumab prevents IL-6 binding to both cell-associated and soluble IL-6 receptors and therefore inhibits both classical and trans-IL-6 signaling. Other cytokine-directed therapies, such as siltuximab, a chimeric anti-IL-6 monoclonal antibody, and anakinra, an IL-1 receptor antagonist, have been used.

Adoptive transfer of CAR-engineered T cells is a promising therapy for cancers. The most common acute toxicity of CAR T cells is CRS. CAR T cell-associated CRS may be associated with cardiac dysfunction and neurotoxicity. The management includes supportive care and tocilizumab.

Severe reactions from rituximab have occurred with high numbers ($>50 \times 10^9$ lymphocytes) of circulating cells bearing the target antigen (CD20) and have been associated with a rapid fall in circulating tumor cells, mild electrolyte evidence of TLS, and, very rarely, death. In addition, increased liver enzymes, -dimer, and LDH and prolongation of the prothrombin time may occur. Diphenhydramine, hydrocortisone, and acetaminophen can often prevent or suppress the infusion-related symptoms. If they occur, the infusion is stopped and restarted at half the initial infusion rate after the symptoms have abated.

■ HEMOLYTIC UREMIC SYNDROME

Hemolytic-uremic syndrome (HUS) and, less commonly, thrombotic thrombocytopenic purpura (TTP) (Chap. 317) may rarely occur after treatment with antineoplastic drugs, including mitomycin, gemcitabine, cisplatin, bleomycin, and proteasome inhibitors, and with VEGF inhibitors. Mitomycin and gemcitabine are the most common offenders. Unlike mitomycin, there is no clear-cut relationship between the cumulative dose of gemcitabine and risk of HUS. It occurs most often in patients with gastric, lung, colorectal, pancreatic, and breast carcinoma. In one series, 35% of patients were without evident cancer at the time this syndrome appeared. Secondary HUS/TTP has also been reported as a rare but sometimes fatal complication of bone marrow transplantation.

HUS usually has its onset 4–8 weeks after the last dose of chemotherapy, but it is not rare to detect it several months later. HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. Dyspnea, weakness, fatigue, oliguria, and purpura are also common initial symptoms and findings. Systemic hypertension and pulmonary edema frequently occur. Severe hypertension, pulmonary edema, and rapid worsening of hemolysis and renal function may occur after a blood or blood product transfusion. Cardiac findings include atrial arrhythmias, pericardial friction rub, and pericardial effusion. Raynaud's phenomenon is part of the syndrome in patients treated with bleomycin.

Laboratory findings include severe to moderate anemia associated with red blood cell fragmentation and numerous schistocytes on peripheral smear. Reticulocytosis, decreased plasma haptoglobin, and an LDH level document hemolysis. The serum bilirubin level is usually normal or slightly elevated. The Coombs test is negative. The white cell count is usually normal, and thrombocytopenia ($<100,000/\mu\text{L}$) is almost always present. Most patients have a normal coagulation profile, although some have mild elevations in thrombin time and in levels of fibrin degradation products. The serum creatinine level is elevated at presentation and shows a pattern of subacute worsening within weeks of the initial azotemia. The urinalysis reveals hematuria, proteinuria, and granular or hyaline casts, and circulating immune complexes may be present.

The basic pathologic lesion appears to be deposition of fibrin in the walls of capillaries and arterioles, and these deposits are similar to those seen in HUS due to other causes. These microvascular abnormalities involve mainly the kidneys and rarely occur in other organs. The pathogenesis of cancer treatment-related HUS is not completely understood, but probably the most important factor is endothelial damage. Primary forms of HUS/TTP are related to a decrease in processing of von Willebrand factor by a protease called ADAMTS13.

The case-fatality rate is high; most patients die within a few months. Optimal treatment for chemotherapy-induced HUS is debated. Immunocomplex removal through plasmapheresis, plasma exchange, immunoabsorption, or exchange transfusion, antiplatelet and anticoagulant therapies, and immunosuppression have all been employed with varying degrees of success.

The outcome with plasma exchange is generally poor, as in many other cases of secondary TTP. Rituximab is successfully used in patients with chemotherapy-induced HUS as well as in ADAMTS13-deficient

TTTP. Eculizumab, a complement inhibitor, is now approved by the U.S. Food and Drug Administration (FDA) and considered first line for the treatment of atypical HUS. Vaccination against *Neisseria meningitis* is mandatory before eculizumab is administered.

■ NEUTROPENIA AND INFECTION

These remain the most common serious complications of cancer therapy. They are covered in detail in Chap. 74.

■ PULMONARY INFILTRATES

Patients with cancer may present with dyspnea associated with diffuse interstitial infiltrates on chest radiographs. Such infiltrates may be due to progression of the underlying malignancy, treatment-related toxicities, infection, and/or unrelated diseases. The cause may be multifactorial; however, most commonly, they occur as a consequence of treatment. Infiltration of the lung by malignancy has been described in patients with leukemia, lymphoma, and breast and other solid cancers. Pulmonary lymphatics may be involved diffusely by neoplasm (pulmonary lymphangitic carcinomatosis), resulting in a diffuse increase in interstitial markings on chest radiographs. The patient is often mildly dyspneic at the onset, but pulmonary failure develops over a period of weeks. In some patients, dyspnea precedes changes on the chest radiographs and is accompanied by a nonproductive cough. This syndrome is characteristic of solid tumors. In patients with leukemia, diffuse microscopic neoplastic peribronchial and peribronchiolar infiltration is frequent but may be asymptomatic. However, some patients present with diffuse interstitial infiltrates, an alveolar capillary block syndrome, and respiratory distress. Thickening of bronchovascular bundles and prominence of peripheral arteries are CT findings suggestive of leukemic infiltration. In these situations, glucocorticoids can provide symptomatic relief, but specific chemotherapy should always be started promptly.

Several cytotoxic agents, such as bleomycin, methotrexate, busulfan, nitrosoureas, gemcitabine, mitomycin, vinorelbine, docetaxel, paclitaxel, fludarabine, pentostatin, and ifosfamide, may cause pulmonary damage. The most frequent presentations are interstitial pneumonitis, alveolitis, and pulmonary fibrosis. Some cytotoxic agents, including methotrexate and procarbazine, may cause an acute hypersensitivity reaction. Cytosine arabinoside has been associated with noncardiogenic pulmonary edema. Administration of multiple cytotoxic drugs, as well as radiotherapy and preexisting lung disease, may potentiate the pulmonary toxicity. Supplemental oxygen may potentiate the effects of drugs and radiation injury. Patients should always be managed with the lowest F_O_2 that is sufficient to maintain hemoglobin saturation.

The onset of symptoms may be insidious, with symptoms including dyspnea, nonproductive cough, and tachycardia. Patients may have bibasilar crepitant rales, end-inspiratory crackles, fever, and cyanosis. The chest radiograph generally shows an interstitial and sometimes an intraalveolar pattern that is strongest at the lung bases and may be symmetric. A small effusion may occur. Hypoxemia with decreased carbon monoxide diffusing capacity is always present. Glucocorticoids may be helpful in patients in whom pulmonary toxicity is related to radiation therapy or to chemotherapy. Treatment is otherwise supportive.

Molecular targeted agents, imatinib, erlotinib, and gefitinib are potent inhibitors of tyrosine kinases. These drugs may cause interstitial lung disease (ILD). In the case of gefitinib, preexisting fibrosis, poor performance status, and prior thoracic irradiation are independent risk factors; this complication has a high fatality rate. In Japan, incidence of ILD associated with gefitinib was ~4.5% compared to 0.5% in the United States. Temsirolimus and everolimus, both esters of rapamycin (sirolimus), are agents that block the effects of mammalian target of rapamycin (mTOR), an enzyme that has an important role in regulating the synthesis of proteins that control cell division. These agents may cause ground-glass opacities (GGO) in the lung with or without diffuse interstitial disease and lung parenchymal consolidation. Patients may be asymptomatic with only radiologic findings or may be symptomatic. Symptoms include cough, dyspnea, and/or hypoxemia, and sometimes patients present with systemic symptoms such as fever and fatigue. The incidence of everolimus-induced ILD also appears to be higher in

Japanese patients. Treatment includes dose reduction or withdrawal and, in some cases, the addition of glucocorticoids.

The FDA-approved immune checkpoint inhibitors (ICI) of the PD-1 and PD-L1 pathway, including nivolumab, pembrolizumab, durvalumab, avelumab, atezolizumab, and cemiplimab, enhance antitumor activity by blocking negative regulators of T-cell function. Immune-mediated pneumonitis is rare (10%) but may be a life-threatening complication of these drugs. Pneumonitis symptoms include cough, shortness of breath, dyspnea, and fever, and often involve only asymptomatic radiographic changes. Pneumonitis shows ground-glass patchy lesions and/or disseminated nodular infiltrates, predominantly in the lower lobes. Identifying the exact cause of a pneumonitis in a patient treated with ICIs could be challenging during the current COVID-19 outbreak (Fig. 75-5A). Chest CT manifestations of COVID-19 include an imaging pattern of pure GGO, consolidation, nodules, fibrous stripes, and mixed patterns, with the distribution slightly predominant in the lower lobe and peripheral areas of the lung. Treatment of immune-mediated pneumonitis includes temporary or permanent withdrawal of drug and the addition of high-dose glucocorticoids (Fig. 75-5B).

Radiation pneumonitis and/or fibrosis are relatively frequent side effects of thoracic radiation therapy. It may be acute or chronic. Radiation-induced lung toxicity is a function of the irradiated lung volume, dose per fraction, and radiation dose. The larger the irradiated lung field, the higher is the risk for radiation pneumonitis. The use of concurrent chemoradiation, particularly regimens including paclitaxel, increases pulmonary toxicity. Radiation pneumonitis usually develops 2–6 months after completion of radiotherapy. The clinical

syndrome, which varies in severity, consists of dyspnea, cough with scanty sputum, low-grade fever, and an initial hazy infiltrate on chest radiographs. The infiltrate and tissue damage usually are confined to the radiation field. The CT scan may show GGOs, consolidation, fibrosis, atelectatic cicatrization, pleural volume loss, or pleural thickening. The patients subsequently may develop a patchy alveolar infiltrate and air bronchograms, which may progress to acute respiratory failure that is sometimes fatal. A lung biopsy may be necessary to make the diagnosis. Asymptomatic infiltrates found incidentally after radiation therapy need not be treated. However, prednisone should be administered to patients with fever or other symptoms. The dosage should be tapered slowly after the resolution of radiation pneumonitis, because abrupt withdrawal of glucocorticoids may cause an exacerbation of pneumonia. Delayed radiation fibrosis may occur years after radiation therapy and is signaled by dyspnea on exertion. Often it is mild, but it can progress to chronic respiratory failure. Therapy is supportive.

Classic radiation pneumonitis that leads to pulmonary fibrosis is due to radiation-induced production of local cytokines such as platelet-derived growth factor β , tumor necrosis factor, interleukins, and transforming growth factor β in the radiation field.

SBRT is a radiotherapy treatment method that has been applied to the treatment of stage I lung cancers in medically inoperable patients. SBRT accurately delivers a high dose of irradiation in one or few treatment fractions to an image-defined lung mass. Most of the acute changes after SBRT occur later than 3 months after treatment, and the shape of the SBRT-induced injury conforms more tightly to the tumor.

Pneumonia is a common problem in patients undergoing treatment for cancer (Chap 74). In patients with pulmonary infiltrates who are



A

B

FIGURE 75-5 A. Computed tomography scan of a 63-year-old female with metastatic adenocarcinoma on nivolumab with immune check point inhibitor pneumonia showing interlobular septal thickening and diffuse ground glass opacity to nivolumab. B. Computed tomography scan of a 68-year-old female with resected adenocarcinoma of lung and COVID 19 pneumonia showing peripheral and basilar predominant patchy groundglass and consolidative opacity consistent with multifocal COVID pneumonia.

afebrile, heart failure and multiple pulmonary emboli are in the differential diagnosis.

■ NEUTROPENIC ENTEROCOLITIS

Neutropenic enterocolitis (typhlitis) is the inflammation and necrosis of the cecum and surrounding tissues that may complicate the treatment of acute leukemia. Nevertheless, it may involve any segment of the gastrointestinal tract including small intestine, appendix, and colon. This complication has also been seen in patients with other forms of cancer treated with taxanes, 5-fluorouracil, irinotecan, vinorelbine, cisplatin, carboplatin, and high-dose chemotherapy (Fig. 75-6). It also has been reported in patients with AIDS, aplastic anemia, cyclic neutropenia, idiosyncratic drug reactions involving antibiotics, and immunosuppressive therapies. The patient develops right lower quadrant abdominal pain, often with rebound tenderness and a tense, distended abdomen, in a setting of fever and neutropenia. Watery diarrhea (often containing sloughed mucosa) and bacteremia are common, and bleeding may occur. Plain abdominal films are generally of little value in the diagnosis; CT scan may show marked bowel wall thickening, particularly in the cecum, with bowel wall edema, mesenteric stranding, and ascites, and may help to differentiate neutropenic colitis from other abdominal disorders such as appendicitis, diverticulitis, and *Clostridium difficile*-associated colitis in this high-risk population. Patients

with bowel wall thickness >10 mm on ultrasonogram have higher mortality rates. However, bowel wall thickening is significantly more prominent in patients with *C. difficile* colitis. Pneumatosis intestinalis is a more specific finding, seen only in those with neutropenic enterocolitis and ischemia. The combined involvement of the small and large bowel suggests a diagnosis of neutropenic enterocolitis. Rapid institution of broad-spectrum antibiotics, bowel rest, and nasogastric suction may reverse the process. Use of myeloid growth factors improved outcome significantly. Surgical intervention is reserved for severe cases of neutropenic enterocolitis with evidence of perforation, peritonitis, gangrenous bowel, or gastrointestinal hemorrhage despite correction of any coagulopathy.

C. difficile colitis is increasing in incidence. Newer strains of *C. difficile* produce ~20 times more of toxins A and B compared to previously studied strains. *C. difficile* risk is also increased with chemotherapy. Antibiotic coverage for *C. difficile* should be added if pseudomembranous colitis cannot be excluded.

■ HEMORRHAGIC CYSTITIS

Hemorrhagic cystitis is characterized by diffuse bladder mucosal bleeding that develops secondary to chemotherapy (mostly cyclophosphamide or ifosfamide), radiation therapy, bone marrow transplantation (BMT), and/or opportunistic infections. Both cyclophosphamide and ifosfamide are metabolized to acrolein, which is a strong chemical irritant that is excreted in the urine. Prolonged contact or high concentrations may lead to bladder irritation and hemorrhage. Symptoms include gross hematuria, frequency, dysuria, burning, urgency, incontinence, and nocturia. The best management is prevention. Maintaining a high rate of urine flow minimizes exposure. In addition, 2-mercaptoethanesulfonate (mesna) detoxifies the metabolites and can be coadministered with the instigating drugs. Mesna usually is given three times on the day of ifosfamide administration in doses that are each 20% of the total ifosfamide dose. If hemorrhagic cystitis develops, the maintenance of a high urine flow may be sufficient supportive care. If conservative management is not effective, irrigation of the bladder with a 0.37–0.74% formalin solution for 10 min stops the bleeding in most cases. *N*-Acetylcysteine may also be an effective irrigant. Prostaglandin (carboprost) can inhibit the process. In extreme cases, ligation of the hypogastric arteries, urinary diversion, or cystectomy may be necessary.

In the BMT setting, early-onset hemorrhagic cystitis is related to drugs in the treatment regimen (e.g., cyclophosphamide), and late-onset hemorrhagic cystitis is usually due to the polyoma virus BKV or adenovirus type 11. BKV load in urine alone or in combination with acute graft-versus-host disease correlates with development of hemorrhagic cystitis. Viral causes are usually detected by polymerase chain reaction (PCR)-based diagnostic tests. Treatment of viral hemorrhagic cystitis is largely supportive, with reduction in doses of immunosuppressive agents, if possible. No antiviral therapy is approved, although cidofovir was reported to be effective in a small series. Hyperbaric oxygen therapy has been used successfully in patients with BKV-associated and cyclophosphamide-induced hemorrhagic cystitis during hematopoietic stem cell transplantation, as well as in hemorrhagic radiation cystitis that occurs in up to 5% of patients after pelvic radiation.

■ HYPERSENSITIVITY REACTIONS TO ANTINEOPLASTIC DRUGS

Many antineoplastic drugs may cause hypersensitivity reaction. These reactions are unpredictable and potentially life threatening. Most reactions occur during or within hours of parenteral drug administration. Taxanes, platinum compounds, asparaginase, etoposide, procarbazine, and biologic agents, including rituximab, bevacizumab, trastuzumab, gemtuzumab, cetuximab, and alemtuzumab, are more commonly associated with acute hypersensitivity reactions than are other agents. Hypersensitivity reactions to some drugs, such as taxanes, occur during the first or second dose administered. Hypersensitivity to platinum compounds occurs after prolonged exposure. Skin testing may identify patients with high risk for hypersensitivity after carboplatin exposure. Premedication with histamine H_1 and H_2 receptor antagonists and glucocorticoids reduces the incidence of hypersensitivity reaction to

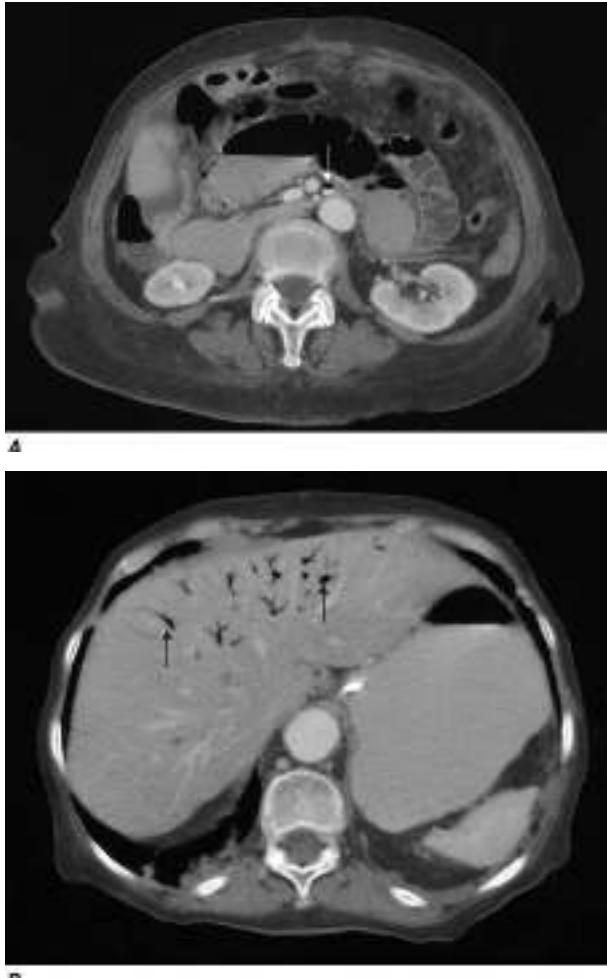


FIGURE 75-6 Abdominal computed tomography (CT) scans of a 72-year-old woman with neutropenic enterocolitis secondary to chemotherapy. A. Air in inferior mesenteric vein (arrow) and bowel wall with pneumatosis intestinalis. B. CT scan of upper abdomen demonstrating air in portal vein (arrows).

taxanes, particularly paclitaxel. Despite premedication, hypersensitivity reactions may still occur. In these cases, rapid desensitization in the intensive care unit setting or re-treatment may be attempted with care, but the use of alternative agents may be required. Skin testing is used to assess the involvement of IgE in the reaction. Tryptase levels measured at the time of the reaction help to explain the mechanism of the reaction and its severity. Increased tryptase levels indicate underlying mast cell activation. Candidate patients for desensitization include those who have mild to severe hypersensitivity type I, with mast cell-mediated and IgE-dependent reactions occurring during a chemotherapy infusion or shortly thereafter.

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RISK FACTORS AND EPIDEMIOLOGY

The epidemiologic patterns seen in melanoma reflect the genetic and biologic features of melanocytes and their response to environmental ultraviolet radiation (UVR). Clinical features that confer an increased risk for melanoma include: (1) vulnerability to sun damage (light/red coloration of skin, hair, or eyes; photodamaged skin; history of exposure to natural or artificial UVR; prior history of skin cancers of any type); (2) abnormal growth of melanocytes (increased absolute number of nevi, increased size of nevi, or atypical features of moles such as multiple colors, speckles, or shapes); and (3) immunosuppression (innate, functional, or drug-induced). Table 76-1 summarizes melanoma risk factors and the relative risk associated with these factors.

The incidence and mortality rates are strongly influenced by ethnic and geographic/environmental factors, which superimpose substantial variability on melanoma rates. Specifically, the incidence of melanoma is 1/100,000 per year in populations with high skin eumelanin content and up to 27/100,000 per year in populations with low skin eumelanin. Men are affected slightly more than women (1.3:1), and the median age at diagnosis is the late fifties. Melanoma is one of the few cancer types with increasing incidence in the United States and is now the fifth leading cancer in men (60,190 new cases estimated in 2020; probability 1:28) and the sixth leading cancer in women (40,160 new cases estimated in 2020; probability 1:41). Although these rankings are based on the total number of new invasive melanoma cases (100,350 in 2020), an additional 95,710 cases of melanoma in situ are expected to occur in 2020. Given the stable or decreasing mortality (see below), it seems likely that new cases include some that represent overdiagnosis of cancers that would not progress to fatal disease.

Mortality rates begin to rise at age 55, with the greatest mortality in men age >65 years. In contrast to the increasing incidence, the mortality rates for melanoma are decreasing, though this trend appears less dramatic outside of the United States. The reasons for the decrease are not entirely clear but have been attributed in part to the recent success of melanoma therapeutics on survival. After U.S. Food and Drug Administration (FDA) approval of ipilimumab and vemurafenib in 2011, the 1-year relative survival rate increased from 42% (2008–2010) to 55% (2013–2015). The mortality rate from 2013 to 2017 dropped annually by 7% in those aged 20–64 years old and dropped 5–6% per year for individuals aged ≥65 years.

GLOBAL CONSIDERATIONS

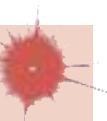
The incidence of both nonmelanoma and melanoma skin cancers around the world has been increasing. Every year, between 2 and 3 million people develop NMSC, and in 2018, there were 300,000 cases of melanoma. A disproportionate number of cases and deaths occur in North America, Europe, Australia, and New Zealand. The highly variable incidence rates of melanoma in different populations are due to the interplay between risk factors, including host genetics and environmental factors, that distribute risk unevenly across these populations and account for the absolute risk in different ethnic groups and geographic areas.

Dark-skinned populations (such as those of India and Puerto Rico), blacks, and East Asians also develop melanoma but at rates 10–20 times lower than those in whites. Cutaneous melanomas in dark-skinned populations are more often diagnosed at a higher stage, and patients tend to have worse outcomes. Surveillance, Epidemiology, and End Results (SEER) data (2000–2004) reveal that whites have the highest incidence of melanoma at 27.2/100,000 and that the incidence drops substantially in Hispanics (4.5/100,000), Native Americans (4.1/100,000), Asians/Pacific Islanders (1.7/100,000), and blacks (1.1/100,000). In nonwhite populations, the frequency of acral (subungual, plantar, palmar) and mucosal melanomas is much higher; the incidence of melanoma in black and Hispanic populations is not associated with ultraviolet (UV) exposure. In China, about 20,000 new melanomas are reported each year, and in contrast to the United States, mortality is increasing. This may be due to the fact that in Asians and dark-skinned populations, more melanomas arise from acral and mucosal areas, which have a different biology and carry a poorer

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Cancer of the Skin

Brendan D. Curti, John T. Vetto,
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MELANOMA

Pigmented lesions are among the most common findings on skin examination. The challenge for the physician is to distinguish benign lesions from cutaneous melanomas and nonmelanoma skin cancers (NMSCs). Both melanoma and NMSC are increasing in frequency, and melanoma accounts for over half of the deaths resulting from skin cancer. Melanoma is an aggressive malignancy of melanocytes, pigment-producing cells that originate from the neural crest and migrate to the skin, meninges, mucous membranes, upper esophagus, and eyes. Melanocytes in each of these locations have the potential for malignant transformation, but the vast majority of melanomas arise in the skin, often permitting detection at a time when complete surgical excision leads to cure. Cutaneous melanoma can occur in people of all ages and all colors. Examples of malignant melanoma of the skin, mucosa, eye, and nail are shown in Fig. 76-1.



FIGURE 76-1 Types of melanoma. *A*, Hypomelanotic melanoma. *B*, Superficial spreading melanoma. *C*, Melanoma arising in a nevus. *D*, Melanoma arising in a nevus. *E*, Nodular melanoma. *F*, Cutaneous melanoma metastases at a surgical margin (also known as melanoma satellites when <2 cm from the primary tumor and in-transit melanoma when >2 cm). *G*, Mucosal melanoma arising in the vulva. *H*, Choroidal melanoma with tumor borders marked by arrowheads, color fundus photograph. *I*, Acral melanoma with Hutchinson's sign on the proximal nail fold.

prognosis than cutaneous melanomas. Little is yet known about the effects of mixed ethnicity on melanoma risk.

■ GENETIC SUSCEPTIBILITY TO MELANOMA

Approximately 20–40% of hereditary melanomas (0.2–2% of all melanomas) are due to germline mutations in the cell cycle regulatory gene cyclin-dependent kinase inhibitor 2A (*CDKN2A*). In fact, 70% of all cutaneous melanomas have mutations or deletions affecting the *CDKN2A* locus on chromosome 9p21. This locus encodes two distinct tumor-suppressor proteins from alternate reading frames: p16 and ARF (p14^{ARF}). The p16 protein inhibits CDK4/6-mediated phosphorylation and inactivation of the retinoblastoma (RB) protein, whereas ARF inhibits MDM2 ubiquitin-mediated degradation of p53. The loss of *CDKN2A* results in inactivation of two critical tumor-suppressor pathways, RB and p53, which control entry of cells into the cell cycle. Several studies have shown an increased risk of pancreatic cancer among melanoma-prone families with *CDKN2A* mutations. A second high-risk locus for melanoma susceptibility, *CDK4*, is located on chromosome 12q13 and encodes the cyclin-dependent kinase inhibited by p16. *CDK4* mutations, which also inactivate the RB pathway, are much rarer than *CDKN2A* mutations. Germline mutations in the melanoma

lineage-specific oncogene microphthalmia-associated transcription factor (*MITF*), BRCA1-associated protein 1 (*BAP-1*), protection of telomeres 1 (*POT-1*), and telomerase reverse transcriptase (*TERT*) also predispose to familial melanoma with a not yet quantified high penetrance, based on families that have been tested.

The melanocortin-1 receptor (*MC1R*) gene is a moderate-risk inherited melanoma susceptibility factor. UVR stimulates the production of melanocortin (α -melanocyte-stimulating hormone [α -MSH]), the ligand for *MC1R*, which is a G-protein-coupled receptor that signals via cyclic AMP and regulates the amount and type of pigment produced by melanocytes. *MC1R* is highly polymorphic, and many among its ~80 variants result in partial or full loss of signaling and lead to the production of non-photoprotective red/yellow pheomelanins, rather than photoprotective brown/black eumelanins. The red hair color (RHC) phenotype produced by *MC1R* mutations includes lightly colored skin, red hair, freckles, increased sun sensitivity, and increased risk of melanoma. In addition to its weak UV-shielding capacity relative to eumelanin, increased pheomelanin production in patients with inactivating polymorphisms of *MC1R* also provides a UV-independent carcinogenic contribution to melanogenesis via oxidative damage and reduced DNA damage repair.

TABLE 76-1 Melanoma Risk Factors and Relative Risk

RISK LEVEL	RISK FACTOR	RELATIVE RISK
Elevated	1 atypical nevus versus 0	1.5
	Total common nevi, 16+ versus <15	1.5
	Blue eye color versus dark	1.5
	Hazel eye color versus dark	1.5
	Green eye color versus dark	1.6
	Light brown hair versus dark	1.6
	Indoor tanning in any gender versus never	1.7
	Fitzpatrick II versus IV	1.8
	Fitzpatrick III versus IV	1.8
	History of sunburn versus no sunburn	2.0
	Blond hair versus dark	2.0
	2 atypical nevi versus 0	2.1
	Fitzpatrick I versus IV	2.1
	High density of freckles versus none	2.1
	Total common nevi 41–60 versus <15	2.2
	Family history of melanoma in 1 or more first-degree relatives	1.7–3.0
	3 atypical nevi versus 0	3.0
	Total common nevi 61–80 versus <15	3.3
Moderately elevated	Red hair versus dark	3.6
	Chronic lymphocytic leukemia	3.9
	History of actinic keratoses and/or keratinocyte carcinoma versus not	4.3
	Indoor tanning in women aged 30–39 versus never	4.3
	4 atypical nevi versus 0	4.4
	Transplant recipient versus not	2.2–4.6
	Indoor tanning in women aged <30 versus never	6.0
	5 atypical nevi versus 0	6.4
	Total common nevi 81–120 versus <15	6.9
	Personal history of melanoma	8.2–13.4
High	CDKN2A mutation carrier	14–28

Other more common, low-penetrance polymorphisms in genes related to pigmentation, nevus count, immune responses, DNA repair, metabolism, and the vitamin D receptor have small effects on melanoma susceptibility. In sum, ~50–60% of the genetic risk for hereditary melanoma can be attributed to known melanoma predisposition genes, with ~40% of the known genetic risk attributable to *CDKN2A*. The other components of inherited risk are most likely due to the presence of additional modifier genes and/or shared environmental exposures of the host.

■ PREVENTION AND EARLY DETECTION

Primary prevention of melanoma and NMSC is based on protection from the sun. Public health initiatives, such as the SunSmart program that started in Australia and is now operative in Europe and the United States, have demonstrated that behavioral change can decrease the incidence of NMSC and melanoma. Preventive measures should start early in life because damage from UV light begins early despite the fact that cancers develop years later. Early episodes of sun burns may be a greater risk than chronic tanning. Some individuals tan compulsively. There is greater understanding of tanning addiction and the cutaneous-neural connections that may give rise to this behavior. Compulsive tanners exhibit differences in dopamine binding and reactivity in reward pathways in the brain, such as the basal striatum, resulting in cutaneous secretion of β -endorphins after UV exposure. Identifying individuals with tanning addiction may be another prevention method. Regular use of broad-spectrum sunscreens that block UVA and UVB

with a sun protection factor (SPF) of at least 30 and protective clothing should be encouraged. Physical blockers such as zinc oxide and titanium dioxide have less likelihood of being absorbed or of generating an allergic reaction than chemical sunscreens. Avoidance of sunburns, tanning beds, and midday sun exposure is recommended.

Secondary prevention comprises education and screening with the goal of early detection and can be individualized based on risk factors. A full-body skin exam is warranted in populations at higher risk for melanoma such as patients with clinically atypical moles (dysplastic nevi) and those with a personal history of melanoma. Surveillance in high-risk patients should be performed by a dermatologist and include total-body photography and dermoscopy where appropriate. Individuals with three or more primary melanomas and families with at least one invasive melanoma and two or more cases of melanoma and/or pancreatic cancer among first- or second-degree relatives on the same side of the family may benefit from genetic testing. Severely atypical nevi and melanoma in situ should be removed. Early detection of small lesions allows the use of simpler treatment modalities with higher cure rates and lower morbidity. Monthly self-screening augments provider-based screening. Patients should be taught to recognize the clinical features of melanoma and advised to report any change in a pigmented lesion. There is evidence supporting the ability of media campaigns to reduce cancer mortality in lung cancer, and results from Australia's skin cancer campaigns demonstrate improvement in attitude and behavior and a reduction in melanoma incidence. A benefit/cost analysis in Australia showed a return of \$3.85 for every \$1 invested. Although the U.S. Preventive Services Task Force states that there is insufficient evidence to recommend skin screening for the general population, additional research is anticipated to find best practices for skin cancer detection and prevention.

■ DIAGNOSIS

The goal is to identify a melanoma before it becomes invasive and life-threatening metastases have occurred. Early detection may be facilitated by applying the ABCDEs: *asymmetry* (benign lesions are usually symmetric); *border irregularity* (most nevi have clear-cut borders); *color variegation* (benign lesions usually have uniform light or dark pigment); *diameter >6 mm* (the size of a pencil eraser); and *evolving* (any change in size, shape, color, or elevation or new symptoms such as bleeding, itching, and crusting). In addition, any nevus that appears atypical and different from the rest of the nevi on that individual (an “ugly duckling”) should be considered suspicious.

The entire skin surface, including the scalp and mucous membranes, as well as the nails should be examined in each patient. Bright room illumination is important, and a hand lens or dermatoscope is helpful for evaluating variation in pigment pattern. Any suspicious lesions should be biopsied, evaluated by a specialist, or recorded by chart and/or photography for follow-up. Dermoscopy employs low-level magnification of the epidermis with polarized light or water interface and permits a more precise visualization of patterns of pigmentation than is possible with the naked eye (Fig. 76-2).

Biopsy Any pigmented cutaneous lesion that has changed in size or shape or has other features suggestive of malignant melanoma is a candidate for biopsy. An excisional biopsy with 1- to 3-mm margins (narrow-margin excision) is suggested. This facilitates histologic assessment of the lesion, permits accurate measurement of thickness if the lesion is melanoma, and constitutes definitive treatment if the lesion is benign. For lesions that are large or on anatomic sites where excisional biopsy may not be feasible (such as the face, hands, and feet), an incisional biopsy (partial biopsy) through the most nodular or darkest area of the lesion is acceptable. Incisional biopsy does not appear to facilitate the spread of melanoma. For suspicious lesions, every attempt should be made to preserve the ability to assess the deep and peripheral margins and to perform immunohistochemistry. Shave, saucerization, or punch biopsies are an acceptable alternative, particularly if the suspicion of malignancy is low. They should be deep enough to include the deepest component of the entire lesion, and any pigment at the base of the lesion should be removed and included with the biopsy specimen.

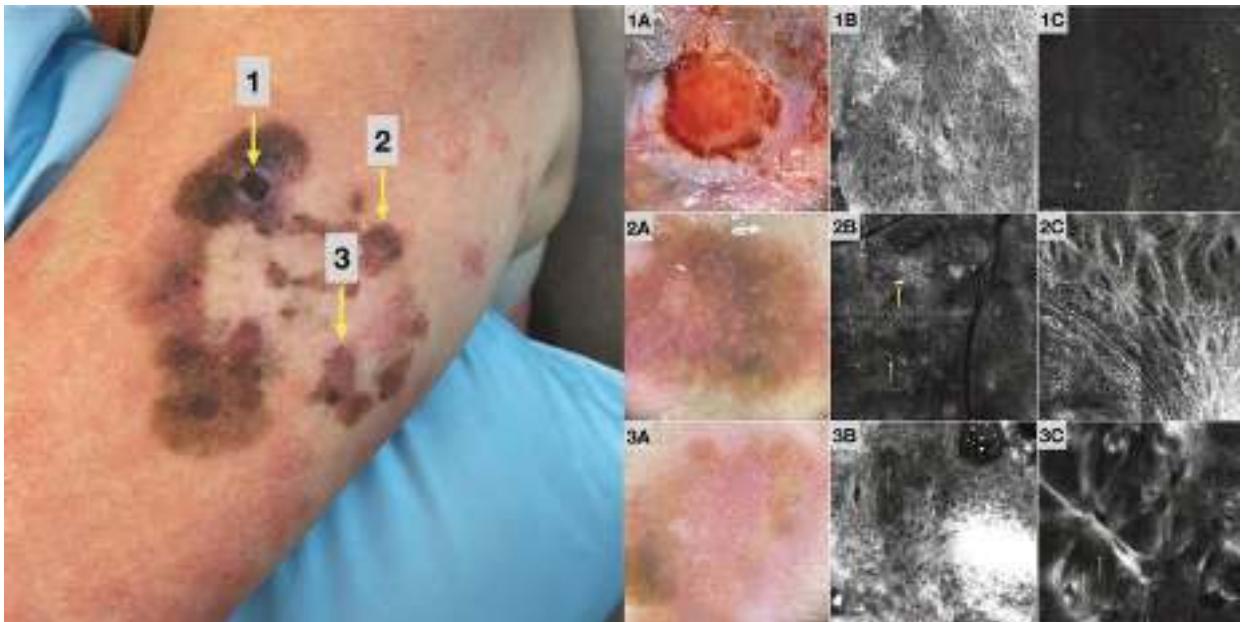


FIGURE 76-2 Clinical and confocal diagnostic findings of melanoma. *Left panel:* A clinical image of a large melanoma of a 60-year-old woman used to illustrate classic features of nodular melanoma (1), superficial spreading melanoma (2), and amelanotic melanoma (3). Panels 1A, 2A, and 3A correspond to the dermoscopy images taken at sites 1, 2, and 3, respectively (Sklip Dermatoscope, Sklip LLC, Las Vegas, NV). Panels 1B, 1C, 2B, 2C, 3B, and 3C are reflectance confocal microscopy images of the epidermis and upper dermis taken at sites 1, 2, and 3, respectively (Vivascope 1500 Gen 4, Caliber I.D., Rochester, NY). 1A. Site 1 dermoscopy shows a pink nodule with polymorphous vessels and ulceration with active bleeding consistent with malignancy. 1B. Site 1 reflectance confocal microscopy of the epidermis shows an atypical enlarged honeycombed pattern frequently seen in melanoma. 1C. Site 1 reflectance confocal microscopy of the upper dermis shows cerebriform nests (*) seen in nodular melanoma. 2A. Site 2 dermoscopy shows a pigmented area with an atypical, thickened network, blue-gray structures, and polymorphous vessels. 2B. Site 2 reflectance confocal microscopy of the epidermis shows an irregular honeycombed pattern and pagetoid cells with nuclei (↑) typically seen in a superficial spreading melanoma. 2C. Site 2 reflectance confocal microscopy of the dermoepidermal junction shows thickened junctional nests with bright reflective linear dendritic cells (*). 3A. Site 3 dermoscopy shows an amelanotic area within the melanoma with milky red areas, polymorphous vessels, atypical network, and blue-gray structures classic for an amelanotic melanoma. 3B. Site 3 reflectance confocal microscopy of the epidermis shows an irregular enlarged honeycombed pattern, dermal nests protruding upward into the epidermis (↑), and artefacts (*). 3C. Site 3 reflectance confocal microscopy image of the dermoepidermal junction shows thickened collagen bundles (*) with atypical polymorphous vessels (↑).

Punch biopsies are more likely to clear the deep margin but more likely to be positive at the radial margins; the opposite is true for shave biopsies. The choice of biopsy type should be guided by which is most likely to remove the entire lesion for histologic evaluation.

The biopsy should be read by a pathologist experienced in pigmented lesions, and the report should include Breslow thickness, mitotic rate, presence or absence of ulceration, lymphatic invasion, regression, microsatellitosis, and the status of the peripheral and deep margins. Breslow thickness is the greatest thickness of a primary cutaneous melanoma measured on the slide from the top of the epidermal granular layer, or from the ulcer base, to the bottom of the tumor. To

distinguish melanomas from benign nevi in challenging cases, fluorescence *in situ* hybridization with multiple probes or comparative genomic hybridization can be helpful. Gene expression profile (GEP) assays have been developed to determine prognosis and are commercially available.

CLASSIFICATION AND PATHOGENESIS

Clinical Five major types of cutaneous melanoma are described in Table 76-2. In *superficial spreading melanoma*, *lentigo maligna melanoma*, and *acral lentiginous melanoma*, the lesion has a period of

TABLE 76-2 Major Histologic Subtypes of Malignant Melanoma

TYPE	SITE	APPEARANCE	ASSOCIATED MUTATIONS
Lentigo maligna	Sun-exposed surfaces, particularly malar region and temple	In flat portions, brown and tan predominate, but whitish gray sometimes present; in nodules, reddish brown, bluish gray, bluish black.	BRAF 28% NRAS 15%
Superficial spreading	Any (more common on upper back and, in women, lower legs)	Brown mixed with bluish red, bluish black, reddish brown, and often whitish pink. The lesion border is often visibly and/or palpably raised.	BRAF 57% NRAS 18%
Nodular	Any	Reddish blue, purple, or bluish black; can be uniform or mixed with brown and black.	BRAF 47% NRAS 33%
Acral lentiginous	Palm, sole, nail bed, mucous membrane	In flat portions, dark brown; in raised lesions (plaques), brown-black or blue-black.	NRAS 25% c-KIT 5-10% BRAF 10%
Desmoplastic	Any (more common on head and neck)	Highly variable; pigmentation is frequently absent. Can mimic nodular basal cell carcinoma.	MAPK and PI3K 73% High tumor mutational burden, BRAF and NRAS uncommon

Driver Mutations

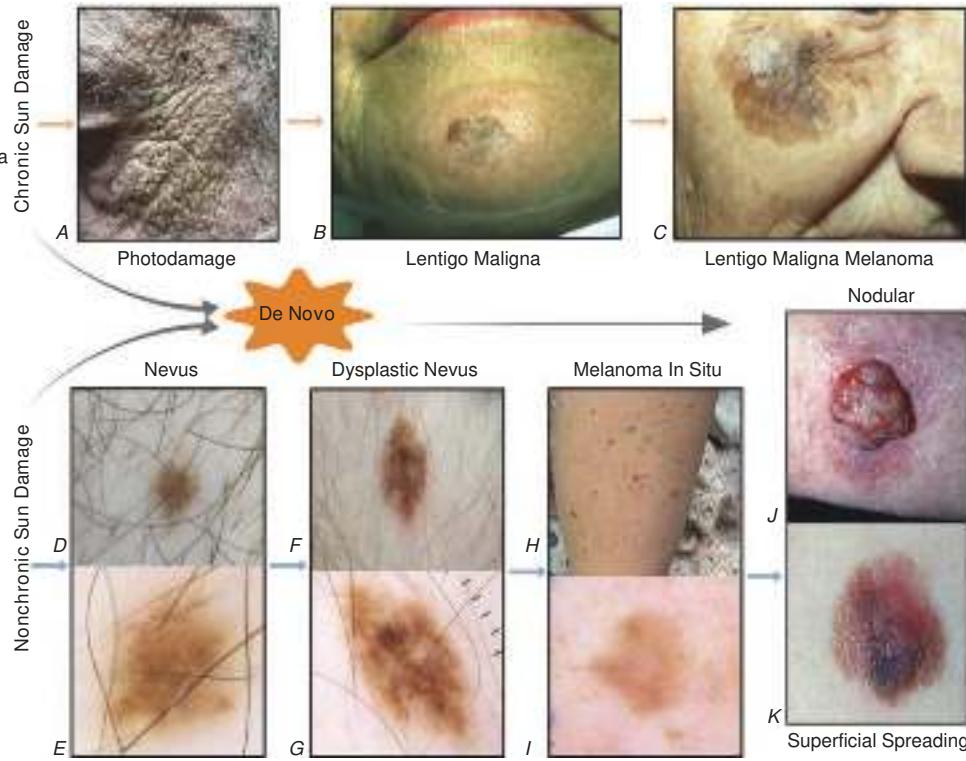
BRAF: 10%*NRAS*: 10%*C-KIT*: 5–10%*NF1*: 48% of *BRAF*and *NRAS* WT melanoma
in older patients*BRAF*: 50%*NRAS*: 20%*C-KIT*: 0%

FIGURE 76-3 Cutaneous melanoma development and associated driver mutations. Chronic sun damage (A) predisposes to a lentigo maligna (in situ) (B), which can evolve into lentigo maligna melanoma (invasive) (C). Similarly, nonchronic sun damage can initiate melanoma de novo or in nevomelanocytes, where clinical and histologic changes of atypia may be seen prior to complete transformation. Nevi (D, E) can evolve into atypical lesions (F, G), in situ melanoma (H, I), and eventually invasive nodular (J) or superficial spreading melanomas (K).

superficial (radial) growth during which it increases in surface area but does not penetrate deeply and is most capable of being cured by surgical excision. Melanomas with a radial growth phase are characterized by irregular and sometimes notched borders, variation in pigment pattern, and variation in color. *Nodular melanoma* does not have a radial growth phase but usually presents with penetration deep into the skin (vertical growth phase). *Desmoplastic melanoma* is associated with a fibrotic response, neural invasion, and a greater tendency for local recurrence. Occasionally, melanomas appear clinically to be amelanotic (not pigmented), in which case the diagnosis is established microscopically after biopsy.

Although these subtypes are clinically distinct, they are primarily of historical interest because this classification has minimal prognostic value and is not part of American Joint Committee on Cancer (AJCC) staging. Characterizing the genomic and mutational profiles of melanoma has become increasingly common, informs prognosis, reflects the mechanisms of tumorigenesis, and may influence surveillance strategies and treatment.

Genomic The advent of next-generation sequencing has led to whole exome sequencing of hundreds of cutaneous melanomas derived from nonglabrous skin. This has revealed very complex genomic changes resulting from both germline (see “Genetic Susceptibility to Melanoma” above) and somatic mutations. Cutaneous melanomas have one of the highest somatic mutation rates (>10 mutations/Mb) among all cancers; the majority (76% of primary tumors and 84% of metastatic melanomas) exhibit mutations indicative of UVR exposure. The mutation rate varies based on body site; melanomas arising in chronic sun-damaged skin harbor substantially more mutations than melanomas from non-sun-damaged skin.

Melanomas can harbor thousands of mutations, but only a few are “driver” mutations that promote cell proliferation or inhibit normal

pathways of apoptosis or DNA repair and confer a growth advantage to the neoplastic cell. Some of the driver mutations for cutaneous melanoma are depicted in Fig. 76-3 along with the clinical evolution of melanoma lesions. Driver mutations are often found in combination with mutations to germline susceptibility genes such as *p16*, which affect cell cycle arrest, and *ARF*, which result in defective apoptotic responses to genotoxic damage. The altered melanocytes accumulate DNA damage and develop the malignant phenotype characterized by invasion, metastasis, and angiogenesis.

A genomic classification of cutaneous melanoma has been proposed based on the pattern of the most prevalent mutated genes, *BRAF*, *RAS*, and *NFI*, along with a triple wild-type (WT) in which no mutations in these three genes are found. The pattern of DNA mutations can vary with the site of origin and is independent of the histologic subtype of the tumor. An important aspect of this classification is that the mutational profile can guide therapy. The proliferative pathways affected by the mutations include the mitogen-activated protein (MAP) kinase and phosphatidylinositol 3' kinase/AKT pathways. *RAS* and *BRAF*, members of the MAP kinase pathway, which mediate the transcription of genes involved in cell proliferation and survival, undergo somatic mutation in melanoma and thereby generate potential therapeutic targets. *NRAS* is mutated in ~20% of melanomas, and somatic activating *BRAF* mutations are found in most benign nevi and 40–50% of cutaneous melanomas. Neither mutation by itself appears to be sufficient to cause melanoma; thus, they often are accompanied by other mutations, such as *TERT*. The *BRAF* mutation is most commonly a T→A point mutation that results in a valine-to-glutamate amino acid substitution (V600E). V600E *BRAF* mutations are more common in younger patients and are present in most melanomas that arise on sites with intermittent sun exposure and are less common in melanomas from chronically sun-damaged skin (i.e., those of older patients).

Melanomas may harbor mutations in *AKT* (primarily in *AKT3*) and *PTEN* (phosphatase and tensin homolog). *AKT* can be amplified, and

PTEN may be deleted or undergo epigenetic silencing that leads to constitutive activation of the PI3K/AKT pathway and enhanced cell survival by antagonizing the intrinsic pathway of apoptosis. A loss-of-function mutation in *NFL*, which can affect both the MAP kinase and PI3K/AKT pathways, has been described in 10–15% of melanomas. In melanoma, these two signaling pathways (MAP kinase and PI3K/AKT) enhance tumorigenesis, chemoresistance, migration, and cell cycle dysregulation.

■ PROGNOSTIC FACTORS

The most important prognostic factors for a newly diagnosed patient are incorporated in the staging classification. The best predictor of recurrence is Breslow thickness, followed by ulceration, which together make up the T stage of the AJCC system for melanoma. The anatomic site of the primary tumor is also prognostic; favorable sites are the forearm and leg, and unfavorable sites include the scalp, hands, feet, and mucous membranes. Women with stage I or II disease have better survival than men, perhaps in part because of earlier diagnosis; women frequently have melanomas on the lower leg, where self-recognition is more likely and the prognosis is better.

Older individuals, especially men >60, have a tendency toward delayed diagnosis (and thus thicker tumors), have more head and neck and acral melanomas (which tend to have earlier vertical growth and distant metastases), and are more likely to develop melanomas in chronically UVR-damaged skin (which are more often *BRAF* wild type, with fewer options for therapy). Other important adverse factors include high mitotic rate, microscopic evidence of regression, and lymphatic/vascular invasion. Clinical features such as microsatellite lesions and/or in-transit metastases, evidence of nodal involvement, elevated serum lactate dehydrogenase (LDH), and presence and site of distant metastases all portend a higher stage and worse prognosis.

GEPs and machine-learning algorithms that associate genomic changes with clinical outcomes have been used to estimate the prognosis of melanoma. A commercially available 31-gene GEP is available that predicts for all-site (particularly distant) relapse and incorporates the increased and decreased expression, as well as the dysregulation, of genes involved in many of the cellular processes leading to melanoma progression described earlier. Although this 31-gene GEP can estimate the probability of distant relapse, it has not supplanted the prognostic estimates derived from surgical staging. It is anticipated that GEPs will be incorporated into future cutaneous melanoma management guidelines, as they have been for uveal melanoma, breast cancer, thyroid cancer, and other malignancies.

■ STAGING

Once the diagnosis of melanoma has been made, the tumor is staged to determine the prognosis and aid in treatment selection. The current melanoma staging criteria and estimated 10-year survival by stage are depicted in Table 76-3. The clinical stage is determined after the microscopic evaluation of the melanoma skin lesion and clinical and radiologic assessment. The pathologic stage also includes microscopic examination of clinically negative regional lymph nodes obtained at sentinel lymph node biopsy (SLNB), any enlarged nodes found on exam or imaging, and any suspected metastases amenable to open or image-guided biopsy.

All patients should have a complete history, with attention to symptoms that suggest metastatic disease, such as new palpable masses, malaise, weight loss, headaches, changes in vision or bowel habits, hemoptysis, and pain. The provider should look for persistent disease at the biopsy site, dermal or subcutaneous nodules that could represent satellite or in-transit metastases, and lymphadenopathy. A complete blood count, complete metabolic panel, and LDH should be performed. Although these tests rarely help uncover occult metastatic disease, a microcytic anemia would raise the possibility of bowel metastases, elevated liver function tests can suggest liver metastases, and LDH is part of the AJCC system for stage IV disease. Abnormal test results should prompt a more extensive evaluation, including computed tomography (CT) scan or a positron emission tomography (PET) scan (or CT/PET combined).

TABLE 76-3 Staging and Survival

STAGE	TNM	10-YEAR MELANOMA-SPECIFIC SURVIVAL ESTIMATE
0	TisN0M0	>99%
IA	T1aN0M0, T1bN0M0	98–96%
IB	T2aN0M0	92%
IIA	T2b-T3aN0M0	88%
IIB	T3b-T4aN0M0	81–83%
IIC	T4bN0M0	75%
IIIA	T1a-T2aN1a-2aM0	88%
IIIB	T2b-T3aN1a-N2bM0	77%
IIIC	T3b-4bN1a-N3cM0	60%
IID	T4bN3a-N3cM0	24%
IVM1a	Any T, any N, skin, soft tissue, or distant nodal sites	50% at 5 years
IVM1b	Any T, any N, lung + any M1a sites	35–50% at 5 years
IVM1c	Any T, any N, skin, non-CNS visceral disease, any M1a or M1b sites	~25% at 5 years
IVM1d	Any T, any N, CNS metastasis + any M1a,b,c sites	<5% at 5 years

Abbreviations: CNS, central nervous system; TNM, tumor-node-metastasis.

Despite all the above considerations, >80% of patients at presentation will have disease confined to the skin and a negative history and physical examination, in which case imaging is not indicated. Although controversial, an exception is sometimes made for very-high-risk primaries (e.g., >4 mm with ulceration) in which the chance for occult distant metastases is higher than that for a positive SLNB.

TREATMENT

Melanoma

MANAGEMENT OF CLINICALLY LOCALIZED MELANOMA (STAGE I, II)

For a newly diagnosed cutaneous melanoma, wide surgical excision of the lesion with a margin of normal skin is necessary to remove all malignant cells and minimize the probability of local recurrence. The National Comprehensive Cancer Network (NCCN), based on data from six randomized trials, recommends the following radial margins for a primary melanoma: *in situ*, 0.5–1.0 cm; invasive up to 1 mm thick, 1 cm; >1.0–2 mm, 1–2 cm; and >2 mm, 2 cm. Smaller margins may be used for special locations such as the face, hands, feet, and genitalia due to the higher likelihood of morbidity in these regions. In all instances, however, inclusion of subcutaneous fat in the surgical specimen facilitates adequate thickness measurement and assessment of surgical margins by the pathologist. When feasible, excision should go down to fascia, with fascial resection for thick (T4) lesions. Topical imiquimod can be used to treat lentigo maligna in cosmetically sensitive locations with narrow resection margins by promoting local immune response resulting in decreased local recurrence.

SLNB is a valuable staging tool providing prognostic information to identify patients at high risk for relapse who may be candidates for adjuvant therapy. The first (sentinel) draining node(s) from the primary site is (are) located by injecting a blue dye and a gamma-emitting radioisotope around the primary site. The sentinel node(s) then is (are) identified using a handheld gamma detector brought sterilely into the operative field. The surgeon makes an incision of the area of uptake and looks for the blue-stained, “hot” node(s), which is (are) removed and subjected to histopathologic analysis with serial sectioning using hematoxylin and eosin and immunohistochemical stains (e.g., S100, HMB45, MART-1, and MelanA) to identify melanocytes.

NCCN guidelines recommend SLNB to patients with a 10% or greater chance of having tumor in the node. This includes patients

with tumors >1 mm thick (T2) or T1 tumors that have ulceration (T1b). Patients with a 5–10% risk of node positivity, such as those with tumors measuring between 0.75 and 1.0 mm, transected tumors, regressed tumors, or lymphovascular invasion, should also be considered for SLNB. The NCCN does not recommend SLNB for patients with a risk of a positive SLNB ≤5% such as those with melanomas ≤0.75 mm thick and no high-risk features. In these patients, wide excision alone is the usual definitive therapy.

Patients whose SLNB is negative can either be followed or considered for a clinical trial if the primary lesion is considered high risk (i.e., stages IIB and IIC). Patients with a positive sentinel lymph node should undergo imaging (CT or PET scanning) to rule out distant metastatic disease, and if none is found (i.e., stage III), adjuvant therapy on or off a clinical study should be offered (see next section). Complete lymphadenectomy for a positive sentinel lymph node has been shown to improve relapse-free but not overall survival, and therefore, it is no longer offered routinely. This avoids the morbidity of regional node dissection in most patients. However, patients not undergoing immediate completion node dissection should have nodal bed surveillance with physical examination and ultrasound at 4- to 6-month intervals for approximately 3 years to rule out isolated nodal bed progression. Complete node dissection is therefore still offered to patients who cannot comply with follow-up and/or forgo adjuvant therapy.

MANAGEMENT OF REGIONALLY METASTATIC MELANOMA (STAGE III)

Patients with a positive sentinel lymph node, resected regional nodal macrometastases, or resected locoregional disease (e.g., recurrences in the wide excision site, within 2 cm of the site [“satellite metastases”], or >2 cm from the site [“in-transit metastases”]) are all considered as having stage III disease. Even after complete resection of stage III disease, the risk for progression to distant metastases (stage IV) may be high, and adjuvant systemic therapy should be offered. Melanomas may recur at the edge of the incision or graft, as satellite metastases, in-transit metastases, or most commonly, regional spread to a draining lymph node basin. Each of these presentations is managed surgically and, increasingly, with post-surgical adjuvant immunotherapy or targeted therapy, after which there is the possibility of long-term disease-free survival. Topical therapy with imiquimod has been useful for patients with low-volume dermal lesions. Talimogene laherparepvec is an engineered, oncolytic herpes simplex virus type 1 that is FDA approved for injection of primary or recurrent melanomas including cutaneous and subcutaneous lesions or lymph node deposits that cannot be completely removed by surgery.

Stage III patients rendered free of disease after surgery are at risk for local or distant recurrence and should be offered adjuvant therapy. Radiotherapy can reduce the risk of local recurrence after lymphadenectomy but does not influence overall survival. Patients with large nodes (>3–4 cm), four or more involved lymph nodes, or extranodal spread on microscopic examination should be considered for radiation. Systemic adjuvant therapy is indicated primarily for patients with stage III disease, but high-risk, node-negative patients (>4 mm thick or ulcerated lesions) and patients with completely resected stage IV disease also may benefit.

Current options for adjuvant therapy include anti-PD-1 (nivolumab or pembrolizumab) or targeted therapy in melanomas that express a *BRAF* V600 mutation. Both anti-PD-1 and targeted therapy have been shown to confer disease-free and overall survival benefits in stage III and stage IV melanoma (see below for further discussion). Other agents such as ipilimumab and interferon α 2b (IFN- α 2b) have been used in the adjuvant setting, but due to a higher percentage of immune-mediated side effects in the case of ipilimumab and limited efficacy in the case of interferon, they have been supplanted by better alternatives. Ongoing clinical trials are comparing systemic therapy before surgery (neoadjuvant) with adjuvant treatment, the optimal sequence of immunotherapy and targeted therapies, and the utility of anti-PD-1 in high-risk stage

II melanoma. GEP may help to identify patients with stage II or III melanoma who are at lower risk of recurrence and could avoid the toxicity and expense of adjuvant therapy.

TREATMENT

Metastatic Disease

At diagnosis, 84% patients with melanoma will have stage I or II disease and 4% will present with metastases. Many others will develop metastases after initial therapy for locoregional disease. The probability of recurrence is related to initial stage, ranging from <5% with stage IA to >90% for subsets of patients with stage IIID disease at presentation. Patients with a history of melanoma who develop signs or symptoms suggesting recurrent disease should undergo restaging as described earlier. Distant metastases (stage IV) commonly involve skin and lymph nodes as well as viscera, bone, or the brain. The prognosis is better for patients with skin and subcutaneous metastases (M1a) than for lung (M1b) and worst for those with metastases to bone or other visceral organs (M1c) or brain (M1d). An elevated serum LDH is a poor prognostic factor and places the patient in stage M1c regardless of the metastatic sites. The 15-year survival of patients with melanoma was <10% before 2010; however, the development of targeted therapy and immunotherapy has improved disease-free and overall survival, especially for patients with M1a and M1b disease, such that currently the 15-year survival exceeds 25%. Even patients with M1c disease may have prolonged survival, and those who are progression-free for >2 years after immunotherapy or targeted therapy have a high probability of living >5 years from the onset of metastasis.

FDA-approved agents since 2011 include three immune T-cell checkpoint inhibitors (ipilimumab, nivolumab, and pembrolizumab), combination immunotherapy (ipilimumab plus nivolumab), six oral agents that target the MAP kinase pathway (the *BRAF* inhibitors vemurafenib, dabrafenib, and encorafenib, and the MEK inhibitors trametinib, cobimetinib, and binimetinib), and the oncolytic virus talimogene laherparepvec (Table 76-4).

Local modalities, such as surgery and stereotactic radiosurgery, should be considered for patients with oligometastatic disease because they may experience long-term disease-free survival after metastectomy or ablative high-dose-per-fraction radiation. Patients with solitary metastases are the best candidates, but local modalities can also be used for patients with metastases at more than one site if a complete resection or treatment of all sites can be achieved with reasonable side effects. Patients rendered free of disease can be considered for adjuvant therapy or a clinical trial because their risk of developing additional metastases remains high. Surgery can also be used as an adjunct to systemic therapy when, for example, a few of many metastatic lesions prove resistant to

TABLE 76-4 Treatment Options for Metastatic Melanoma

Immunotherapy

Immune checkpoint blockade

Anti-PD-1: pembrolizumab or nivolumab

Anti-CTLA-4: ipilimumab

Combined ipilimumab and nivolumab

Cytokine-based immunotherapy

High-dose interleukin 2

Oncolytic virus

Talimogene laherparepvec

Targeted therapies

BRAF inhibitors: vemurafenib, dabrafenib, encorafenib

MEK inhibitors: trametinib, cobimetinib, binimetinib

Local modalities

Surgery

Stereotactic radiation

immunotherapy; it may also be helpful to obtain tumor to establish the mutational profile of the recurrent melanoma.

IMMUNOTHERAPY

Checkpoint Blockade Immunotherapies are based on an understanding of the control mechanisms of the normal immune response. Inhibitory receptors or checkpoints, including CTLA-4 and PD-1, are upregulated on T cells after engagement of the T-cell receptor by cognate tumor antigen in the context of the appropriate class I or II HLA molecules during the interaction between a T cell and antigen-presenting cell. Immune checkpoints are an absolute requirement to ensure proper regulation of a normal immune response; however, the continued expression of inhibitory receptors during chronic infection (hepatitis, HIV) and in cancer patients leads to exhausted T cells with limited potential for proliferation, cytokine production, or cytotoxicity. Checkpoint blockade with an antagonistic monoclonal antibody results in improved T-cell function and eradication of tumor cells in preclinical animal models. Ipilimumab, a fully human IgG1 antibody that binds CTLA-4 and blocks inhibitory signals, was the first drug shown in a randomized trial to improve survival in patients with metastatic melanoma. Although response rates are low (about 10%), overall survival is improved. Anti-CTLA-4 monotherapy has been supplanted by combination anti-CTLA-4 plus anti-PD-1 or anti-PD-1 monotherapy due to enhanced survival and, in the case of anti-PD-1 monotherapy, better patient tolerance, as detailed below.

Chronic T-cell activation also leads to induction of PD-1 on the surface of T cells. Expression of one of its ligands, PD-L1, on tumor cells can protect them from immune destruction. Blockade of the PD-1:PD-L1 axis by intravenous (IV) administration of anti-PD-1 or anti-PD-L1 has substantial clinical activity, including cure, in some patients with advanced melanoma and other solid tumors with significantly less toxicity than ipilimumab. The PD-1 blockers, nivolumab and pembrolizumab, have been approved to treat patients with advanced melanoma. Combination T-cell checkpoint therapy, blocking both inhibitory pathways with ipilimumab and nivolumab, leads to superior antitumor activity compared to treatment with either agent alone. Combined therapy with IV ipilimumab and nivolumab is administered in the outpatient setting every 3 weeks for four doses (induction), followed by nivolumab given every 2–4 weeks (maintenance) for up to 1 year, and is associated with an objective response rate of 56% and enhanced survival compared to ipilimumab monotherapy. There may be subsets of patients, specifically those who have >5% expression of PD-1 on T cells in a melanoma biopsy sample, who derive a similar level of clinical benefit from nivolumab monotherapy, although using PD-1 expression to select therapy remains problematic as some patients whose melanoma has no detectable PD-1 expression can still respond to immunotherapy.

T-cell checkpoint antibodies can also interfere with normal immune regulatory mechanisms, which may produce a novel spectrum of side effects. The most common immune-related adverse events were skin rash and diarrhea (sometimes severe, life-threatening colitis), but toxicity can involve almost any organ (e.g., thyroiditis, hypophysitis, hepatitis, nephritis, pneumonitis, myocarditis, neuritis). The severity and frequency of toxicity are greatest with combination T-cell checkpoint antibody therapy, followed by anti-CTLA-4 and then anti-PD-1 monotherapies. Vigilance, interruption of therapy, and early intervention with steroids or other immunosuppressive agents, such as anti-tumor necrosis factor antibodies or mycophenolate mofetil, can mitigate toxicity and prevent permanent organ damage. The management of drug-induced toxicity with immunosuppressive agents does not appear to interfere with antitumor activity, and benefit is manifest even in patients who have to discontinue immunotherapy due to immune-mediated toxicity. The use of T-cell checkpoint antibodies for metastatic melanoma has become commonplace, but there is controversy about whether all patients need combined anti-CTLA-4 and anti-PD-1, whether biomarkers can be used to select patients who may benefit

from anti-PD-1 alone, and the best sequence of targeted therapy and immunotherapy in patients whose melanomas have a *BRAF* mutation. There is also a significant economic impact with any anticancer therapy, which must be placed in the context of the survival benefit.

TARGETED THERAPY

The RAS-RAF-MEK-ERK pathway delivers proliferation and survival signals from the cell surface to the cytoplasm and nucleus and is mutated in approximately 50% of melanomas. Inhibitors of BRAF and MEK can induce regression of melanomas that harbor a *BRAF* mutation. Three BRAF inhibitors, vemurafenib, dabrafenib, and encorafenib, have been approved for the treatment of patients whose stage IV melanomas harbor a mutation at position 600 in *BRAF*. Monotherapy with BRAF inhibitors has been supplanted with combined BRAF and MEK inhibition to address the rapid adaptation of the majority of melanomas that use MAP kinase pathway reactivation to facilitate growth when BRAF is inhibited. Combined therapy with BRAF and MEK inhibitors (dabrafenib and trametinib, vemurafenib with cobimetinib, or encorafenib and binimetinib) improved progression-free and overall survival compared to monotherapy with a BRAF inhibitor. Long-term results of inhibition of the MAP kinase pathway confirm that some patients achieve long intervals of disease control, yet the major limitation of both monotherapy and combined therapy appears to be the acquisition of resistance; the majority of patients relapse and eventually die. The mechanisms of resistance are diverse and reflect the genomic heterogeneity of melanoma; however, most instances involve reactivation of the MAPK pathway, often through *RAS* mutations or mutant *BRAF* amplification. Patients who develop resistance to BRAF and MEK inhibition are candidates for immunotherapy or clinical trials.

Targeted therapy is accompanied by manageable side effects that differ from those experienced during immunotherapy or chemotherapy. A class-specific side effect of BRAF inhibitor monotherapy is the development of hyperproliferative skin lesions, some of which are well-differentiated squamous cell skin cancers (SCCs) occurring in up to 25% of patients. Paradoxical activation of the MAP kinase pathway occurs from BRAF inhibitor-mediated changes in *BRAF* wild-type cells, and the activation is blocked by MEK inhibitor, which explains why these lesions occur much less frequently during combined therapy. Metastases of the treatment-induced SCCs have not been reported, and BRAF and MEK inhibitors can be continued safely following simple excision of the SCCs. Cardiac and ocular toxicities, although infrequent, can occur with BRAF and MEK inhibitors and require medical evaluation, management, and usually discontinuation of targeted therapy.

Activating mutations in the c-kit receptor tyrosine kinase are found in a minority of cutaneous melanomas with chronic sun damage but are more common in mucosal and acral lentiginous subtypes. Overall, the number of patients with *c-kit* mutations is small, but when present, they are similar to those found in gastrointestinal stromal tumors and melanomas with activating *c-kit* mutations and can have clinically meaningful responses to imatinib. The probability of objective response in patients whose melanomas harbor a *c-kit* mutation is 29%. *N-RAS* mutations occur in 15–20% of melanomas. At present, there are no effective targeted agents for these patients, but *N-RAS* inhibitors are being investigated in clinical trials.

Other systemic therapies used to treat stage IV melanoma patients include high-dose interleukin 2, which is also associated with durable remissions in some patients. Chemotherapy with dacarbazine or taxanes is infrequently used, and clinical trials remain an important option for patients with advanced melanoma.

INITIAL APPROACH TO PATIENT WITH METASTATIC DISEASE

Upon diagnosis of stage IV disease, a sample of the patient's tumor should be submitted for molecular testing to determine whether a *BRAF* or *c-kit* mutation is present. Analysis of a metastatic lesion biopsy (if possible) is preferred, but any sample will suffice because

there is little discordance between primary and metastatic lesions. Treatment algorithms start with the tumor's *BRAF* status. For *BRAF* wild-type tumors, immunotherapy is recommended. For patients whose tumors harbor a *BRAF* mutation, initial therapy with either combination *BRAF* and MEK inhibitors or immunotherapy is acceptable. Combined therapy with *BRAF* and MEK inhibitors is recommended for patients with rapidly growing and symptomatic disease when a *BRAF* mutation is present. The sequence of immunotherapy and targeted therapy that confers the greatest survival benefit in patients with minimally symptomatic melanoma is not yet known, but ongoing randomized phase III trials should answer this important question. Despite improvements in therapy, the majority of patients with metastatic melanoma will not be cured, so enrollment in a clinical trial is always an important consideration, even for previously untreated patients.

Clinical trials should be considered for patients with stage IV disease who experience tumor progression despite current therapy. Many will be poor candidates for therapy because of extensive disease burden, poor performance status, or concomitant illness; thus, the timely integration of palliative care and hospice remains an important element of care.

FOLLOW-UP

Skin examination and surveillance at least once a year are recommended for all patients with melanoma. Routine blood work and imaging for patients with stage IA-IIA disease is not recommended unless symptoms are present. Surveillance diagnostic imaging can be considered in patients with stage III high-risk disease but is mainly reserved for patients with signs or symptoms of recurrent disease or to follow response to therapy. For stage-specific recommendations, please consult the NCCN guidelines (see ‘Further Reading’).

NONMELANOMA SKIN CANCERS

NMSCs (mostly SCCs and basal cell cancer [BCC]) are the most common cancers in the United States. Although tumor registries do not routinely gather data on the incidence of NMSCs, it is estimated that the annual incidence is more than 5.3 million cases in the United States; SCCs and BCCs account for 80% and 18%, respectively. While less common, the incidence of Merkel cell carcinoma (MCC) has tripled over the past 20 years. There are now an estimated 1600 cases per year with an annual increase in incidence of 8%. While all forms of NMSCs can metastasize, MCCs do this most commonly, with sentinel lymph node positivity rates of 25% (compared to 12–19% for melanoma) and mortality rates approaching 33% at 3 years. SCCs, particularly those with high-risk features, can also metastasize and account for 2400 deaths annually.

PATHOPHYSIOLOGY AND ETIOLOGY

Similar to melanoma, the most significant cause of NMSCs is UVR, with a dose-response relationship between tanning bed use and the incidence of NMSC. As few as four tanning bed visits per year confers a 15% increase in BCC and an 11% increase in SCC. The risk of lip or oral SCC is increased with cigarette smoking and, like SCC of the ear, has a worse prognosis than that seen on other body sites. Human papillomaviruses and UVR may act as co-carcinogens. Inherited disorders of DNA repair, such as xeroderma pigmentosum, are associated with a greatly increased incidence of skin cancer and help to establish the link between UV-induced DNA damage, inadequate DNA repair, and skin cancer.

The genes damaged most commonly by UV in SCC include *p53* and *N-RAS*, whereas BCC is associated with damage to hedgehog signaling pathway (Hh) genes, which lead to basal cell proliferation. This is usually the result of loss of function of the tumor-suppressor patched homolog 1 (*PTCH1*), which normally inhibits the signaling of smoothened homolog (*SMO*). Two oral *SMO* inhibitors, vismodegib and sonidegib, have been approved by the FDA to treat advanced inoperable or metastatic BCC and locally advanced BCC that has recurred following surgery or radiotherapy, respectively. Vismodegib

also reduces the incidence of BCC in patients with basal cell nevus syndrome who have *PTCH1* mutations, affirming the importance of Hh in the onset of BCC.

Immunosuppression has also been associated with the development of NMSCs; chronically immunosuppressed solid organ transplant recipients have a 65-fold increase in SCC and a 10-fold increase in BCC. The frequency of skin cancer is proportional to the level and duration of immunosuppression and the extent of sun exposure before and after transplantation. SCCs in this population are particularly aggressive, demonstrating higher rates of local recurrence, metastasis, and mortality. Tumor necrosis factor (TNF) antagonist therapy of inflammatory bowel disease and autoimmune disorders, such as rheumatoid and psoriatic arthritis, may also confer an increased risk of NMSC.

Other risk factors for NMSCs include HIV infection, ionizing radiation, thermal burn scars, *BRAF* inhibitor monotherapy, and chronic ulcerations. Albinism, xeroderma pigmentosum, Muir-Torre syndrome, Rombo's syndrome, Bazex-Dupré-Christol syndrome, dyskeratosis congenita, and basal cell nevus syndrome (Gorlin syndrome) also increase the incidence of NMSC.

Although MCC is also clearly related to UV exposure, age, and immunosuppression, this neural crest-derived cancer also appears to have a viral etiology; an oncogenic Merkel cell polyomavirus (MCPyV) is present in 80% of tumors. In patients with MCPyV-positive tumors, there is inactivation of tumor-suppressor genes, specifically the *p53* transcription factor and retinoblastoma protein (*Rb*). In addition, the viral large T antigen is expressed on tumor cells, and many patients have detectable cellular or humoral immune responses to polyoma viral proteins, although this immune response is insufficient to eradicate the malignancy.

CLINICAL PRESENTATION

Basal Cell Carcinoma BCC arises from epidermal basal cells. The least invasive of BCC subtypes, superficial BCC, consists of often subtle, erythematous scaling plaques that slowly enlarge and are most commonly seen on the trunk and proximal extremities (Fig. 76-4). This subtype may be confused with benign inflammatory dermatoses, especially nummular eczema and psoriasis or premalignant actinic keratoses. BCC also can present as a small, slowly growing, pearly nodule, often with tortuous telangiectatic vessels on its surface, rolled borders, and a central crust (nodular BCC). The occasional presence of melanin in this variant of nodular BCC (pigmented BCC) may lead to confusion with melanoma. Morpheaform (fibrosing), infiltrative, and micronodular BCC, the most invasive and potentially aggressive subtypes, manifest as solitary, flat or slightly depressed, indurated whitish, yellowish, or pink scar-like plaques. Borders are typically indistinct, and lesions can be subtle; thus, delay in treatment is common, and tumors can be more extensive than expected clinically. An archaic name for this tumor is “rodent ulcer.”

Squamous Cell Carcinoma Primary *cutaneous SCC* is a malignant neoplasm of keratinizing epidermal cells that has a variable clinical course, ranging from indolent to rapid growth, with the potential to metastasize to regional and distant sites. Commonly, SCC appears as an ulcerated erythematous nodule or superficial erosion on sun-exposed skin of the head, neck, trunk, and extremities (Fig. 76-5). It may also appear as a banal, firm, dome-shaped papule or rough textured plaque. It is commonly mistaken for a wart or callosus when the inflammatory response to the lesion is minimal. Dotted or coiled vessels are a hallmark of SCC when viewed through a dermatoscope. The margins of this tumor may be ill defined, and fixation to underlying structures may occur (“tethering”).

A very rapidly growing low-grade form of SCC, called keratoacanthoma (KA), typically appears as a large dome-shaped papule with a central keratotic crater. Some KAs regress spontaneously without therapy, but because progression to metastatic SCC has been documented, KAs should be treated in the same manner as other types of cutaneous SCC. KAs occur in 15–25% of patients receiving monotherapy with a *BRAF* inhibitor.



FIGURE 76-4 Clinical and confocal diagnostic findings of basal cell carcinoma. *A*, Typical basal cell carcinoma with skin-colored, slightly translucent rolled borders and a small central erosion on chronically sun-damaged skin of the lateral posterior shoulder. *B*, Dermoscopic image of the same lesion as in panel *A* clearly revealing the central erosion and classic gray, nonreticular globular structures of melanophages that characterize BCC. *C*, In vivo reflectance confocal microscopy of the same lesion as in panel *A* showing typical nests of dermal basaloid cells (*) with classic cleft formation around the nests.

Actinic keratoses and cheilitis (actinic keratoses on the lip), both premalignant forms of SCC, present as hyperkeratotic papules on sun-exposed areas. Malignant transformation occurs in 0.25–20% of untreated lesions. SCC in situ, also called *Bowen's disease*, is the intraepidermal form of SCC and usually presents as a scaling, erythematous plaque. SCC in situ most commonly arises on sun-damaged skin but can occur anywhere on the body. Bowen's disease occurring secondary to infection with human papillomavirus can arise on skin

with minimal or no prior sun exposure, such as the buttock or posterior thigh. Treatment of premalignant and in situ lesions reduces the subsequent risk of invasive disease.

Merkel Cell Carcinoma MCC, also known as cutaneous apudoma, primary neuroendocrine carcinoma of the skin, primary small cell carcinoma of the skin, and trabecular carcinoma of the skin, arises from Merkel cells, which are neuroendocrine skin cells that act as



FIGURE 76-5 Progression of squamous cell carcinoma (SCC). *A*, Actinic keratoses (AKs). *B*, Actinic cheilitis (AK of the lip). *C*, Bowen's disease (SCC in situ). *D*, Keratoacanthoma (well-differentiated SCC). *E*, SCC. *F*, Metastatic SCC.

pressure receptors. Like other skin cancers, MCCs most commonly arise as visible skin lesions, usually as raised, flesh-colored nodules or masses; they can also be red or blue in color and vary in size from 0.5 to >5 cm in diameter and may enlarge rapidly. Although MCCs may arise almost anywhere on the body, they are most commonly found in sun-exposed areas such as the head, neck, or extremities. They can also be found around the anus and on eyelids. The common clinical features of MCC can be summarized by the acronym AEIOU: asymptomatic/nontender, expand rapidly, immune suppression, older than 50 years, and ultraviolet-exposed site.

NATURAL HISTORY

Basal Cell Carcinoma The natural history of BCC is that of a slowly enlarging, locally invasive neoplasm. The degree of local destruction and risk of recurrence vary with the size, duration, location, and histologic subtype of the tumor. Location on the central face, ears, or scalp may portend a higher risk. Small nodular, pigmented, cystic, or superficial BCCs respond well to most treatments. Large lesions and micronodular, infiltrative, and morphaeform subtypes may be more aggressive. The metastatic potential of BCC is low (0.1%) in immunocompetent patients, but the risk of recurrence or a new primary NMSC is about 40% over 5 years.

Squamous Cell Carcinoma The natural history of SCC depends on tumor and host characteristics. Tumors arising on sun-damaged skin have a lower metastatic potential than do those on non-sun-exposed areas. Cutaneous SCC metastasizes in 0.3–5.2% of individuals, most frequently to regional lymph nodes. Tumors occurring on the lower lip and ear develop regional metastases in 13 and 11% of patients, respectively, whereas the metastatic potential of SCC arising in scars, chronic ulcerations, and genital or mucosal surfaces is higher. Recurrent SCC has a 30% probability for metastatic spread. Large, poorly differentiated, deep tumors with perineural or lymphatic invasion, multifocal tumors, and those arising in immunosuppressed patients often behave aggressively.

Merkel Cell Carcinoma MCCs have clinical features of both skin cancers and neuroendocrine tumors (particularly small cell lung cancer [SCLC]); thus, they can present locally and develop spread to lymph nodes and distant sites. Molecular markers of neuroendocrine origin such as synaptophysin or chromogranin A are useful to diagnose MCC. Unlike other neuroendocrine tumors, MCCs are not associated with measurable hormone secretion or endocrine syndromes.

Survival with MCC depends on extent of disease: 90% of patients with local disease are cured, whereas 52% with nodal involvement and 10% with distant disease survive. MCC has its own tumor-node-metastasis (TNM) staging system, which incorporates tumor size (<2 cm vs. >2 cm), nodal status (which can be determined by SLNB for clinically negative nodes), and the presence of distant metastases.

Independent of stage, the prognosis of MCC is improved if the tumor cells contain virus, RB protein expression, and intratumoral CD8+ T lymphocyte infiltration, whereas p63 expression, lymphovascular infiltrative pattern, and the presence of immunosuppression (e.g., organ transplant, HIV infection, certain cancers) portend a worse prognosis.

TREATMENT

Basal Cell, Squamous Cell, and Merkle Cell Carcinoma

BASAL CELL CARCINOMA

Treatment for BCC includes electrodesiccation and curettage (ED&C), excision, cryosurgery, radiation therapy (RT), laser therapy, Mohs micrographic surgery (MMS), topical 5-fluorouracil, photodynamic therapy (PDT), and topical immunomodulators, such as imiquimod. The choice of therapy depends on tumor characteristics including depth and location, patient age, medical status, and patient preference. ED&C remains the most commonly employed treatment for superficial, minimally invasive nodular

BCCs and low-risk tumors (e.g., a small tumor of a less aggressive subtype in a favorable location). Wide local excision with standard margins is usually selected for invasive, ill-defined, and more aggressive subtypes of tumors or for cosmetic reasons. MMS, a specialized type of surgical excision that provides the best method for tumor removal while preserving uninvolved tissue, is associated with cure rates $>98\%$. It is the preferred modality for lesions that are recurrent, in high-risk or cosmetically sensitive locations (including recurrent tumors in these locations), and for which maximal tissue conservation is critical (e.g., the eyelids, lips, ears, nose, and digits). RT can cure patients not considered surgical candidates and can be used as a surgical adjunct in high-risk tumors. Imiquimod can be used to treat superficial and smaller nodular BCCs, although it is not FDA approved for nodular BCC. Topical 5-fluorouracil therapy should be limited to superficial BCC. PDT, which uses selective activation of a photoactive drug by visible light, has been used in patients with numerous tumors. Intralesional therapy (5-fluorouracil or IFN) can also be employed. Like RT, it remains an option for selected patients who cannot or will not undergo surgery. Systemic therapy with an SMO inhibitor, vismodegib or sonidegib, is indicated for patients with metastatic or advanced BCC that has recurred after local therapy and who are not candidates for surgery or RT. Targeted therapy with SMO antagonists does not cure patients with BCC but induces regression in approximately 50% of patients with a median duration of response >9 months.

SQUAMOUS CELL CARCINOMA

The principles for surgical management of SCC are the same as for BCC. Previously, advanced disease was treated with cisplatin-containing chemotherapy, intralesional 5-fluorouracil, or cetuximab. These regimens have been supplanted by cemiplimab, a monoclonal antibody targeting PD-1, which causes tumor regression in 47% of patients with advanced disease. SCC and KAs that develop in patients receiving BRAF-targeted therapy should be excised, after which BRAF therapy can be continued.

MERKEL CELL CARCINOMA

The epidemiology, clinical features, and treatments for MCC overlap those for melanoma and NMSC. Early-stage MCCs may be cured with wide local excision of the primary tumor and nodal staging with SLNB. Like SCLCs, MCC is sensitive to radiation, PD-1-directed immunotherapy, and platinum-based chemotherapy. RT is often used as postoperative adjuvant therapy at both the primary excision and SLNB sites, although its use may be withheld around sensitive areas such as the eyelids and hands and after a negative SLNB. For nonsensitive areas, RT may allow for primary excision margins smaller than the traditionally recommended 2-cm radial margins. Similar to melanoma, completion node dissection is now uncommonly used for a positive sentinel node. Adjuvant RT, close observation, and clinical trials investigating immunotherapy based on anti-PD-1 agents are favored.

For patients with metastatic disease, immunotherapy has supplanted chemotherapy. Avelumab (anti-PD-L1) therapy led to objective responses in 33% of patients with advanced MCC; 82% of the responses were durable.

Follow-up of patients with MCC is based on stage and risk. Routine skin exams by a dermatologist familiar with MCC and regular examinations of the nodal basins are recommended. A serum titer of monoclonal antibody to MCPyV should be obtained in newly diagnosed MCC patients. The test can be used to follow patients for relapse if the titer is elevated at baseline and returns to normal after treatment. Conversely, if the titer is elevated but does not return to normal after treatment, imaging should be obtained to look for occult metastases.

PREVENTION

The principles for prevention are those described for melanoma earlier. Unique strategies for NMSC include active surveillance for patients on immunosuppressive medications or BRAF-targeted therapy.



FIGURE 76-6 Other malignant cutaneous tumors. *A*. Patch stage mycosis fungoides (variant of cutaneous T-cell lymphoma). *B*. Tumor stage mycosis fungoides. *C*. Extramammary Paget's disease. *D*. Merkel cell carcinoma. *E*. Dermatofibrosarcoma protuberans. *F*. Kaposi's sarcoma. *G*. Kaposi's sarcoma.

Chemoprophylaxis using synthetic retinoids and immunosuppression reduction when possible may be useful in controlling new lesions and managing patients with multiple tumors. Field therapy with topical 5-fluorouracil, ingenol mebutate, or imiquimod can reduce transformation to SCC in patients with severely sun-damaged skin and numerous premalignant actinic keratoses. Older, immunosuppressed patients should be managed with the lowest doses of immunosuppression possible and encouraged to be particularly careful to minimize UV exposure. Earlier biopsy of unusual-appearing skin lesions may lead to better control of aggressive lesions.

■ OTHER NONMELANOMA CUTANEOUS MALIGNANCIES

Neoplasms of cutaneous adnexae and sarcomas of fibrous, mesenchymal, fatty, and vascular tissues make up the remaining 1–2% of NMSCs (Fig. 76-6). Lymphomas of B- or T-cell origin can also manifest in the skin and can mimic benign conditions such as psoriasis and eczema.

Extramammary Paget's disease is an uncommon apocrine malignancy arising from stem cells of the epidermis that is characterized histologically by the presence of Paget cells. These tumors present as moist erythematous patches on anogenital or axillary skin of the elderly.

Outcomes are generally good with surgery, and 5-year disease-specific survival is 95% with localized disease. Advanced age and extensive disease at presentation confer poorer prognosis. RT or topical imiquimod can be considered for more extensive disease. Local management may be challenging because these tumors often extend far beyond clinical margins; surgical excision with MMS has the highest cure rates. Similarly, MMS is the treatment of choice in other rare cutaneous tumors with extensive subclinical extension such as *dermatofibrosarcoma protuberans*.

Kaposi's sarcoma (KS) is a soft tissue sarcoma of vascular origin that is induced by the human herpesvirus 8. The incidence of KS increased

dramatically during the AIDS epidemic, but has now decreased tenfold with the institution of highly active antiretroviral therapy.

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Epithelial carcinomas of the head and neck arise from the mucosal surfaces in the head and neck and typically are squamous cell in origin. This category includes tumors of the paranasal sinuses, the oral cavity, and the nasopharynx, oropharynx, hypopharynx, and larynx. Tumors of the salivary glands differ from the more common carcinomas of the head and neck in etiology, histopathology, clinical presentation, and therapy. They are rare and histologically highly heterogeneous. Thyroid malignancies are described in [Chap. 385](#).

■ INCIDENCE AND EPIDEMIOLOGY

The number of new cases of head and neck cancers (oral cavity, pharynx, and larynx) in the United States was estimated at 65,630 in 2020, accounting for about 4% of adult malignancies; estimated deaths were 14,500. The worldwide incidence exceeds half a million cases annually. In North America and Europe, the tumors usually arise from the oral cavity, oropharynx, or larynx. The incidence of oropharyngeal cancers is increasing in recent years, especially in Western countries. Nasopharyngeal cancer is more commonly seen in the Mediterranean countries and in the Far East, where it is endemic in some areas.

■ ETIOLOGY AND GENETICS

Alcohol and tobacco use are the most significant environmental risk factors for head and neck cancer, and when used together, they act synergistically. Smokeless tobacco is an etiologic agent for oral cancers. Other potential carcinogens include marijuana and occupational exposures such as nickel refining, exposure to textile fibers, and woodworking.

Some head and neck cancers have a viral etiology. Epstein-Barr virus (EBV) infection is frequently associated with nasopharyngeal cancer, especially in endemic areas of the Mediterranean and Far East. EBV antibody titers can be measured to screen high-risk populations and are under investigation to monitor treatment response. Nasopharyngeal cancer has also been associated with consumption of salted fish and indoor pollution.

In Western countries, the human papillomavirus (HPV) is associated with a rising incidence of tumors arising from the oropharynx, that is, the tonsillar bed and base of tongue. Over 50% of oropharyngeal tumors are caused by HPV in the United States, and in many urban centers, this proportion is higher. HPV 16 is the dominant viral subtype, although HPV 18 and other oncogenic subtypes are seen as well. Alcohol- and tobacco-related cancers, on the other hand, have decreased in incidence. HPV-related oropharyngeal cancer frequently occurs in a younger patient population and is associated with increased numbers of sexual partners and oral sexual practices. It is associated with a better prognosis, especially for nonsmokers. Vaccination with the nine-valent HPV vaccine may prevent the disease in high-risk populations.

Dietary factors may contribute. The incidence of head and neck cancer is higher in people with the lowest consumption of fruits and vegetables. Certain vitamins, including carotenoids, may be protective if included in a balanced diet. Supplements of retinoids, such as *cis*-retinoic acid, have not been shown to prevent head and neck cancers (or lung cancer) and may increase the risk in active smokers. No specific risk factors or environmental carcinogens have been identified for salivary gland tumors.

■ HISTOPATHOLOGY, CARCINOGENESIS, AND MOLECULAR BIOLOGY

Squamous cell head and neck cancers are divided into well-differentiated, moderately well-differentiated, and poorly differentiated categories. Poorly differentiated tumors have a worse prognosis than well-differentiated tumors. For nasopharyngeal cancers, the less

common differentiated squamous cell carcinoma is distinguished from nonkeratinizing and undifferentiated carcinoma (lymphoepithelioma) that contains infiltrating lymphocytes and is commonly associated with EBV.

Salivary gland tumors can arise from the major (parotid, submandibular, sublingual) or minor salivary glands (located in the submucosa of the upper aerodigestive tract). Most parotid tumors are benign, but half of submandibular and sublingual gland tumors and most minor salivary gland tumors are malignant. Malignant tumors include mucoepidermoid and adenoid cystic carcinomas and adenocarcinomas.

The mucosal surface of the entire pharynx is exposed to alcohol- and tobacco-related carcinogens and is at risk for the development of a premalignant or malignant lesion. Erythroplakia (a red patch) or leukoplakia (a white patch) can be histopathologically classified as hyperplasia, dysplasia, carcinoma in situ, or carcinoma. However, most head and neck cancer patients do not present with a known history of premalignant lesions. Multiple synchronous or metachronous cancers can also be observed. In fact, over time, patients with treated early-stage tobacco- and alcohol-related head and neck cancer are at greater risk of dying from a second malignancy than from a recurrence of the primary disease.

Second head and neck malignancies are usually not therapy induced; they reflect the exposure of the upper aerodigestive mucosa to the same carcinogens that caused the first cancer. These second primaries develop in the head and neck area, the lung, or the esophagus. Thus, computed tomography (CT) screening for lung cancer in heavy smokers who have already developed a head and neck cancer is recommended. Rarely, patients can develop a radiation therapy-induced sarcoma after having undergone prior radiotherapy for a head and neck cancer.

Much progress has been made in describing the molecular features of head and neck cancer. These features have allowed investigators to describe the genetic and epigenetic alterations and the mutational spectrum of these tumors. Early reports demonstrated frequent overexpression of the epidermal growth factor receptor (EGFR). Overexpression was shown to correlate with poor prognosis. However, it has not proved to be a good predictor of tumor response to EGFR inhibitors, which are active in only about 10–15% of patients as single agents. Complex genetic analyses, including those by The Cancer Genome Atlas project, have been performed. *p53* mutations are found frequently with other major affected oncogenic driver pathways including the mitotic signaling and Notch pathways and cell cycle regulation in HPV-negative tumors. HPV oncogenes act through direct inhibition of the *p53* and *RB* tumor-suppressor genes, thereby initiating the carcinogenic process. *HRAS* appears to be emerging as a potentially targetable mutation in a small patient subset. While overall mutation rates are similar in HPV-positive and carcinogen-induced tumors, the specific mutational signature of HPV-positive tumors differs, with frequent alteration of the PI3K pathway and occasional mutations in *KRAS*. Overall, these alterations affect mitogenic signaling, genetic stability, cellular proliferation, and differentiation.

■ CLINICAL PRESENTATION AND DIFFERENTIAL DIAGNOSIS

Most tobacco-related head and neck cancers occur in patients older than age 60 years. HPV-related malignancies are frequently diagnosed in younger patients, usually in their forties or fifties, whereas EBV-related nasopharyngeal cancer can occur at all ages, including in teenagers. The manifestations vary according to the stage and primary site of the tumor. Patients with nonspecific signs and symptoms in the head and neck area should be evaluated with a thorough otolaryngologic examination, particularly if symptoms persist longer than 2–4 weeks. Males are more frequently affected than women by head and neck cancers, including HPV-positive tumors.

Cancer of the nasopharynx typically does not cause early symptoms. However, it may cause unilateral serous otitis media due to obstruction of the eustachian tube, unilateral or bilateral nasal obstruction, or epistaxis. Advanced nasopharyngeal carcinoma causes neuropathies of the cranial nerves due to skull base involvement.

Carcinomas of the oral cavity present as nonhealing ulcers, changes in the fit of dentures, or painful lesions and masses. Tumors of the tongue base or oropharynx can cause decreased tongue mobility and alterations in speech. Cancers of the oropharynx or hypopharynx rarely cause early symptoms, but they may cause sore throat and/or otalgia. HPV-related tumors frequently present with neck lymphadenopathy as the first sign.

Hoarseness may be an early symptom of laryngeal cancer, and persistent hoarseness requires referral to a specialist for indirect laryngoscopy and/or radiographic studies. If a head and neck lesion treated initially with antibiotics does not resolve in a short period, further workup is indicated; to simply continue the antibiotic treatment may be to lose the chance of early diagnosis of a malignancy.

Advanced head and neck cancers in any location can cause severe pain, otalgia, airway obstruction, cranial neuropathies, trismus, odynophagia, dysphagia, decreased tongue mobility, fistulas, skin involvement, and massive cervical lymphadenopathy, which may be unilateral or bilateral. Some patients have enlarged lymph nodes even though no primary lesion can be detected by endoscopy or biopsy; these patients are considered to have carcinoma of unknown primary (Fig. 77-1). Tonsillectomy and directed biopsies of the base of tongue can frequently identify a small primary tumor that frequently will be HPV related. If the enlarged nodes are located in the upper neck and the tumor cells are of squamous cell histology, the malignancy probably arose from a mucosal surface in the head or neck. Tumor cells in supraclavicular lymph nodes may also arise from a primary site in the chest or abdomen.

The physical examination should include inspection of all visible mucosal surfaces and palpation of the floor of the mouth and of the tongue and neck. In addition to tumors themselves, leukoplakia (a white mucosal patch) or erythroplakia (a red mucosal patch) may be observed; these “ premalignant” lesions can represent hyperplasia, dysplasia, or carcinoma in situ and require biopsy. Further examination should be performed by a specialist. Additional staging procedures include CT or MRI of the head and neck to identify the extent of the disease. Patients with lymph node involvement should have CT scan of the chest and upper abdomen to screen for distant metastases. In heavy smokers, the CT scan of the chest can also serve as a screening tool to rule out a second lung primary tumor. A positron emission tomography (PET) scan can help to identify or exclude distant metastases. CT and PET scans may also be useful in evaluating response to therapy. The definitive staging procedure is an endoscopic examination under anesthesia, which may include laryngoscopy, esophagoscopy,

and bronchoscopy; during this procedure, multiple biopsy samples are obtained to establish a primary diagnosis, define the extent of primary disease, and identify any additional premalignant lesions or second primaries.

Head and neck tumors are classified according to the tumor-node-metastasis (TNM) system of the American Joint Committee on Cancer (AJCC) (Fig. 77-2). This classification varies according to the specific anatomic subsite. In general, primary tumors are classified as T1 to T3 by increasing size, whereas T4 usually represents invasion of another structure such as bone, muscle, or root of tongue. Lymph nodes are staged by size, number, and location (ipsilateral vs contralateral to the primary). Distant metastases are found in <10% of patients at initial diagnosis and are more common in patients with advanced lymph node stage; microscopic involvement of the lungs, bones, or liver is more common, particularly in patients with advanced neck lymph node disease. Modern imaging techniques may increase the number of patients with clinically detectable distant metastases in the future. HPV-related oropharyngeal malignancies have consistently been shown to have a better prognosis, and in the eighth edition of the AJCC staging manual, a separate staging system that takes into account the more favorable outlook of these patients will be included. According to this system, patients with advanced nodal stage can still be considered to have an overall early stage (and associated good prognosis), especially if the patient is a nonsmoker or has limited lifelong tobacco exposure.

In patients with lymph node involvement and no visible primary, the diagnosis should be made by lymph node excision (Fig. 77-1). If the results indicate squamous cell carcinoma, a panendoscopy should be performed, with biopsy of all suspicious-appearing areas and directed biopsies of common primary sites, such as the nasopharynx, tonsil, tongue base, and pyriform sinus. HPV-positive tumors especially can have small primary tumors that spread early to locoregional lymph nodes.

TREATMENT

Head and Neck Cancer

Patients with head and neck cancer can be grossly categorized into three clinical groups: those with localized disease, those with locally or regionally advanced disease (lymph node positive), and those with recurrent and/or metastatic disease below the neck. Comorbidities associated with tobacco and alcohol abuse can affect treatment outcome and define long-term risks for patients who are cured of their disease.

LOCALIZED DISEASE

Nearly one-third of patients have localized disease, that is, T1 or T2 (stage I or stage II) lesions without detectable lymph node involvement or distant metastases. These patients are treated with curative intent by either surgery or radiation therapy. The choice of modality differs according to anatomic location and institutional expertise. Radiation therapy is often preferred for laryngeal cancer to preserve voice function, and surgery is preferred for small lesions in the oral cavity to avoid the long-term complications of radiation, such as xerostomia and osteoradionecrosis and dental decay. Randomized data have shown that a prophylactic staging neck dissection should be part of the surgical procedure to eliminate occult nodal metastatic disease. Overall 5-year survival is 60–90%. Most recurrences occur within the first 2 years following diagnosis and are usually local.

LOCALLY OR REGIONALLY ADVANCED DISEASE

Locally or regionally advanced disease—disease with a large primary tumor and/or lymph node metastases—is the stage of presentation for >50% of patients. Such patients can also be treated with curative intent, but not usually with surgery or radiation therapy alone. Combined-modality therapy, including surgery and/or radiation therapy and chemotherapy, is most successful. Chemotherapy can be administered as induction chemotherapy (chemotherapy before surgery and/or radiotherapy) or as concomitant (simultaneous)

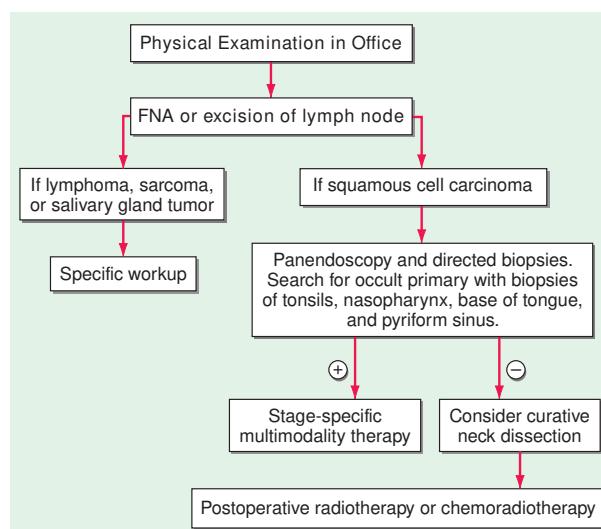


FIGURE 77-1 Evaluation of a patient with cervical adenopathy without a primary mucosal lesion; a diagnostic workup. FNA, fine-needle aspiration.

Definition of TNM			Stage groupings		
Stage I  T1	Tumor ≤2 cm in greatest dimension ≤5 mm depth of invasion (DOI)	N0	 N0- No regional lymph node metastasis	T1	N0 M0
Stage II  T2	Tumor ≥2 cm but not more than 4 cm in greatest dimension OR DOI >5 mm and ≤10 mm	N0	 N0- No regional lymph node metastasis	T2	N0 M0
Stage III  T3	Tumor ≥4 cm OR DOI >10 mm	N1  ≤3 cm	N1- Metastasis in a single ipsilateral lymph node, ≤3 cm in greatest dimension	T3 T1 T2 T3	N0 M0 N1 M0 N1 M0 N1 M0
Stage IVA  T4a	Tumor invades skin, mandible, ear canal, fascial nerve, and/or floor of mouth	N2  ≤6 cm	N2a- Metastasis in a single ipsilateral lymph node, >3 cm but ≤6 cm N2b- Metastasis in multiple ipsilateral lymph nodes, none >6 cm N2c- Metastasis in bilateral or contralateral lymph nodes, none >6 cm	T4a T4a T1 T2 T3 T4a	N0 M0 N1 M0 N2 M0 N2 M0 N2 M0 N2 M0
Stage IVB  T4b	Tumor invades skull base and/or pterygoid plates and/or encases carotid artery	N3  >6 cm	N3- Metastasis in a lymph node >6 cm in greatest dimension or clinically overt extranodal extension	T4b Any T	Any N M0 N3 M0
Stage IVC		M1		Any T	Any N M1

FIGURE 77-2 Tumor-node-metastasis (TNM) staging system. (Figure based on the AJCC Cancer Staging Manual, 8th edition.)

chemotherapy and radiation therapy. The latter is currently most commonly used and supported by the best evidence. Five-year survival rates exceed 50% in many trials, but part of this increased survival may be due to an increasing fraction of study populations with HPV-related tumors who carry a better prognosis. HPV testing of newly diagnosed tumors should be performed for patients with oropharyngeal tumors at the time of diagnosis. Clinical trials for HPV-related tumors are focused on exploring reductions in

treatment intensity, especially radiation dose, in order to ameliorate long-term toxicities (fibrosis, swallowing dysfunction).

In patients with intermediate-stage tumors (stage III and early stage IV), concomitant chemoradiotherapy can be administered as a primary treatment for patients with unresectable disease, to pursue an organ-preserving approach especially for patients with laryngeal cancer (omission of surgery), or in the postoperative setting for smaller resectable tumors with adverse prognostic features.

Induction Chemotherapy In this strategy, patients receive chemotherapy (current standard is a three-drug regimen of docetaxel, cisplatin, and fluorouracil [5-FU]) before surgery and radiation therapy. Most patients who receive three cycles show tumor reduction, and the response is clinically “complete” in up to half of patients. This “sequential” multimodality therapy allows for organ preservation in patients with laryngeal and hypopharyngeal cancer and results in higher cure rates compared with radiotherapy alone.

Concomitant Chemoradiotherapy With the concomitant strategy, chemotherapy and radiation therapy are given simultaneously rather than in sequence. Tumor recurrences from head and neck cancer develop most commonly locoregionally (in the head and neck area of the primary and draining lymph nodes). The concomitant approach is aimed at enhancing tumor cell killing by radiation therapy in the presence of chemotherapy (radiation enhancement) and is a conceptually attractive approach for bulky tumors. Toxicity (especially mucositis, grade 3 or 4, in 70–80%) is increased with concomitant chemoradiotherapy. However, meta-analyses of randomized trials document an improvement in 5-year survival of 8% with concomitant chemotherapy and radiation therapy. Cisplatin is preferentially given weekly during a course of daily radiotherapy over a 6- to 7-week course. In addition, concomitant chemoradiotherapy produces better laryngectomy-free survival (organ preservation) than radiation therapy alone in patients with advanced larynx cancer. The use of radiation therapy together with cisplatin produces improved survival in patients with advanced nasopharyngeal cancer. The outcome of HPV-related cancers seems to be especially favorable following cisplatin-based chemoradiotherapy. Trials substituting cisplatin with the EGFR inhibitor cetuximab in that patient population have shown inferior survival.

The success of concomitant chemoradiotherapy in patients with unresectable disease has led to the testing of a similar approach in patients with resected intermediate-stage disease as a postoperative therapy. Concomitant chemoradiotherapy produces a significant improvement over postoperative radiation therapy alone for patients whose tumors demonstrate higher risk features, such as extracapsular spread beyond involved lymph nodes, involvement of multiple lymph nodes, or positive margins at the primary site following surgery.

A monoclonal antibody to EGFR (cetuximab) increases survival rates when administered during radiotherapy. EGFR blockade results in radiation sensitization and has milder systemic side effects than traditional chemotherapy agents, although an acneiform skin rash is commonly observed. Nevertheless, the addition of cetuximab to current standard chemoradiotherapy regimens has failed to show further improvement in survival and is not recommended.

TREATMENT APPROACHES FOR HPV-RELATED HEAD AND NECK CANCERS

Given consistent observations of high survival rates for patients with advanced HPV-related oropharyngeal tumors using combined-modality treatment strategies, de-escalation protocols have attracted widespread interest. The goal here is to decrease the long-term morbidity resulting from high-dose radiation therapy, including extensive neck fibrosis, swallowing problems, and osteoradionecrosis of the jaw. Current studies are investigating the use of lower radiation doses, the use of induction chemotherapy and subsequent omission of chemotherapy or administration of significantly reduced chemoradiation doses in very good responders, and other strategies. In addition, interest has increased in surgical approaches using robotic surgery, which allows better visualization of the base of tongue and tonsil. While technically feasible, this approach remains investigational because a large number of patients with disease involving multiple lymph nodes will still require postoperative chemoradiotherapy, thus negating the goal of treatment de-escalation. It is expected that distinct treatment guidelines from carcinogen-induced tumors will be defined in the coming years.

RECURRENT AND/OR METASTATIC DISEASE

Five to ten percent of patients present with metastatic disease, and 30–50% of patients with locoregionally advanced disease experience recurrence, frequently outside the head and neck region. Patients with recurrent and/or metastatic disease are, with few exceptions, treated with palliative intent. Some patients may require local or regional radiation therapy for pain control, but most are given systemic therapy.

Combination chemotherapy formerly was the first-line systemic therapeutic approach to patients with recurrent disease after prior curative intent surgery and/or chemoradiotherapy or those presenting initially with metastatic disease. In particular, a combination of cisplatin with 5-FU and cetuximab (the EXTREME regimen) was frequently used.

However, immunotherapies have proven to be of value in this setting. In particular, inhibitors of the immunosuppressive lymphocyte surface receptor (PD-1) pathway have shown activity in squamous cell cancers of the head and neck. A randomized trial evaluating the PD-1 inhibitor nivolumab versus traditional chemotherapy in the second-line treatment of patients with recurrent or metastatic disease showed a significant increase in 1-year survival rates with fewer severe treatment-related toxicities. In addition, some responses were of long duration, allowing a cohort of patients to live far beyond the historical median of <1 year. The PD-1 inhibitor pembrolizumab also demonstrated activity in a similarly designed randomized trial.

Following establishment of second-line activity, pembrolizumab was compared as single-agent therapy or in combination with cisplatin and 5-FU with prior standard chemotherapy alone (cisplatin, 5-FU, and cetuximab). In this trial, overall survival was improved with pembrolizumab versus chemotherapy as well as with the combination of chemotherapy plus pembrolizumab. No statistically significant impact on progression-free survival was noted. In addition, expression of PD-L1 in the tumor tissue was shown to be of importance. Patients with tumors high in expression (PD-L1 score >20%; i.e., expression of PD-L1 on 20% of tumor cells) had a marked survival benefit with pembrolizumab as single agent, whereas patients with lower PD-L1 expression had a less impressive but still statistically significant survival benefit. However, for the group expressing lower levels of PD-L1, the combination of pembrolizumab with chemotherapy showed more substantial benefit. Current standard treatment therefore frequently consists of combination chemoimmunotherapy for patients with a low combined positive score (CPS; the fraction of tumor cells expressing PD-L1), whereas those with higher CPS scores can be treated with immunotherapy alone, especially if overall tumor burden is limited. Patients who experience progression after first-line chemoimmunotherapy or immunotherapy can then be treated with additional single-agent or combination chemotherapy.

EGFR-directed therapies, including monoclonal antibodies (e.g., cetuximab) and tyrosine kinase inhibitors (TKIs) of the EGFR signaling pathway (e.g., erlotinib or gefitinib), have single-agent activity of 10%. Side effects are usually limited to an acneiform rash and diarrhea (for the TKIs). The addition of cetuximab to standard combination chemotherapy with cisplatin or carboplatin and 5-FU results in a significant increase in median survival. Drugs targeting specific mutations are under investigation, and patients with *HRAS* mutations have tumor shrinkage with the farnesyltransferase inhibitor tipifarnib.

COMPLICATIONS

Complications from treatment of head and neck cancer are usually correlated to the extent of surgery and exposure of normal tissue structures to radiation. Currently, the extent of surgery has been limited or completely replaced by chemotherapy and radiation therapy as the primary approach. Acute complications of radiation include mucositis and dysphagia. Long-term complications include xerostomia, loss of taste, decreased tongue mobility, second malignancies, dysphagia, and neck fibrosis. The complications of

chemotherapy vary with the regimen used but usually include myelosuppression, mucositis, nausea and vomiting, and nephrotoxicity (with cisplatin).

The mucosal side effects of therapy can lead to malnutrition and dehydration. Many centers address issues of dentition before starting treatment, and some place feeding tubes to ensure control of hydration and nutrition intake. About 50% of patients develop hypothyroidism from the treatment; thus, thyroid function should be monitored.

SALIVARY GLAND TUMORS

Most benign salivary gland tumors are treated with surgical excision, and patients with invasive salivary gland tumors are treated with surgery and radiation therapy. These tumors may recur regionally; adenoid cystic carcinoma has a tendency to recur along the nerve tracks. Distant metastases may occur as late as 10–20 years after the initial diagnosis. For metastatic disease, therapy is given with palliative intent, usually chemotherapy with doxorubicin and/or cisplatin. Identification of novel agents with activity in these tumors is a high priority. It is hoped that comprehensive genomic characterization of these rare tumors will facilitate these efforts.

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established in the mid-twentieth century and codified with the release of the U.S. Surgeon General's 1964 report on the health effects of tobacco smoking. Following the report, cigarette use started to decline in North America and parts of Europe, and with it, so did the incidence of lung cancer. Although tobacco smoking remains the primary cause of lung cancer worldwide, approximately 60% of new lung cancers in the United States occur in former smokers (smoked ≥100 cigarettes per lifetime, quit ≥1 year), many of whom quit decades ago, or never smokers (smoked <100 cigarettes per lifetime). Moreover, one in five women and one in 12 men diagnosed with lung cancer have never smoked.

EPIDEMIOLOGY

Lung cancer is the most common cause of cancer death among American men and women. Approximately 228,000 individuals will be diagnosed with lung cancer in the United States in 2020, and >135,000 individuals will die from the disease. Lung cancer is uncommon below age 40, with rates increasing until age 80, after which the rate tapers off. The projected lifetime probability of developing lung cancer is estimated to be 8% among males and 6% among females. The incidence of lung cancer varies by racial and ethnic group, with the highest age-adjusted incidence rates among African Americans. The excess in age-adjusted rates among African Americans occurs only among men, but examinations of age-specific rates show that below age 50, mortality from lung cancer is >25% higher among African American than Caucasian women. Incidence and mortality rates among Hispanics and Native and Asian Americans are 40–50% those of whites.

RISK FACTORS

Cigarette smokers have a 10-fold or greater increased risk of developing lung cancer compared to those who have never smoked. A large-scale genomic study suggested that one genetic mutation is induced for every 15 cigarettes smoked. The risk of lung cancer is lower among persons who quit smoking than among those who continue smoking. The size of the lung cancer risk reduction increases with the length of time the person has quit smoking, although even long-term former smokers have higher risks of lung cancer than those who never smoked. Cigarette smoking has been shown to increase the risk of all major types of lung cancer. Environmental tobacco smoke (ETS) or second-hand smoke is also an established cause of lung cancer. The risk from ETS is less than from active smoking, with about a 20–30% increase in lung cancer observed among never smokers married for many years to smokers, in comparison to the 2000% increase among continuing active smokers. The impact on the development of lung cancer among users of alternate nicotine delivery devices (e-cigarettes or vaping) is undefined. While one large randomized study demonstrated the superiority of e-cigarettes compared to traditional nicotine replacement therapy in aiding smoking cessation, e-cigarette- or vaping-associated lung injury (EVALI) is an emerging phenomenon that poses risks that may counterbalance the potential benefit in helping patients reduce traditional cigarette consumption and lung cancer risk.

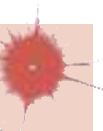
Although cigarette smoking is the cause of the majority of lung cancers, several other risk factors have been identified, including occupational exposure to asbestos, arsenic, bischloromethyl ether, hexavalent chromium, mustard gas, nickel (as in certain nickel-refining processes), and polycyclic aromatic hydrocarbons.

Ionizing radiation is also an established lung carcinogen, most convincingly demonstrated from studies showing increased rates of lung cancer among survivors of the atom bombs dropped on Hiroshima and Nagasaki and large excesses among workers exposed to alpha irradiation from radon in underground uranium mining. Prolonged exposure to low-level radon in homes might impart a risk of lung cancer equal to or greater than that of ETS. Prior lung diseases such as chronic bronchitis, emphysema, and tuberculosis have been linked to increased risks of lung cancer as well. The risk of lung cancer appears to be higher among individuals with low fruit and vegetable intake during adulthood. This observation led to hypotheses that specific nutrients, in particular retinoids and carotenoids, might have chemopreventative effects for lung cancer. However, randomized trials failed to validate this hypothesis.

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Neoplasms of the Lung

Leora Horn, Wade T. Iams



Lung cancer, which was rare before 1900 with fewer than 400 cases described in the medical literature, is considered a disease of modern man, killing over three times as many men as prostate cancer and nearly twice as many women as breast cancer. Although lung cancer remains the number one cause of cancer-related mortality, a decline in lung cancer deaths has emerged, attributed to improvements in testing and therapeutic strategies and a decline in tobacco usage. Tobacco consumption is the primary cause of lung cancer, a reality firmly

Smoking Cessation Given the undeniable link between cigarette smoking and lung cancer, physicians must promote tobacco abstinence. Stopping tobacco use before middle age avoids >90% of the lung cancer risk attributable to tobacco. Importantly, smoking cessation can even be beneficial in individuals with an established diagnosis of lung cancer, as it is associated with improved survival, fewer side effects from therapy, and an overall improvement in quality of life. Consequently, it is important to promote smoking cessation even *after* the diagnosis of lung cancer is established.

Physicians need to understand the essential elements of smoking cessation therapy. The individual must want to stop smoking and must be willing to work hard to achieve the goal of smoking abstinence. Self-help strategies alone only marginally affect quit rates, whereas individual and combined pharmacotherapies in combination with counseling can significantly increase rates of cessation. Therapy with an antidepressant (e.g., bupropion) and nicotine replacement therapy (varenicline, a $\alpha_4\beta_2$ nicotinic acetylcholine receptor partial agonist) are approved by the U.S. Food and Drug Administration (FDA) as first-line treatments for nicotine dependence. In a randomized trial, varenicline was shown to be more efficacious than bupropion or placebo. Prolonged use of varenicline beyond the initial induction phase proved useful in maintaining smoking abstinence. Clonidine and nortriptyline are recommended as second-line treatments. A role for e-cigarettes has not been definitively established (*Chap. 454*).

Inherited Predisposition to Lung Cancer Exposure to environmental carcinogens, such as those found in tobacco smoke, induce or facilitate the transformation from bronchoepithelial cells to a malignant phenotype. The contribution of carcinogens to transformation is modulated by polymorphic variations in genes that affect aspects of carcinogen metabolism. Certain genetic polymorphisms of the P450 enzyme system, specifically CYP1A1, and chromosome fragility are associated with the development of lung cancer. These genetic variations occur at relatively high frequency in the population, but their contribution to an individual's lung cancer risk is generally low. However, because of their population frequency, the overall impact on lung cancer risk could be high.

First-degree relatives of lung cancer probands have a two- to three-fold excess risk of lung cancer and other cancers, many of which are not smoking-related. These data suggest that specific genes and/or genetic variants may contribute to susceptibility to lung cancer. However, very few such genes have yet been identified. Individuals with inherited mutations in *RB* (patients with retinoblastoma living to adulthood) and *p53* (Li-Fraumeni syndrome) genes may develop lung cancer. Common gene variants involved in lung cancer have identified three separate loci that are associated with lung cancer (5p15, 6p21, and 15q25) and include genes that regulate acetylcholine nicotinic receptors and telomerase production. A rare germline mutation (T790M) involving the epidermal growth factor receptor (EGFR) maybe be linked to lung cancer susceptibility in never smokers. Likewise, a susceptibility locus on chromosome 6q greatly increases lung cancer risk among light and never smokers. Although progress has been made, there is a significant amount of work that remains to be done in identifying heritable risk factors for lung cancer. Currently no molecular criteria are suitable to select patients for more intense screening programs or for specific chemopreventive strategies.

PATHOLOGY

The World Health Organization (WHO) defines lung cancer as tumors arising from the respiratory epithelium (bronchi, bronchioles, and alveoli). The WHO classification system divides epithelial lung cancers into four major cell types: small-cell lung cancer (SCLC), adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma; the latter three types are collectively known as non-small-cell carcinomas (NSCLCs) (*Fig. 78-1*). Small-cell carcinomas consist of small cells with scant cytoplasm,

ill-defined cell borders, finely granular nuclear chromatin, absent or inconspicuous nucleoli, and a high mitotic count. SCLC may be distinguished from NSCLC by the presence of neuroendocrine markers including CD56, neural cell adhesion molecule (NCAM), synaptophysin, and chromogranin. Adenocarcinomas possess glandular differentiation or mucin production and may show acinar, papillary, lepidic, or solid features or a mixture of these patterns. Squamous cell carcinomas of the lung are morphologically identical to extrapulmonary squamous cell carcinomas and cannot be distinguished by immunohistochemistry alone. Squamous cell tumors show keratinization and/or intercellular bridges that arise from bronchial epithelium. The tumor consists of sheets of cells rather than the three-dimensional groups of cells characteristic of adenocarcinomas. Large-cell carcinomas compose <10% of lung carcinomas. These tumors lack the cytologic and architectural features of small-cell carcinoma and glandular or squamous differentiation. Together, these four histologic types account for 90% of all epithelial lung cancers.

All histologic types of lung cancer can develop in current and former smokers, although squamous and small-cell carcinomas are most commonly associated with tobacco use. With the decline in cigarette consumption, adenocarcinoma has become the most frequent histologic subtype of lung cancer in the United States. In lifetime never smokers or former light smokers (<10 pack-year history), women, and younger adults (<60 years), adenocarcinoma tends to be the most common form of lung cancer.

In addition to distinguishing between SCLC and NSCLC, because these tumors have quite different natural histories and therapeutic approaches (see below), it is necessary to classify whether NSCLC is squamous or nonsquamous. The classification system, developed jointly by the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society, provides an integrated approach to the classification of lung adenocarcinoma that includes clinical, molecular, radiographic, and pathologic information.

It is recognized that most lung cancers present in an advanced stage and are often diagnosed based on small biopsies or cytologic specimens, rendering clear histologic distinctions difficult, if not impossible. In such cases, particularly in patients with advanced-stage disease, a repeat biopsy is recommended to obtain additional tissue for further clarification. The distinction between squamous and nonsquamous lung cancer is viewed as critical to optimal therapeutic decision making, and a diagnosis of *non-small-cell carcinoma, not otherwise specified* is no longer considered acceptable. This distinction can be achieved using a single marker for adenocarcinoma (thyroid transcription factor-1 or napsin-A) plus a squamous marker (p40 or p63) and/or mucin stains. If tissue is limited and a clear morphologic pattern is evident, a diagnosis can be made without immunohistochemistry staining. In addition to determining histologic subtype, preservation of sufficient specimen material for appropriate molecular testing and PD-L1 testing necessary to help guide therapeutic decision making is recommended (see below).

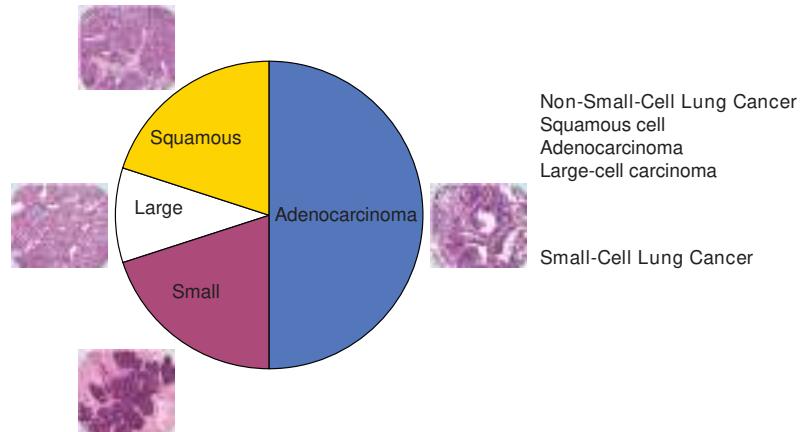


FIGURE 78-1 Histologic subsets of lung cancer.

The terms *adenocarcinoma in situ* and *minimally invasive adenocarcinoma* are now recommended for small solitary adenocarcinomas (≤ 3 cm) with either pure lepidic growth (term used to describe single-layered growth of atypical cuboidal cells coating the alveolar walls) or predominant lepidic growth with ≤ 5 mm invasion. Individuals with these entities experience 100% or near 100% 5-year disease-free survival with complete tumor resection. *Invasive adenocarcinomas*, representing more than 70–90% of surgically resected lung adenocarcinomas, are now classified by their predominant pattern: lepidic, acinar, papillary, and solid patterns. Lepidic-predominant subtype has a favorable prognosis, acinar and papillary have an intermediate prognosis, and solid-predominant has a poor prognosis. The terms *signet ring* and *clear cell adenocarcinoma* have been eliminated from the variants of invasive lung adenocarcinoma, whereas the term *micropapillary*, a subtype with a particularly poor prognosis, has been added. Because of prognostic implications, squamous cell carcinoma has also been modified to consist of keratinizing, nonkeratinizing, and basaloid, analogous to head and neck cancers.

■ IMMUNOHISTOCHEMISTRY

The diagnosis of lung cancer most often rests on the morphologic or cytologic features correlated with clinical and radiographic findings. Immunohistochemistry may be used to verify neuroendocrine differentiation within a tumor, with markers such as neuron-specific enolase (NSE), CD56 or NCAM, synaptophysin, chromogranin, and Leu7. Immunohistochemistry is also helpful in differentiating primary from metastatic adenocarcinomas; thyroid transcription factor-1 (TTF-1), identified in tumors of thyroid and pulmonary origin, is positive in >70% of pulmonary adenocarcinomas and is a reliable indicator of primary lung cancer, provided a thyroid primary has been excluded. A negative TTF-1, however, does not exclude the possibility of a lung primary. TTF-1 is also positive in neuroendocrine tumors of pulmonary and extrapulmonary origin. Napsin-A (Nap-A) is an aspartic protease that plays an important role in maturation of surfactant B7 and is expressed in cytoplasm of type II pneumocytes. In several studies, Nap-A has been reported in >90% of primary lung adenocarcinomas. Notably, a combination of Nap-A and TTF-1 is useful in distinguishing primary lung adenocarcinoma (Nap-A positive, TTF-1 positive) from primary lung squamous cell carcinoma (Nap-A negative, TTF-1 negative) and primary SCLC (Nap-A negative, TTF-1 positive). Cytokeratins 7 and 20 used in combination can help narrow the differential diagnosis; nonsquamous NSCLC, SCLC, and mesothelioma may stain positive for CK7 and negative for CK20, whereas squamous cell lung cancer often will be both CK7 and CK20 negative. p63 is a useful marker for the detection of NSCLCs with squamous differentiation when used in cytologic pulmonary samples. Mesothelioma can be easily identified ultrastructurally, but it has historically been difficult to differentiate from adenocarcinoma through morphology and immunohistochemical staining. Several markers in the past few years have proven to be more helpful including CK5/6, calretinin, and Wilms tumor gene-1 (*WT-1*), all of which show positivity in mesothelioma.

■ MOLECULAR PATHOGENESIS

As proposed by Hanahan and Weinberg, virtually all cancer cells acquire six hallmark capabilities: self-sufficiency in growth signals, insensitivity to antigrowth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. The order in which these hallmark capabilities are acquired is variable. Events leading to acquisition of these hallmarks vary widely, although broadly, cancers arise as a result of accumulations of gain-of-function mutations in oncogenes and loss-of-function mutations in tumor-suppressor genes. Further complicating

the study of lung cancer, the sequence of events that leads to disease is clearly different for the various histopathologic entities.

For cancers in general, one theory holds that a small subset of the cells within a tumor (i.e., “stem cells”) are responsible for the full malignant behavior of the tumor. As part of this concept, the large bulk of the cells in a cancer are “offspring” of these cancer stem cells. While clonally related to the cancer stem cell subpopulation, most cells by themselves cannot regenerate the full malignant phenotype. The stem cell concept may explain the failure of standard medical therapies to eradicate lung cancers, even when there is a clinical complete response. Disease recurs because therapies do not eliminate the stem cell component, which may be more resistant to therapy. Precise human lung cancer stem cells have yet to be identified.

Among lung cancer histologies, adenocarcinomas have been the most extensively catalogued for recurrent genomic gains and losses as well as for somatic mutations (Fig. 78-2, Table 78-1). While multiple different kinds of aberrations have been found, a major class involves “driver mutations,” which are mutations that occur in genes encoding signaling proteins that, when aberrant, drive initiation and maintenance of tumor cells. Importantly, driver mutations can serve as a potential Achilles’ heels for tumors, if their gene products can be targeted appropriately. These genes encode cell-surface receptors consisting of an extracellular ligand-binding domain, a transmembrane structure, and an intracellular tyrosine kinase (TK) domain. The binding of ligand to receptor activates receptor dimerization and TK autoprophosphorylation, initiating a cascade of intracellular events, and leading to increased cell proliferation, angiogenesis, metastasis, and a decrease in apoptosis. Lung adenocarcinomas can arise when normal alveolar type II cells develop mutations in *EGFR*, *BRAF*, *MET*, *KRAS*, and *PIK3CA*. These same tumors display high sensitivity to small-molecule TK inhibitors (TKIs). Additional subsets of lung adenocarcinoma have been identified as defined by the presence of specific chromosomal rearrangements, resulting in the aberrant activation of the TKs *ALK*, *ROS1*, *NTRK*, and *RET*. Notably, most driver mutations in lung cancer appear to be mutually exclusive, suggesting that acquisition of one of these mutations is sufficient to drive tumorigenesis. Although driver mutations have mostly been found in adenocarcinomas, three

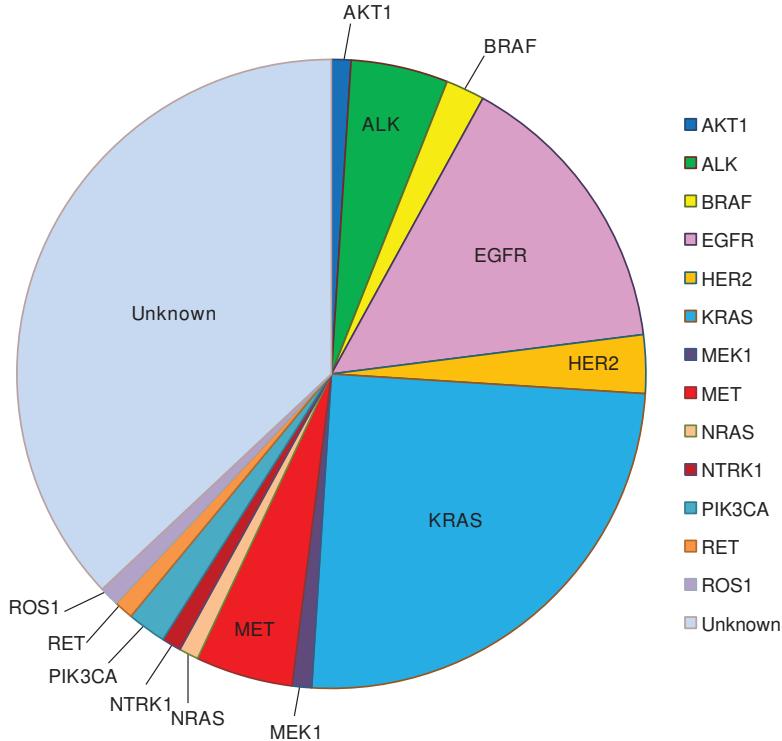


FIGURE 78-2 Driver mutations in lung adenocarcinomas.

TABLE 78-1 Driver Mutations in Non-Small-Cell Lung Cancer (NSCLC)

GENE	ALTERATION	FREQUENCY IN NSCLC	TYPICAL HISTOLOGY
AKT1	Mutation	1%	Adenocarcinoma, squamous
ALK	Rearrangement	3–7%	Adenocarcinoma
BRAF	Mutation	1–3%	Adenocarcinoma
DDR2	Mutation	4%	Squamous
EGFR	Mutation	10–35%	Adenocarcinoma
FGFR1	Amplification	20%	Squamous
HER2	Mutation	2–4%	Adenocarcinoma
KRAS	Mutation	15–25%	Adenocarcinoma
MEK1	Mutation	1%	Adenocarcinoma
MET	Amplification	2–4%	Adenocarcinoma
NRAS	Mutation	1%	Adenocarcinoma
NTRK	Rearrangement	1–2%	Adenocarcinoma
PIK3CA	Mutation	1–3%	Squamous
PTEN	Mutation	4–8%	Squamous
ROS1	Rearrangement	1–2%	Adenocarcinoma

potential molecular targets have been identified in squamous cell lung carcinomas: *FGFR1* amplification, *DDR2* mutations, and *PIK3CA* mutations/*PTEN* loss as well as *BRAF* and *MET* (Table 78-1).

A large number of tumor-suppressor genes have also been identified that are inactivated during the pathogenesis of lung cancer. These include *TP53*, *RBI*, *RASSF1A*, *CDKN2A/B*, *LKB1* (*STK11*), and *FHIT*. Nearly 90% of SCLCs harbor mutations in *TP53* and *RBI*. Several tumor-suppressor genes on chromosome 3p appear to be involved in nearly all lung cancers. Allelic loss of this region occurs very early in lung cancer pathogenesis, including in histologically normal smoking-damaged lung epithelium.

EARLY DETECTION AND SCREENING

In lung cancer, clinical outcome is related to the stage at diagnosis, and hence, it is generally assumed that early detection of occult tumors will lead to improved survival. Early detection is a process that involves screening tests, surveillance, diagnosis, and early treatment. Screening refers to the use of tests across a healthy population in order to identify individuals who harbor asymptomatic disease. For a screening program to be successful, the target population must have a high burden of disease; the test must be sensitive, specific, accessible, and cost effective; and effective treatment must be available that can reduce mortality. With any screening procedure, it is important to consider the possible influence of *lead-time bias* (detecting the cancer earlier without an effect on survival), *length-time bias* (indolent cancers are detected on screening and may not affect survival, whereas aggressive cancers are likely to cause symptoms earlier in patients and are less likely to be detected), and *overdiagnosis* (diagnosing cancers so slow growing that they are unlikely to cause the death of the patient).

Because a majority of lung cancer patients present with advanced disease beyond the scope of surgical resection, the value of screening for this condition is debated. Indeed, randomized controlled trials conducted in the 1960s to 1980s using screening chest x-rays (CXR), with or without sputum cytology, reported no impact on lung cancer-specific mortality in patients characterized as high risk (males age ≥ 45 years with a smoking history). These studies have been criticized for their design, statistical analyses, and outdated imaging modalities. In contrast to CXR, low-dose, noncontrast, thin-slice spiral chest computed tomography (LDCT) has emerged as an effective tool to screen for lung cancer. In nonrandomized studies conducted in the 1990s, LDCT scans were shown to detect more lung nodules and cancers than standard CXR in selected high-risk populations (e.g., age ≥ 60 years and a smoking history of ≥ 10 pack-years). Notably, up to 85% of the lung cancers discovered in these trials were classified as stage I disease and therefore considered potentially curable with surgical resection.

These data prompted the National Cancer Institute (NCI) to initiate the National Lung Screening Trial (NLST), a randomized study designed to determine if LDCT screening could reduce mortality from lung cancer in high-risk populations as compared with standard posterior anterior CXR. High-risk patients were defined as individuals between 55 and 74 years of age, with a ≥ 30 pack-year history of cigarette smoking; former smokers must have quit within the previous 15 years. Excluded from the trial were individuals with a previous lung cancer diagnosis, a history of hemoptysis, an unexplained weight loss of >15 lb in the preceding year, or a chest CT within 18 months of enrollment. A total of 53,454 persons were enrolled and randomized to annual screening yearly for 3 years (LDCT screening, n = 26,722; CXR screening, n = 26,732). Any noncalcified nodule measuring ≥ 4 mm in any diameter found on LDCT and CXR images with any noncalcified nodule or mass were classified as “positive.” Participating radiologists had the option of not calling a final screen positive if a noncalcified nodule had been stable on the three screening examinations. Overall, 39.1% of participants in the LDCT group and 16% in the CXR group had at least one positive screening result. Of those who screened positive, the false-positive rate was 96.4% in the LDCT group and 94.5% in the CXR group. This was consistent across all three rounds. In the LDCT group, 1060 cancers were identified compared with 941 cancers in the CXR group (645 vs 572 per 100,000 person-years; relative risk [RR], 1.13; 95% confidence interval [CI], 1.03–1.23). Nearly twice as many stage IA cancers were detected in the LDCT group compared with the CXR group (40% vs 21%). The overall rates of lung cancer death were 247 and 309 deaths per 100,000 participants in the LDCT and CXR groups, respectively, representing a 20% reduction in lung cancer mortality in the LDCT-screened population (95% CI, 6.8–26.7%; p = .004). Compared with the CXR group, the rate of death in the LDCT group from *any* cause was reduced by 6.7% (95% CI, 1.2–13.6%; p = .02). The number needed to screen (NNTS) to prevent one lung cancer death was calculated to be 320.

The Nelson study was a second randomized trial comparing no screening to CT scans at baseline and in years 1, 3, and 5.5 in 13,195 men and 2594 women. Participants were 50–75 years of age and were current and former smokers with 10 years or less of cessation who smoked >15 cigarettes a day for >25 years or >10 cigarettes daily for >30 years. Participants were selected from four regions in the Netherlands or Belgium and were excluded if they were in moderate or bad self-reported health, were unable to climb two flights of stairs, had a body weight >140 kg, had a CT of the chest within the past year or a history of lung cancer <5 years ago or were still under treatment, or had current or past renal cell carcinoma, melanoma, or breast cancer. The hazard ratio for lung cancer mortality at 10 years was 0.74 (95% CI, 0.60–0.91; p = .003) and 0.61 (95% CI, 0.35–1.04; p = .0543) in men and women, respectively. These two trials have validated the use of annual CT scans for early detection of lung cancer in high-risk populations.

LDCT screening for lung cancer comes with known risks including a high rate of false-positive results, false-negative results, potential for unnecessary follow-up testing, radiation exposure, overdiagnosis, changes in anxiety level and quality of life, and substantial financial costs. By far, the biggest challenge confronting the use of CT screening is the high false-positive rate. False positives can have a substantial impact on patients through the expense and risk of unneeded further evaluation and emotional stress. The management of these patients usually consists of serial CT scans over time to see if the nodules grow, attempted fine-needle aspirates, or surgical resection. At \$300 per scan (NCI estimated cost), the outlay for initial LDCT alone could run into the billions of dollars annually, an expense that only further escalates when factoring in various downstream expenditures an individual might incur in the assessment of positive findings. A formal cost-effectiveness analysis of the NLST demonstrated differences between sex, age, and current smoking status and the method of follow-up. Despite some questions, LDCT screening has been recommended for all patients meeting criteria for enrollment on NLST. When discussing the option of LDCT screening, use of absolute risks rather than relative risks is helpful because studies indicate the public can process absolute terminology more effectively than relative risk projections. A useful

TABLE 78-2 The Benefits and Harms of LDCT Screening for Lung Cancer Based on NLST Data

	LDCT	CXR
Benefits: How did CT scans help compared to CXR?		
4 in 1000 fewer died from lung cancer	13 in 1000	17 in 1000
5 in 1000 fewer died from all causes	70 in 1000	75 in 1000
Harms: What problems did CT scans cause compared to CXR?		
223 in 1000 had at least 1 false alarm	365 in 1000	142 in 1000
18 in 1000 had a false alarm leading to an invasive procedure	25 in 1000	7 in 1000
2 in 1000 had a major complication from an invasive procedure	3 in 1000	1 in 1000

Abbreviations: CXR, chest x-ray; LDCT, low-dose computed tomography; NLST, National Lung Screening Trial.

Source: From S Woloshin: Cancer screening campaigns getting past uninformative persuasion. *N Engl J Med* 367:1167, 2012. Copyright © (2012) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

guide has been developed by the NCI to help patients and physicians assess the benefits and harms of LDCT screening for lung cancer (Table 78-2).

CLINICAL MANIFESTATIONS

Over half of all patients diagnosed with lung cancer present with locally advanced or metastatic disease at the time of diagnosis. The majority of patients present with signs, symptoms, or laboratory abnormalities that can be attributed to the primary lesion, local tumor growth, invasion or obstruction of adjacent structures, growth at distant metastatic sites, or a paraneoplastic syndrome (Tables 78-3 and 78-4). The prototypical lung cancer patient is a current or former smoker of either sex, usually in the seventh decade of life. A history of chronic cough with or without hemoptysis in a current or former smoker with chronic obstructive pulmonary disease (COPD) age 40 years or older should prompt a thorough investigation for lung cancer even in the face of a normal CXR. A persistent pneumonia without constitutional symptoms and unresponsive to repeated courses of antibiotics also should prompt an evaluation for the underlying cause. Lung cancer arising in a lifetime never smoker is more common in women and East Asians. Such patients also tend to be younger than their smoking counterparts at the time of diagnosis. The clinical presentation of lung cancer in never smokers tends to mirror that of current and former smokers.

Patients with central or endobronchial growth of the primary tumor may present with cough, hemoptysis, wheeze, stridor, dyspnea, or

TABLE 78-3 Presenting Signs and Symptoms of Lung Cancer

SYMPTOM AND SIGNS	RANGE OF FREQUENCY
Cough	8–75%
Weight loss	0–68%
Dyspnea	3–60%
Chest pain	20–49%
Hemoptysis	6–35%
Bone pain	6–25%
Clubbing	0–20%
Fever	0–20%
Weakness	0–10%
Superior vena cava obstruction	0–4%
Dysphagia	0–2%
Wheezing and stridor	0–2%

Source: Reproduced with permission from MA Beckles: Initial evaluation of the patient with lung cancer. Symptoms, sign, laboratory tests, and paraneoplastic syndromes. *Chest* 123:97, 2003.

TABLE 78-4 Clinical Findings Suggestive of Metastatic Disease

Symptoms elicited in history	<ul style="list-style-type: none"> Constitutional: weight loss >10 lb Musculoskeletal: pain Neurologic: headaches, syncope, seizures, extremity weakness, recent change in mental status
Signs found on physical examination	<ul style="list-style-type: none"> Lymphadenopathy (>1 cm) Hoarseness, superior vena cava syndrome Bone tenderness Hepatomegaly (>13 cm span) Focal neurologic signs, papilledema Soft-tissue mass
Routine laboratory tests	<ul style="list-style-type: none"> Hematocrit, <40% in men; <35% in women Elevated alkaline phosphatase, GGT, SGOT, and calcium levels

Abbreviations: GGT, gamma-glutamyltransferase; SGOT, serum glutamic-oxaloacetic transaminase.

Source: Reproduced with permission from GA Silvestri et al: The noninvasive staging of non-small cell lung cancer. *Chest* 123:147S, 2003.

postobstructive pneumonia. Peripheral growth of the primary tumor may cause pain from pleural or chest wall involvement, dyspnea on a restrictive basis, and symptoms of a lung abscess resulting from tumor cavitation. Regional spread of tumor in the thorax (by contiguous growth or by metastasis to regional lymph nodes) may cause tracheal obstruction, esophageal compression with dysphagia, recurrent laryngeal nerve paralysis with hoarseness, phrenic nerve palsy with elevation of the hemidiaphragm and dyspnea, and sympathetic nerve paralysis with Horner's syndrome (enophthalmos, ptosis, miosis, and anhidrosis). Malignant pleural effusions can cause pain, dyspnea, or cough. Pancoast (or superior sulcus tumor) syndromes result from local extension of a tumor growing in the apex of the lung with involvement of the eighth cervical and first and second thoracic nerves, and present with shoulder pain that characteristically radiates in the ulnar distribution of the arm, often with radiologic destruction of the first and second ribs. Often Horner's syndrome and Pancoast syndrome coexist. Other problems of regional spread include superior vena cava syndrome from vascular obstruction; pericardial and cardiac extension with resultant tamponade, arrhythmia, or cardiac failure; lymphatic obstruction with resultant pleural effusion; and lymphangitic spread through the lungs with hypoxemia and dyspnea. In addition, lung cancer can spread transbronchially, producing tumor growth along multiple alveolar surfaces with impairment of gas exchange, respiratory insufficiency, dyspnea, hypoxemia, and sputum production. Constitutional symptoms may include anorexia, weight loss, weakness, fever, and night sweats. Apart from the brevity of symptom duration, these parameters fail to clearly distinguish SCLC from NSCLC or even from neoplasms metastatic to lungs.

Extrathoracic metastatic disease is found at autopsy in >50% of patients with squamous carcinoma, 80% of patients with adenocarcinoma and large-cell carcinoma, and >95% of patients with SCLC. Approximately one-third of patients present with symptoms as a result of distant metastases. Lung cancer metastases may occur in virtually every organ system, and the site of metastatic involvement largely determines other symptoms. Patients with brain metastases may present with headache, nausea and vomiting, seizures, or neurologic deficits. Patients with bone metastases may present with pain, pathologic fractures, or spinal cord compression. The latter may also occur with epidural metastases. Individuals with bone marrow invasion may present with cytopenias or leukoerythroblastosis. Those with liver metastases may present with hepatomegaly, right upper quadrant pain, fever, anorexia, and weight loss. Liver dysfunction and biliary obstruction are rare. Adrenal metastases are common but rarely cause pain or adrenal insufficiency unless they are large.

Paraneoplastic syndromes are common in patients with lung cancer, especially those with SCLC, and may be the presenting finding or the first sign of recurrence. In addition, paraneoplastic syndromes may mimic metastatic disease and, unless detected, lead to inappropriate

palliative rather than curative treatment. Often the paraneoplastic syndrome may be relieved with successful treatment of the tumor. In some cases, the pathophysiology of the paraneoplastic syndrome is known, particularly when a hormone with biologic activity is secreted by a tumor. However, in many cases, the pathophysiology is unknown. Systemic symptoms of anorexia, cachexia, weight loss (seen in 30% of patients), fever, and suppressed immunity are paraneoplastic syndromes of unknown etiology or at least not well defined. Weight loss >10% of total body weight is considered a bad prognostic sign. Endocrine syndromes are seen in 12% of patients; hypercalcemia resulting from ectopic production of parathyroid hormone (PTH) or, more commonly, PTH-related peptide is the most common life-threatening metabolic complication of malignancy, primarily occurring with squamous cell carcinomas of the lung. Clinical symptoms include nausea, vomiting, abdominal pain, constipation, polyuria, thirst, and altered mental status.

Hyponatremia may be caused by the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) or possibly atrial natriuretic peptide (ANP) (Chap. 93). SIADH resolves within 1–4 weeks of initiating chemotherapy in the vast majority of cases. During this period, serum sodium can usually be managed and maintained above 128 mEq/L via fluid restriction. Demeclocycline can be a useful adjunctive measure when fluid restriction alone is insufficient. Vasopressin receptor antagonists like tolvaptan also have been used in the management of SIADH. However, the use of tolvaptan has significant limitations including liver injury and overly rapid correction of the hyponatremia, which can lead to irreversible neurologic injury. Likewise, the cost of tolvaptan may be prohibitive (as high as \$300 per tablet in some areas). Of note, patients with ectopic ANP may have worsening hyponatremia if sodium intake is not concomitantly increased. Accordingly, if hyponatremia fails to improve or worsens after 3–4 days of adequate fluid restriction, plasma levels of ANP should be measured to determine the causative syndrome.

Ectopic secretion of ACTH by SCLC and pulmonary carcinoids usually results in additional electrolyte disturbances, especially hypokalemia, rather than the changes in body habitus that occur in Cushing's syndrome from a pituitary adenoma (Chap. 93). Treatment with standard medications, such as metyrapone and ketoconazole, is largely ineffective due to extremely high cortisol levels. The most effective strategy for management of the Cushing's syndrome is effective treatment of the underlying SCLC. Bilateral adrenalectomy may be considered in extreme cases.

Skeletal-connective tissue syndromes include clubbing in 30% of cases (usually NSCLCs) and hypertrophic primary osteoarthropathy in 1–10% of cases (usually adenocarcinomas). Patients may develop periostitis, causing pain, tenderness, and swelling over the affected bones and a positive bone scan. Neurologic-myopathic syndromes are seen in only 1% of patients but are dramatic and include the myasthenic Eaton-Lambert syndrome and retinal blindness with SCLC, whereas peripheral neuropathies, subacute cerebellar degeneration, cortical degeneration, and polymyositis are seen with all lung cancer types. Many of these are caused by autoimmune responses such as the development of anti-voltage-gated calcium channel antibodies in Eaton-Lambert syndrome. Patients with this disorder present with proximal muscle weakness, usually in the lower extremities, occasional autonomic dysfunction, and rarely, cranial nerve symptoms or involvement of the bulbar or respiratory muscles. Depressed deep tendon reflexes are frequently present. In contrast to patients with myasthenia gravis, strength improves with serial effort. Some patients who respond to chemotherapy will have resolution of the neurologic abnormalities. Thus, chemotherapy is the initial treatment of choice. Paraneoplastic encephalomyelitis and sensory neuropathies, cerebellar degeneration, limbic encephalitis, and brainstem encephalitis occur in SCLC in association with a variety of antineuronal antibodies such as anti-Hu, anti-CRMP5, and ANNA-3. Paraneoplastic cerebellar degeneration may be associated with anti-Hu, anti-Yo, or P/Q calcium channel autoantibodies. Coagulation or thrombotic or other hematologic manifestations occur in 1–8% of patients and include migratory venous thrombophlebitis (Trousseau's syndrome), nonbacterial thrombotic (marantic) endocarditis with arterial emboli, and disseminated

intravascular coagulation with hemorrhage, anemia, granulocytosis, and leukoerythroblastosis. Thrombotic disease complicating cancer is usually a poor prognostic sign. Cutaneous manifestations such as dermatomyositis and acanthosis nigricans are uncommon (1%), as are the renal manifestations of nephrotic syndrome and glomerulonephritis (<1%).

DIAGNOSING LUNG CANCER

Tissue sampling is required to confirm a diagnosis in all patients with suspected lung cancer. In patients with suspected metastatic disease, a biopsy of a distant site of disease is preferred for tissue confirmation. Given the greater emphasis placed on molecular and PD-L1 testing for NSCLC patients, a core biopsy is preferred to ensure adequate tissue for analysis. Tumor tissue may be obtained via minimally invasive techniques such as bronchial or transbronchial biopsy during fiberoptic bronchoscopy, by fine-needle aspiration (FNA) or percutaneous biopsy using image guidance, or via endobronchial ultrasound (EBUS)-guided biopsy. Depending on the location, lymph node sampling may occur via transesophageal endoscopic ultrasound (EUS)-guided biopsy, EBUS-guided biopsy, or blind biopsy. In patients with suspected metastatic disease, a diagnosis may be confirmed by bronchoscopy, percutaneous biopsy of a soft tissue mass, lytic bone lesion, bone marrow, pleural or liver lesion, or an adequate cell block obtained from a malignant pleural effusion. In patients with a suspected malignant pleural effusion, if the initial thoracentesis is negative, a repeat thoracentesis is warranted. Although the majority of pleural effusions are due to malignant disease, particularly if they are exudative or bloody, some may be parapneumonic. In the absence of distant disease, such patients should be considered for possible curative treatment.

The diagnostic yield of any biopsy depends on several factors including location (accessibility) of the tumor, tumor size, tumor type, and technical aspects of the diagnostic procedure including the experience level of the bronchoscopist and pathologist. In general, central lesions such as squamous cell carcinomas, small-cell carcinomas, or endobronchial lesions such as carcinoid tumors are more readily diagnosed by bronchoscopic examination, whereas peripheral lesions such as adenocarcinomas and large-cell carcinomas are more amenable to transthoracic biopsy.

Sputum cytology is inexpensive and noninvasive but has a lower yield than other specimen types due to poor preservation of the cells and more variability in acquiring a good-quality specimen. The yield for sputum cytology is highest for larger and centrally located tumors such as squamous cell carcinoma and small-cell carcinoma histology. The specificity for sputum cytology averages close to 100%, although sensitivity is generally <70%. The accuracy of sputum cytology improves with increased numbers of specimens analyzed. Consequently, analysis of at least three sputum specimens is recommended. However, the quality of the specimen may not be adequate for histologic subclassification and PD-L1 and molecular testing.

STAGING LUNG CANCER

Lung cancer staging consists of two parts: first, a determination of the location of the tumor and possible metastatic sites (anatomic staging), and second, an assessment of a patient's ability to withstand various antitumor treatments (physiologic staging). All patients with lung cancer should have a complete history and physical examination, with evaluation of all other medical problems, determination of performance status, and history of weight loss. Staging with regard to a patient's potential for surgical resection is principally applicable to NSCLC.

■ ANATOMIC STAGING OF PATIENTS WITH LUNG CANCER

The accurate staging of patients with NSCLC is essential for determining the appropriate treatment in patients with resectable disease and for avoiding unnecessary surgical procedures in patients with advanced disease. All patients with NSCLC should undergo initial radiographic imaging with CT scan, positron emission tomography (PET), or preferably CT-PET. PET scanning attempts to identify sites of malignancy based on glucose metabolism by measuring the uptake

of ^{18}F -fluorodeoxyglucose (FDG). Rapidly dividing cells, presumably in the lung tumors, will preferentially take up ^{18}F -FDG and appear as a ‘hot spot.’ To date, PET has been mostly used for staging and detection of metastases in lung cancer and in the detection of nodules >15 mm in diameter. Combined ^{18}F -FDG PET-CT imaging has been shown to improve the accuracy of staging in NSCLC compared to visual correlation of PET and CT or either study alone. CT-PET has been found to be superior in identifying pathologically enlarged mediastinal lymph nodes and extrathoracic metastases. A standardized uptake value (SUV) of >2.5 on PET is highly suspicious for malignancy. False negatives can be seen in diabetes, in lesions <8 mm, and in slow-growing tumors (e.g., carcinoid tumors or well-differentiated adenocarcinoma). False positives can be seen in certain infections and granulomatous disease (e.g., tuberculosis). Thus, PET should never be used alone to diagnose lung cancer, mediastinal involvement, or metastases. Confirmation with tissue biopsy is required. For brain metastases, magnetic resonance imaging (MRI) is the most effective method. MRI can also be useful in selected circumstances, such as superior sulcus tumors to rule out brachial plexus involvement, but in general, MRI does not play a major role in NSCLC staging.

In patients with NSCLC, the following are contraindications to potential curative resection: extrathoracic metastases, superior vena cava syndrome, vocal cord and, in most cases, phrenic nerve paralysis, malignant pleural effusion, cardiac tamponade, tumor within 2 cm of the carina (potentially curable with combined chemoradiotherapy), metastasis to the contralateral lung, metastases to supraclavicular lymph nodes, contralateral mediastinal node metastases (potentially curable with combined chemoradiotherapy), and involvement of the main pulmonary artery. In situations where it will make a difference in treatment, abnormal scan findings require tissue confirmation of malignancy so that patients are not precluded from having potentially curative therapy.

The best predictor of metastatic disease remains a careful history and physical examination. If signs, symptoms, or findings from the physical examination suggest the presence of malignancy, then sequential imaging starting with the most appropriate study should be performed. If the findings from the clinical evaluation are negative, then imaging studies beyond CT-PET are unnecessary and the search for metastatic disease is complete. In patients in whom distant metastatic disease has been ruled out, lymph node status needs to be assessed via minimally invasive techniques such as those mentioned above and/or invasive techniques such as mediastinoscopy, mediastinotomy, thoracoscopy, or thoracotomy. Approximately one-quarter to one-half of patients diagnosed with NSCLC will have mediastinal lymph node metastases at the time of diagnosis. Lymph node sampling is recommended in all patients with enlarged nodes detected by CT or PET scan and in patients with large tumors or tumors occupying the inner third of the lung. The extent of mediastinal lymph node involvement is

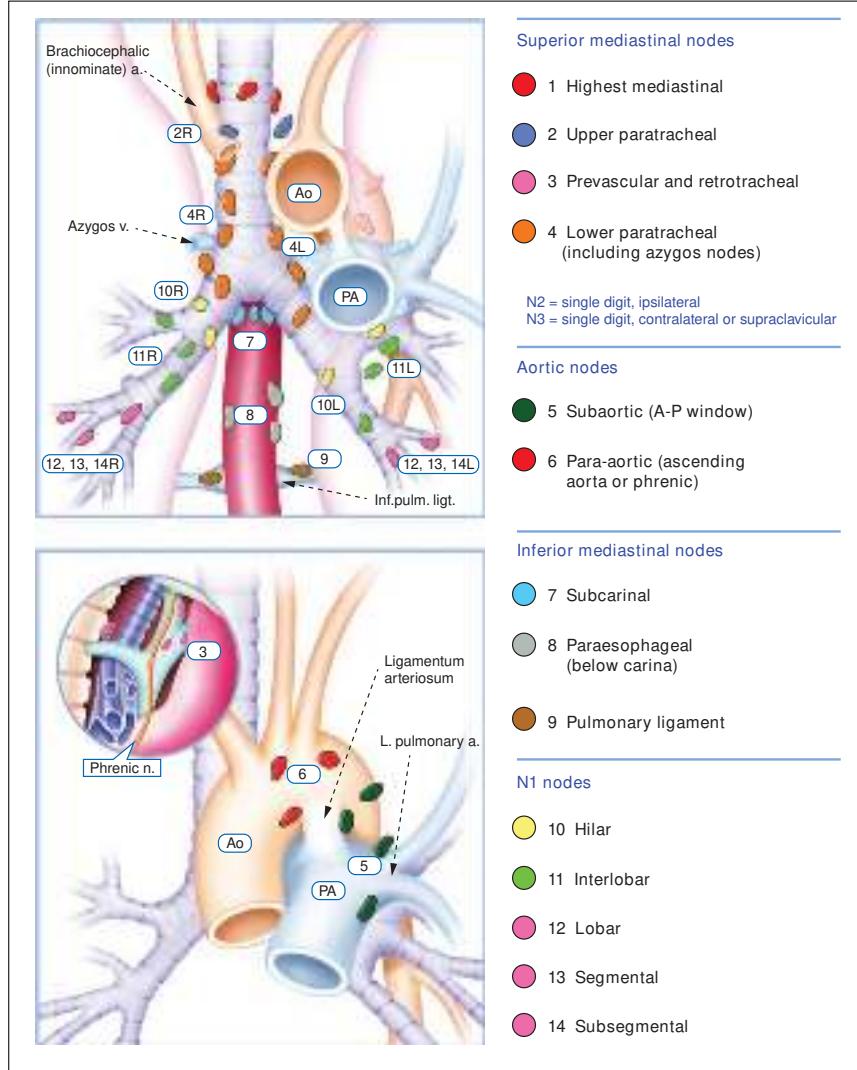


FIGURE 78-3 Lymph node stations in staging non-small-cell lung cancer. The International Association for the Study of Lung Cancer (IASLC) lymph node map, including the proposed grouping of lymph node stations into “zones” for the purposes of prognostic analyses. a., artery; Ao, aorta; Inf. pulm. ligt., inferior pulmonary ligament; n., nerve; PA, pulmonary artery; v., vein.

important in determining the appropriate treatment strategy: surgical resection followed by adjuvant chemotherapy versus combined chemoradiation followed by immunotherapy (durvalumab) (see below). A standard nomenclature for referring to the location of lymph nodes involved with lung cancer has evolved (Fig. 78-3).

In SCLC patients, current staging recommendations include a PET-CT scan and MRI of the brain (positive in 10% of asymptomatic patients). Bone marrow biopsies and aspirations are rarely performed now given the low incidence of isolated bone marrow metastases. Confirmation of metastatic disease, ipsilateral or contralateral lung nodules, or metastases beyond the mediastinum may be achieved by the same modalities recommended earlier for patients with NSCLC.

If a patient has signs or symptoms of spinal cord compression (pain, weakness, paralysis, urinary retention), a spinal CT or MRI scan should be performed. If metastases are evident on imaging, a neurosurgeon should be consulted for possible palliative surgical resection and/or a radiation oncologist should be consulted for palliative radiotherapy to the site of compression. If signs or symptoms of leptomeningeal disease develop at any time in a patient with lung cancer, an MRI of the brain and spinal cord should be performed, as well as a spinal tap, for

detection of malignant cells. If the spinal tap is negative, a repeat spinal tap should be considered. There is currently no approved therapy for the treatment of leptomeningeal disease.

■ STAGING SYSTEM FOR NON SMALL CELL LUNG CANCER

The tumor-node-metastasis (TNM) international staging system provides useful prognostic information and is used to stage all patients with NSCLC. The various T (tumor size), N (regional node involvement), and M (presence or absence of distant metastasis) stages are combined to form different stage groups (Tables 74-5 and 74-6). The eighth edition of the TNM staging system went into effect in 2018. T1 tumors are divided into tumors ≤ 1 cm (T1a), >1 cm and ≤ 2 cm (T1b), and >2 cm and ≤ 3 cm (T1c). T2 tumors are those that are >3 cm but ≤ 5 cm, involve the visceral pleura or main bronchus, or are associated with atelectasis; T2a tumors are >3 cm and ≤ 4 cm, and T2b are >4 cm and ≤ 5 cm. T3 tumors are >5 cm and ≤ 7 cm. T3 tumors also include tumors with invasion into local structures such as the chest wall and diaphragm and with additional nodules in the same lobe. T4 tumors include tumors >7 cm or tumors of any size with invasion into mediastinum, heart, great vessels, trachea, esophagus, or spine

TABLE 78-6 TNM Stage Groupings, Eighth Edition

Stage IA1	T1a	N0	M0
Stage IA2	T1b	N0	M0
Stage IA3	T1c	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T2b	N0	M0
Stage IIB	T1a-T2b T3	N1 N0	M0 M0
Stage IIIA	T1-2b T3 T4	N2 N1 N0/N1	M0 M0 M0
Stage IIIB	T1-2b T3/T4 T3/T4	N3 N0/N1 N3	M0 M0 M0
Stage IVA	Any T	Any N	M1a/M1b
Stage IVB	Any T	Any N	M1c

TABLE 78-5 TNM Staging System for Lung Cancer (Eighth Edition)

Primary Tumor (T)	
T1	Tumor ≤ 3 cm diameter, surrounded by lung or visceral pleura, without invasion more proximal than lobar bronchus
T1mi	Minimally invasive adenocarcinoma (pure lepidic pattern, <3 cm in greatest dimension and <5 mm invasion)—T1a (size <1 cm)—T1b (1 cm $<$ size <2 cm)—T1c (2 cm $<$ size <3 cm)
T2	Tumor >3 cm but ≤ 7 cm, or tumor with any of the following features: Involves main bronchus ≥ 2 cm distal to carina Invades visceral pleura Associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung
T2a	Tumor >3 cm but ≤ 5 cm
T2b	Tumor >5 cm but ≤ 7 cm
T3	Tumor >7 cm or any of the following: Directly invades any of the following: chest wall, diaphragm, phrenic nerve, mediastinal pleura, parietal pericardium, main bronchus <2 cm from carina (without involvement of carina) Atelectasis or obstructive pneumonitis of the entire lung Separate tumor nodules in the same lobe
T4	Tumor of any size that invades the mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, or carina, or with separate tumor nodules in a different ipsilateral lobe
Nodal Stage (N)	
N0	No regional lymph node metastases
N1	Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2	Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
N3	Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)
Metastases (M)	
M0	No distant metastasis
M1	Distant metastasis
M1a	Separate tumor nodule(s) in a contralateral lobe; tumor with pleural nodules or malignant pleural or pericardial effusion
M1b	Distant metastasis (in extrathoracic organs)
M1c	

Abbreviation: M1b, distant metastasis in single organ outside chest; M1c, multiple extrathoracic metastases to one or more organs; TNM, tumor-node-metastasis.

or with multiple nodules in the ipsilateral lung. Lymph node staging depends on metastasis to ipsilateral pulmonary or hilar nodes (N1), mediastinal or subcarinal nodes (N2), or contralateral mediastinal, hilar, or supraclavicular nodes (N3). Patients with metastasis may be classified as M1a (malignant pleural or pericardial effusion, pleural nodules, or nodules in the contralateral lung), M1b (single distant metastasis to a single organ; e.g., bone, liver, adrenal, or brain metastasis), or M1c (multiple metastases to a single organ or metastases to multiple organs). The effect of stage on survival is illustrated in Fig. 78-4. Approximately 15% of patients have localized disease that can be treated with curative attempt (surgery or radiotherapy), about a quarter have local or regional disease that may or may not be amenable to a curative attempt, and half have metastatic disease at the time of diagnosis. In 10%, the extent of disease is undefined.

■ STAGING SYSTEM FOR SMALL CELL LUNG CANCER

In patients with SCLC, it is now recommended that both the Veterans Administration system and the American Joint Committee on Cancer/International Union Against Cancer eighth edition system (TNM) be used to classify the tumor stage. The Veterans Administration system is a distinct two-stage system dividing patients into those with limited- or extensive-stage disease. Patients with limited-stage disease (LD) have cancer that is confined to the ipsilateral hemithorax and can be encompassed within a tolerable radiation port. Thus, contralateral supraclavicular nodes, recurrent laryngeal nerve involvement, and superior vena caval obstruction can all be part of LD. Patients with extensive-stage disease (ED) have overt metastatic disease by imaging or physical examination. Cardiac tamponade, malignant pleural effusion, and bilateral pulmonary parenchymal involvement generally qualify disease as ED, because the involved organs cannot be encompassed safely or effectively within a single radiation therapy port. Sixty to 70% of patients are diagnosed with ED at presentation. The TNM staging system is preferred in the rare SCLC patient presenting with what appears to be clinical stage I disease (see above).

■ PHYSIOLOGIC STAGING

Patients with lung cancer often have other comorbid conditions related to smoking including cardiovascular disease and COPD. To improve their preoperative condition, correctable problems (e.g., anemia, electrolyte and fluid disorders, infections, cardiac disease, and arrhythmias) should be addressed, appropriate chest physical therapy should be instituted, and patients should be encouraged to stop smoking. Patients with a forced expiratory volume in 1 s (FEV₁) of >2 L or $>80\%$ of predicted can tolerate a pneumonectomy, and those with an FEV₁ >1.5 L have adequate reserve for a lobectomy. In patients with borderline lung function but a resectable tumor, cardiopulmonary exercise testing could be performed as part of the physiologic evaluation. This test allows an estimate of the maximal oxygen consumption ($V_{\text{O}_{\text{max}}}$). A $V_{\text{O}_{\text{max}}}$

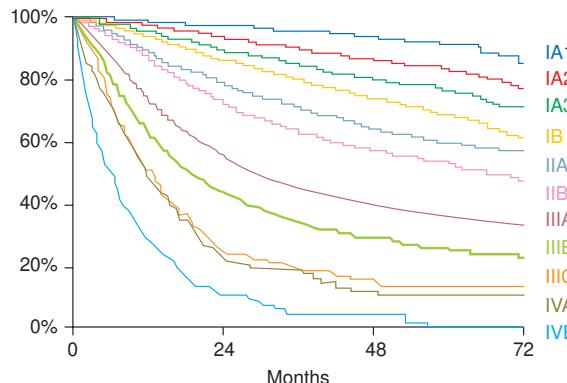


FIGURE 78-4 Influence of non-small-cell lung cancer stage on survival.

<15 mL/(kg·min) predicts for a higher risk of postoperative complications. Patients deemed unable to tolerate lobectomy or pneumonectomy from a pulmonary functional standpoint may be candidates for more limited resections, such as wedge or anatomic segmental resection, although such procedures are associated with significantly higher rates of local recurrence and a trend toward decreased overall survival. All patients should be assessed for cardiovascular risk using American College of Cardiology and American Heart Association guidelines. A myocardial infarction within the past 3 months is a contraindication to thoracic surgery because 20% of patients will die of reinfarction. An infarction in the past 6 months is a relative contraindication. Other major contraindications include uncontrolled arrhythmias, an FEV₁ of <1 L, CO₂ retention (resting P_{CO₂} >45 mmHg), DL <40%, and severe pulmonary hypertension.

TREATMENT

Non-Small-Cell Lung Cancer

The overall treatment approach to patients with NSCLC is shown in Fig. 78-5.

OCCULT AND STAGE 0 CARCINOMAS

Patients with severe atypia on sputum cytology have an increased risk of developing lung cancer compared to those without atypia. In the uncommon circumstance where malignant cells are identified in a sputum or bronchial washing specimen but the chest imaging appears normal (TX tumor stage), the lesion must be localized. More than 90% of tumors can be localized by meticulous examination of the bronchial tree with a fiberoptic bronchoscope under general anesthesia and collection of a series of differential brushings and biopsies. Surgical resection following bronchoscopic localization has been shown to improve survival compared to no treatment. Close follow-up of these patients is indicated because of the high incidence of second primary lung cancers (5% per patient per year).

SOLITARY PULMONARY NODULE AND "GROUND-GLASS" OPACITIES

A solitary pulmonary nodule is defined as an x-ray density completely surrounded by normal aerated lung with circumscribed margins, of any shape, usually 1–6 cm in greatest diameter. The approach to a patient with a solitary pulmonary nodule is based on an estimate of the probability of cancer, determined according to the patient's smoking history, age, and characteristics on imaging (Table 78-7). Prior CXRs and CT scans should be obtained if available for comparison. A PET scan may be useful if the lesion is greater than 7–8 mm in diameter. If no diagnosis is apparent, Mayo investigators reported that clinical characteristics (age, cigarette

Stage	24 months	60 months
IA1	97%	92%
IA2	94%	83%
IA3	90%	77%
IB	87%	68%
IIA	79%	60%
IIB	72%	53%
IIIA	55%	36%
IIIB	44%	26%
IIIC	24%	13%
IVA	23%	10%
IVB	10%	0%

smoking status, and prior cancer diagnosis) and three radiologic characteristics (nodule diameter, spiculation, and upper lobe location) were independent predictors of malignancy. At present, only two radiographic criteria are thought to predict the benign nature of a solitary pulmonary nodule: lack of growth over a period >2 years and certain characteristic patterns of calcification. Calcification alone, however, does not exclude malignancy; a dense central nidus, multiple punctate foci, and "bull's eye" (granuloma) and "popcorn ball" (hamartoma) calcifications are highly suggestive of a benign lesion. In contrast, a relatively large lesion, lack of or asymmetric calcification, chest symptoms, associated atelectasis, pneumonitis, or growth of the lesion revealed by comparison with an old x-ray or CT scan or a positive PET scan may be suggestive of a malignant process and warrant further attempts to establish a histologic diagnosis. An algorithm for assessing these lesions is shown in Fig. 78-6.

Since the advent of screening CTs, small "ground-glass" opacities (GGOs) have often been observed, particularly as the increased sensitivity of CTs enables detection of smaller lesions. Many of these GGOs, when biopsied, are found to be atypical adenomatous hyperplasia (AAH), adenocarcinoma in situ (AIS), or minimally invasive adenocarcinoma (MIA). AAH is usually a nodule of <5 mm and is minimally hazy, also called nonsolid or ground glass (i.e., hazy slightly increased attenuation, no solid component, and preservation of bronchial and vascular margins). On thin-section CT, AIS is usually a nonsolid nodule and tends to be slightly more opaque than AAH. MIA is mainly solid, usually with a small (<5 mm) central solid component. However, overlap exists among the imaging features of the preinvasive and minimally invasive lesions in the lung adenocarcinoma spectrum. Lepidic adenocarcinomas are usually solid but may be nonsolid. Likewise, the small invasive adenocarcinomas also are usually solid but may exhibit a small nonsolid component.

MANAGEMENT OF STAGES I AND II NSCLC

Surgical Resection of Stage I and II NSCLC Surgical resection, ideally by an experienced thoracic surgeon, is the treatment of choice for patients with clinical stage I and II NSCLC who are able to tolerate the procedure. Operative mortality rates for patients resected by thoracic or cardiothoracic surgeons are lower compared to general surgeons. Moreover, survival rates are higher in patients who undergo resection in facilities with a high surgical volume compared to those performing fewer than 70 procedures per year, even though the higher-volume facilities often serve older and less socioeconomically advantaged populations. The improvement in survival is most evident in the immediate postoperative period. In patients with stage I NSCLC, lobectomy is superior to wedge

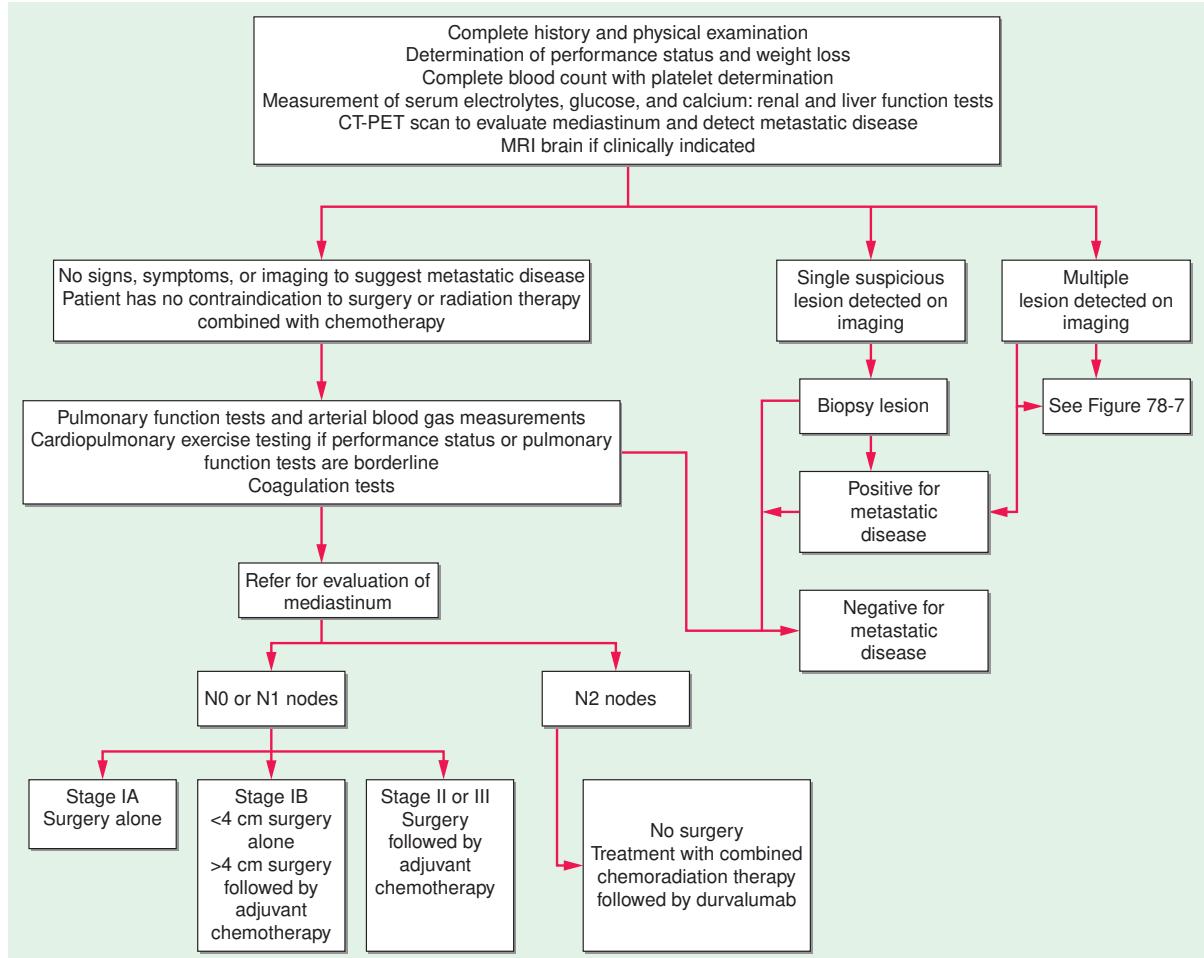


FIGURE 78-5 Algorithm for management of non-small-cell lung cancer. MRI, magnetic resonance imaging; PET, positron emission tomography.

resection with respect to rates of local recurrence. There is also a trend toward improvement in overall survival. In patients with comorbidities, compromised pulmonary reserve, and small peripheral lesions, a limited resection, wedge resection, or segmentectomy (potentially by video-assisted thoracoscopic surgery) may be reasonable surgical options. Pneumonectomy is reserved for patients with central tumors and should be performed only in patients with excellent pulmonary reserve. The 5-year survival rates are 68–92% for patients with stage I NSCLC and 53–60% for patients with stage II NSCLC.

TABLE 78-7 Assessment of Risk of Cancer in Patients with Solitary Pulmonary Nodules

VARIABLE	RISK		
	LOW	INTERMEDIATE	HIGH
Diameter (cm)	<1.5	1.5–2.2	≥2.3
Age (years)	<45	45–60	>60
Smoking status	Never smoker	Current smoker (<20 cigarettes/d)	Current smoker (>20 cigarettes/d)
Smoking cessation status	Quit ≥7 years ago or quit	Quit <7 years ago	Never quit
Characteristics of nodule margins	Smooth	Scalloped	Corona radiata or spiculated

Source: From D Ost et al: The solitary pulmonary nodule. N Engl J Med 348:2535, 2003. Copyright © (2003) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

Accurate pathologic staging requires adequate segmental, hilar, and mediastinal lymph node sampling. Ideally, this includes a mediastinal lymph node dissection. On the right side, mediastinal stations 2R, 4R, 7, 8R, and 9R should be dissected; on the left side, stations 5, 6, 7, 8L, and 9L should be dissected. Hilar lymph nodes are typically resected and sent for pathologic review, although it is helpful to specifically dissect and label level 10 lymph nodes when possible. On the left side, level 2 and sometimes level 4 lymph nodes are generally obscured by the aorta. Although the therapeutic benefit of nodal dissection versus nodal sampling is controversial, a pooled analysis of three trials involving patients with stages I to IIIA NSCLC demonstrated a superior 4-year survival in patients undergoing resection and a complete mediastinal lymph node dissection compared with lymph node sampling. Moreover, complete mediastinal lymphadenectomy added little morbidity to a pulmonary resection for lung cancer when carried out by an experienced thoracic surgeon.

Radiation Therapy in Stages I and II NSCLC There is currently no role for postoperative radiation therapy in patients following resection of stage I or II NSCLC with negative margins. However, patients with stage I and II disease who either refuse or are not suitable candidates for surgery should be considered for radiation therapy with *curative* intent. Stereotactic body radiation therapy (SBRT) is a technique used to treat patients with isolated pulmonary nodules (≤ 5 cm) who are not candidates for or refuse surgical resection. Treatment is typically administered in three to five

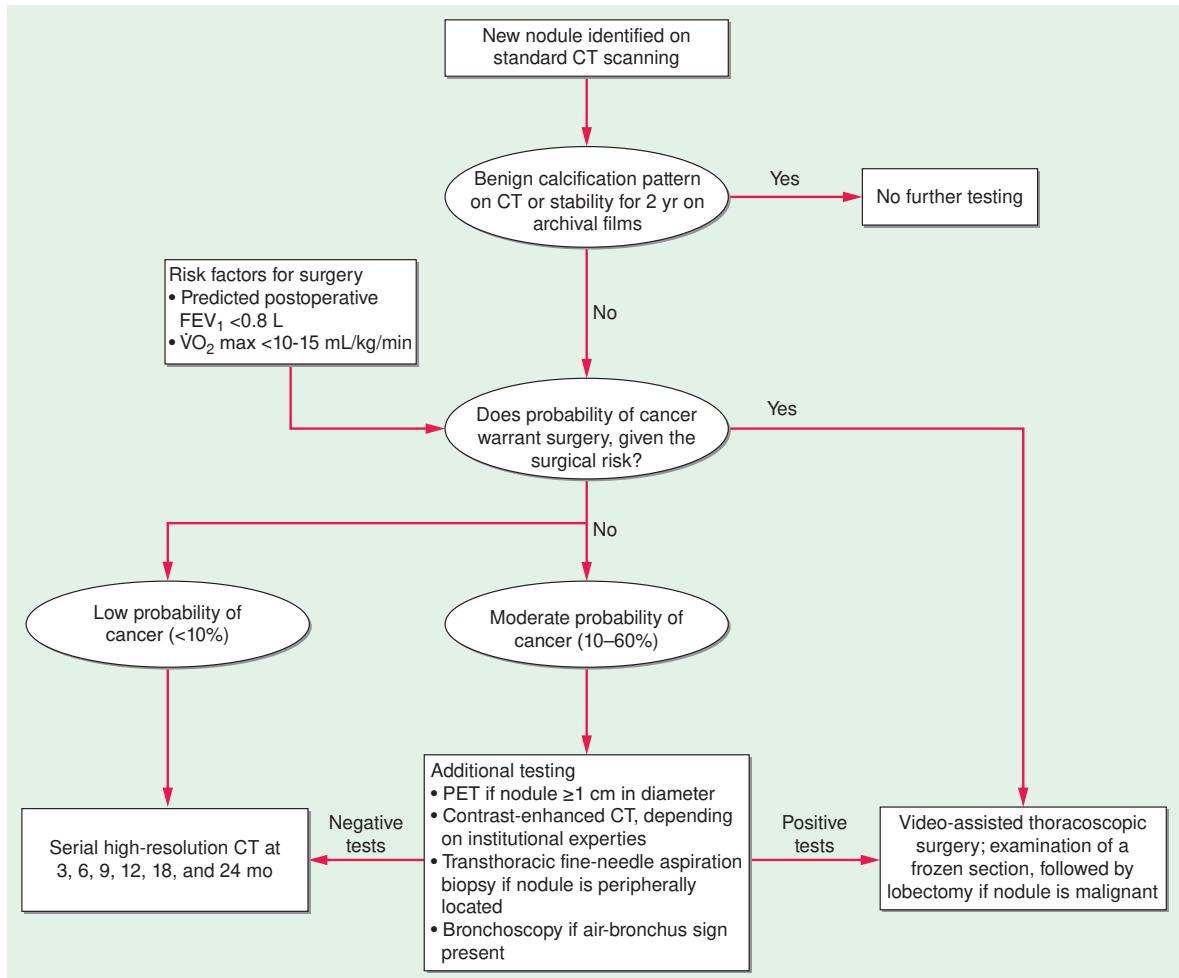


FIGURE 78-6 Approach to the solitary pulmonary nodule. FEV₁, forced expiratory volume in 1 s; PET, positron emission tomography.

fractions delivered over 1–2 weeks. In uncontrolled studies, disease control rates are >90%, and 5-year survival rates of up to 60% have been reported with SBRT. By comparison, survival rates typically range from 13 to 39% in patients with stage I or II NSCLC treated with standard external-beam radiotherapy. Cryoablation is another technique occasionally used to treat small, isolated tumors (i.e., ≤3 cm). However, very little data exist on long-term outcomes with this technique.

Chemotherapy in Stages I and II NSCLC Although a landmark meta-analysis of cisplatin-based adjuvant chemotherapy trials in patients with resected stages I to IIIA NSCLC (the Lung Adjuvant Cisplatin Evaluation [LACE] Study) demonstrated a 5.4% improvement in 5-year survival for adjuvant chemotherapy compared to surgery alone, the survival benefit was seemingly confined to patients with stage II or III disease (Table 78-8). By contrast, survival was actually worsened in stage IA patients with the application of adjuvant therapy. In stage IB, there was a modest improvement in survival of questionable clinical significance. Adjuvant chemotherapy was also detrimental in patients with poor performance status (Eastern Cooperative Oncology Group [ECOG] performance status = 2). These data suggest that adjuvant chemotherapy is best applied in patients with resected stage II or III NSCLC. There is no apparent role for adjuvant chemotherapy in patients with resected stage IA or IB NSCLC. A possible exception to the prohibition of adjuvant therapy in this setting is the stage IB patient with a resected

lesion ≥4 cm. Osimertinib, an EGFR TKI, demonstrated improved disease-free survival for patients with EGFR mutation (exon 19 deletion or L858R)-positive NSCLC treated for 3 years following chemotherapy. However, the effect on overall survival is unknown.

As with any treatment recommendation, the risks and benefits of adjuvant chemotherapy should be considered on an individual patient basis. If a decision is made to proceed with adjuvant chemotherapy, in general, treatment should be initiated 6–12 weeks after surgery, assuming the patient has fully recovered, and should be administered for no more than four cycles. Although cisplatin-based chemotherapy is the preferred treatment regimen, carboplatin can be substituted for cisplatin in patients who are unlikely to tolerate cisplatin for reasons such as reduced renal function, presence of neuropathy, or hearing impairment. A large cooperative group trial compared cisplatin-based chemotherapy with vinorelbine, pemetrexed, gemcitabine, or docetaxel with or without antiangiogenic therapy. While adding antiangiogenic therapy to platinum-based chemotherapy offered no benefit, the trial also demonstrated no difference in survival among the four chemotherapy regimens. Therefore, no specific chemotherapy regimen is considered superior in this setting, and treatment selection may be based on cost and patient comorbidities.

Neoadjuvant chemotherapy, which is the application of chemotherapy administered before an attempted surgical resection, has been advocated by some experts on the assumption that such an approach will more effectively extinguish occult micrometastases compared to

TABLE 78-8 Adjuvant Chemotherapy Trials in Non-Small-Cell Lung Cancer

TRIAL	STAGE	TREATMENT	NO. OF PATIENTS	5-YEAR SURVIVAL (%)	P
IALT	I–III	Cisplatin-based	932	44.5	<.03
		Control	835	40.4	
BR10	IB–II	Cisplatin + vinorelbine	242	69	.03
		Control	240	54	
ANITA	IB–IIIA	Cisplatin + vinorelbine	407	60	.017
		Control	433	58	
ALPI	I–III	MVP	548	50	.49
		Control	540	45	
BLT	I–III	Cisplatin-based	192	60	.90
		Control	189	58	
CALGB	IB	Carboplatin + paclitaxel	173	59	.10
			171	57	
ECOG1505	IB > 4c – IIIA	Cisplatin – based	749	NR	.90
		+ bevacizumab	752	NR	

Abbreviations: ALPI, Adjuvant Lung Cancer Project Italy; ANITA, Adjuvant Navelbine International Trialist Association; BLT, Big Lung Trial; CALGB, Cancer and Lung Cancer Group B; ECOG, Eastern Cooperative Oncology Group; IALT, International Adjuvant Lung Cancer Trial; MVP, mitomycin, vinodesine, and cisplatin; NR, not reported.

postoperative chemotherapy. In addition, it is thought that preoperative chemotherapy might render an inoperable lesion resectable. A meta-analysis of 15 randomized controlled trials involving more than 2300 patients with stage I to III NSCLC suggested there may be a modest 5-year survival benefit (i.e., 5%) that is virtually identical to the survival benefit achieved with postoperative chemotherapy. Accordingly, neoadjuvant therapy may prove useful in selected cases (see below). A decision to use neoadjuvant chemotherapy should always be made in consultation with an experienced surgeon.

All patients with resected NSCLC are at high risk of developing a second primary lung cancer or recurrence, most of which occur within 18–24 months of surgery. Thus, it is reasonable to follow these patients with periodic imaging studies. Given the results of the NLST, periodic CT scans appear to be the most appropriate screening modality. Based on the timing of most recurrences, some guidelines recommend a contrasted chest CT scan every 6 months for the first 3 years after surgery, followed by yearly CT scans of the chest without contrast thereafter.

MANAGEMENT OF STAGE III NSCLC

Management of patients with stage III NSCLC usually requires a combined-modality approach. Patients with stage IIIB disease commonly are stratified into those with “nonbulky” or “bulky” mediastinal lymph node (N2) disease. Although the definition of “bulky” N2 disease varies somewhat in the literature, the usual criteria include the size of a dominant lymph node (i.e., >2–3 cm in short-axis diameter as measured by CT), groupings of multiple smaller lymph nodes, evidence of extracapsular nodal involvement, or involvement of more than two lymph node stations. The distinction between nonbulky and bulky stage IIIB disease is mainly used to select potential candidates for *upfront* surgical resection or for resection after neoadjuvant therapy. Many aspects of therapy of patients with stage III NSCLC remain controversial, and the optimal treatment strategy has not been clearly defined. Furthermore, because stage III disease is highly heterogeneous, no single treatment approach can be recommended for all patients. Key factors guiding treatment choices include the particular combination of tumor (T) and nodal (N) disease, the ability to achieve a complete surgical resection if indicated, and the patient’s

overall physical condition and preferences. For example, in carefully selected patients with limited stage IIIB disease where involved mediastinal lymph nodes can be completely resected, initial surgery followed by postoperative chemotherapy (with or without radiation therapy) may be indicated. By contrast, for patients with clinically evident bulky mediastinal lymph node involvement, the standard approach to treatment is concurrent chemoradiotherapy followed by a year of immunotherapy with durvalumab or other PD-L1-directed antibody.

Absent and Nonbulky Mediastinal (N2, N3) Lymph Node Disease

For the subset of stage IIIB patients initially thought to have clinical stage I or II disease (i.e., pathologic involvement of mediastinal [N2] lymph nodes is *not* detected preoperatively), surgical resection is often the treatment of choice. This is followed by adjuvant chemotherapy in patients with microscopic lymph node involvement in a resection specimen. Postoperative radiation therapy (PORT) may also have a role for those with close or positive surgical margins. Patients with tumors exceeding 7 cm in size or involving the chest wall or proximal airways within 2 cm of the carina with hilar lymph node involvement (but not N2 disease) are classified as having T3N1 stage IIIB disease. They too are best managed with surgical resection, if technically feasible, followed by adjuvant chemotherapy if completely resected. Patients with T3N0 or T3N1 disease due to the presence of satellite nodules within the same lobe as the primary tumor are also candidates for surgery, as are patients with ipsilateral nodules in another lobe and negative mediastinal nodes (IIIA, T4N0 or T4N1). Although data regarding adjuvant chemotherapy in the latter subsets of patients are limited, it is often recommended.

Patients with T4N0–1 may have involvement of the carina, superior vena cava, or a vertebral body and yet still be candidates for surgical resection in selected circumstances. The decision to proceed with an attempted resection must be made in consultation with an experienced thoracic surgeon often in association with a vascular or cardiac surgeon and an orthopedic surgeon depending on tumor location. However, if an incomplete resection is inevitable or if there is evidence of N2 involvement (stage IIIB), surgery for T4 disease is contraindicated. Most T4 lesions are best treated with concurrent chemoradiotherapy followed by durvalumab.

The role of PORT in patients with completely resected stage III NSCLC is controversial. To a large extent, the use of PORT is dictated by the presence or absence of N2 involvement and, to a lesser degree, by the biases of the treating physician. Using the Surveillance, Epidemiology, and End Results (SEER) database, a recent meta-analysis of PORT identified a significant increase in survival in patients with N2 disease but not in patients with N0 or N1 disease. An earlier analysis by the PORT Meta-analysis Trialist Group using an older database produced similar results.

Known Mediastinal (N2, N3) Lymph Node Disease When pathologic involvement of mediastinal lymph nodes is documented preoperatively, a combined-modality approach is recommended assuming the patient is a candidate for treatment with curative intent. These patients are at high risk for both local and distant recurrence if managed with resection alone. For patients with stage III disease who are not candidates for surgical resection, *concurrent* chemoradiotherapy is most commonly used as the initial treatment followed by durvalumab. Concurrent chemoradiotherapy has been shown to produce superior survival compared to *sequential* chemoradiotherapy; however, it also is associated with greater host toxicities (including fatigue, esophagitis, and neutropenia). Therefore, for patients with a good performance status, concurrent chemoradiotherapy is the preferred treatment approach, whereas sequential chemoradiotherapy may be more appropriate for patients with a performance status that is not as good. For patients who are *not* candidates for a combined-modality treatment approach, typically due to a poor performance status or a comorbidity that makes chemotherapy untenable, radiotherapy alone may provide a modest survival benefit in addition to symptom palliation.

For patients with potentially resectable N2 disease, it remains uncertain whether surgery after neoadjuvant chemoradiotherapy improves survival. In an NCI-sponsored Intergroup randomized trial comparing concurrent chemoradiotherapy alone to concurrent chemoradiotherapy followed by attempted surgical resection, no survival benefit was observed in the trimodality arm compared to the bimodality therapy. In fact, patients subjected to a pneumonectomy had a worse survival outcome. By contrast, those treated with a lobectomy appeared to have a survival advantage based on a retrospective subset analysis. Thus, in carefully selected, otherwise healthy patients with nonbulky mediastinal lymph node involvement, surgery may be a reasonable option if the primary tumor can be fully resected with a lobectomy. This is not the case if a pneumonectomy is required to achieve complete resection.

Superior Sulcus Tumors (Pancoast Tumors) Superior sulcus tumors represent a distinctive subset of stage III disease. These tumors arise in the apex of the lung and may invade the second and third ribs, the brachial plexus, the subclavian vessels, the stellate ganglion, and adjacent vertebral bodies. They also may be associated with Pancoast syndrome, characterized by pain that may arise in the shoulder or chest wall or radiate to the neck. Pain characteristically radiates to the ulnar surface of the hand. Horner's syndrome (enophthalmos, ptosis, miosis, and anhidrosis) due to invasion of the paravertebral sympathetic chain may be present as well. Patients with these tumors should undergo the same staging procedures as all patients with stage II and III NSCLC. Neoadjuvant chemotherapy or combined chemoradiotherapy followed by surgery is reserved for those without N2 involvement. This approach yields excellent survival outcomes (>50% 5-year survival in patients with an R0 resection). Patients with N2 disease are less likely to benefit from surgery and can be managed with chemoradiotherapy followed by durvalumab. Patients presenting with metastatic disease can be treated with radiation therapy (with or without chemotherapy) for symptom palliation.

MANAGEMENT OF METASTATIC NSCLC

Approximately 40% of NSCLC patients present with advanced, stage IV disease at the time of diagnosis. In addition, a significant number of patients who first presented with early-stage NSCLC will eventually relapse with distant disease. Patients who have recurrent disease have a better prognosis than those presenting with metastatic disease at the time of diagnosis. Standard medical management, the judicious use of pain medications, and the appropriate use of radiotherapy and systemic therapy—which may consist of targeted therapy, immunotherapy, and/or traditional cytotoxic chemotherapy depending on the specific diagnosis as well as PD-L1 tumor proportion score (TPS) and molecular subtype—form the cornerstone of management. Systemic therapy palliates symptoms, improves quality of life, and improves survival in patients with metastatic NSCLC, particularly in patients with good performance status. Of note, the early application of palliative care in conjunction with chemotherapy in patients with advanced NSCLC is associated with both improved survival and quality of life.

Targeted Therapies for Select Molecular Cohorts of NSCLC For a cohort of NSCLC patients, the presence of an oncogenic driver mutation allows the use of oral therapies with significant antitumor activity and improved survival compared to cytotoxic chemotherapy. These driver mutations occur in genes encoding signaling proteins that, when aberrant, promote the uncontrolled growth and metastasis of tumor cells. Importantly, driver mutations can serve as Achilles' heels for tumors, if their gene products can be targeted therapeutically with small-molecule inhibitors. All patients with advanced NSCLC should undergo molecular testing with broad panel-based testing techniques such as next-generation sequencing (NGS) to look for oncogenic drivers. Mutations, fusions, and deletions have been reported in a number of genes including *EGFR*, *ALK*, *ROS1*, *BRAF*, *RET*, *MET*, *NTRK*, *KRAS*, *PIK3CA*, *NRAS*, *AKT1*, and *MEK1* (*MAP2K1*); however, not all are considered

actionable at this time. As our treatment armamentarium expands, knowledge of these mutations is critical for selection of appropriate therapy.

EGFR mutations have been detected in 10–15% of North American patients diagnosed with NSCLC. *EGFR* mutations are associated with younger age, light (<10 pack-year) and nonsmokers, and adenocarcinoma histology. Approximately 90% of these mutations are exon 19 deletions or exon 21 L858R point mutations within the EGFR TK domain, resulting in hyperactivation of both EGFR kinase activity and downstream signaling. Lung tumors that harbor activating mutations within the EGFR kinase domain display high sensitivity to small-molecule EGFR TKIs. Osimertinib, erlotinib, gefitinib, afatinib and dacomitinib are FDA-approved oral small-molecule TKIs that inhibit EGFR. Several large, international, phase 3 studies have demonstrated improved response rates and progression-free survival in patients with *EGFR* mutation-positive NSCLC treated with an EGFR TKI as compared with standard first-line chemotherapy regimens (Table 78-9). Osimertinib was shown in a randomized phase 3 trial to have superior progression-free and overall survival in patients with *EGFR*-mutant NSCLC compared to earlier-generation EGFR TKIs (erlotinib or gefitinib) and to chemotherapy.

Chromosomal rearrangements involving the anaplastic lymphoma kinase (*ALK*) gene on chromosome 2 have been found in 3–7% of patients with NSCLC. *ALK* rearrangements lead to hyperactivation of the ALK TK domain. Similar to EGFR, *ALK* rearrangements are typically (but not exclusively) associated with younger age, light (<10 pack-year) and nonsmokers, and adenocarcinoma histology. Crizotinib is a first-generation ALK TKI, whereas alectinib, brigatinib and ceritinib are second-generation ALK TKIs approved as first-line treatment options for patients with lung tumors harboring *ALK* rearrangements. Both alectinib and brigatinib have been found to have superior progression-free survival to crizotinib, whereas lorlatinib, a third-generation ALK TKI, is approved in patients who progress on a second-generation ALK TKI (Table 78-10). *ALK* testing may be performed via fluorescence

TABLE 78-9 Phase 3 Trials of EGFR TKIs in EGFR-Positive Non-Small-Cell Lung Cancer

TRIAL	THERAPY	NO. OF PATIENTS	ORR (%)	PFS (MONTHS)
IPASS	CbP	129	47	6.3
	Gefitinib	132	71	9.3
EURTAC	CG	87	15	5.2
	Erlotinib	86	58	9.7
OPTIMAL	CG	72	36	4.6
	Erlotinib	82	83	13.1
NEJOO2	CG	114	31	5.4
	Gefitinib	114	74	10.8
WJTOG3405	CD	89	31	6.3
	Gefitinib	88	62	9.2
LUX LUNG 3	CP	115	23	6.9
	Afatinib	230	56	11.1
LUX LUNG 6	CG	122	23	5.6
	Afatinib	242	67	11.0
LUX LUNG 7	Erlotinib	159	58	10.9
	Afatinib	160	73	11.0
ARCHER 1050	Gefitinib	225	72	9.2
	Dacomitinib	227	75	14.7
FLAURA	Erlotinib or Gefitinib	277	76	8.5
	Osimertinib	279	80	17.2

Abbreviations: CbP, carboplatin and paclitaxel; CD, cisplatin and docetaxel; CG, cisplatin and gemcitabine; CP, cisplatin and paclitaxel; ORR, overall response rate; PFS, progression-free survival.

TABLE 78-10 Results of Phase 3 Trials Comparing First-Line ALK Inhibitors in ALK-Positive NSCLC

TRIAL	THERAPY	NO. OF PATIENTS	ORR (%)	PFS (MONTHS)
Profile 1014	Crizotinib	172	74	10.9
	Platinum-chemotherapy	171	45	7.0
ALEX	Alectinib	152	82.9	25.7
	Crizotinib	151	75.5	10.4
J-ALEX	Alectinib	103	92	34.1
	Crizotinib	104	79	10.2
ALTA1L	Brigatinib	137	71	67% at 1 year
	Crizotinib	138	60	43% at 1 year

Abbreviations: NSCLC, non-small-cell lung cancer; ORR, overall response rate; PFS, progression-free survival.

in situ hybridization (FISH), immunohistochemistry (IHC), or NGS.

ROS1 fusions, detected by FISH or NGS, have been identified in 1% of patients with NSCLC, and similar to *EGFR* mutations and *ALK* fusions, *ROS1* rearrangements are typically associated with younger age and light or never smoking status. Crizotinib and lorlatinib, which inhibit both *ALK* and *ROS1* kinases, and entrectinib have been FDA approved for patients whose tumors harbor a *ROS1* fusion.

NTRK fusions occur in members of the *NTRK* gene family (*NTRK1*, *NTRK2*, *NTRK3*) and result in constitutive protein kinase activation. *NTRK* fusions are rare, occurring in <1% of patients with NSCLC. Similar to the above mutations, they more commonly occur in never smokers; however, patients with *NTRK* fusions are often older patient compared to those with *ROS1* and *ALK* alterations. Entrectinib and larotrectinib have demonstrated durable antitumor efficacy and are currently approved for *NTRK*-positive NSCLC.

MET exon 14 skipping mutations have also been identified in approximately 3–5% of patients with NSCLC. Unlike the above-mentioned mutations, *MET* exon 14 skipping mutations occur in both squamous and nonsquamous NSCLC patients and those with a history of smoking. Pharmacologic inhibition of the overactive *MET* pathway with capmatinib or tepotinib resulted response rates >70%, particularly in treatment-naïve NSCLC patients.

Oncogenic mutations in *BRAF* have been observed in ~2% of patients with NSCLC and, similar to *MET*, occur in both squamous and nonsquamous NSCLC and with an equal prevalence in patients

with a history of smoking. This mutation is typically most targetable when it occurs at the 600th amino acid valine (V600). Combined inhibition with a *BRAF* and MEK inhibitor, dabrafenib plus trametinib, is a first-line or later therapeutic option in patients with *BRAF* V600–mutant NSCLC and appears to be superior to *BRAF* or MEK inhibition alone.

RET alterations typically occur as chromosomal rearrangements resulting in constitutive TKI activation. *RET* rearrangements may be detected by either FISH or NGS in ~1% of NSCLC patients. Analogous to capmatinib, selpercatinib has demonstrated an excellent response rate; as many as 85% of treatment-naïve NSCLC patients with *RET* alterations responded. All National Comprehensive Cancer Network–supported targetable oncogenic driver mutations and potential therapeutic options are summarized in Fig. 78-7.

Mutations within the *KRAS* GTPase are found in 20% of lung adenocarcinomas. Agents targeting *KRAS* G12C are in development. Each of the other driver mutations occurs in <1–3% of lung adenocarcinomas. The great majority of the driver mutations are mutually exclusive. Most cancers have just one main driver. Defining mechanisms of acquired resistance to small-molecule inhibitors is a high research priority.

Immunotherapy Immune checkpoint inhibitors have significantly improved the quality of life and survival for a group of patients with locally advanced and metastatic NSCLC. These agents are used primarily in patients whose tumors do not express a targetable genetic lesion (Fig. 78-8). Immune checkpoint inhibitors work by blocking interactions between T cells and antigen presenting cells (APCs) or tumor cells that lead to T-cell inactivation. By inhibiting this interaction, the immune system is effectively upregulated and T cells become activated against tumor cells. Several randomized studies have demonstrated superior overall survival in patients treated with pembrolizumab or atezolizumab monotherapy or nivolumab plus ipilimumab combination immunotherapy compared to chemotherapy in patients with metastatic NSCLC with PD-L1 expression in ≥50% of tumor cells (Keynote 024, IMPOWER 110) and ≥1% of tumor cells (Keynote 042, CheckMate 227) (Table 78-11). The evidence supporting the use of single-agent immunotherapy in patients with tumor PD-L1 <50% remains unclear; current recommendations suggest the use of chemotherapy plus immunotherapy or immunotherapy combinations as the first-line treatment strategy in patients with metastatic NSCLC with tumor PD-L1 <50%. As discussed below, specific regimens vary by tumor histology (adenocarcinoma vs squamous cell carcinoma). Although PD-L1 has been identified as a biomarker that can predict response to immune checkpoint inhibitors, responses are observed in patients who do

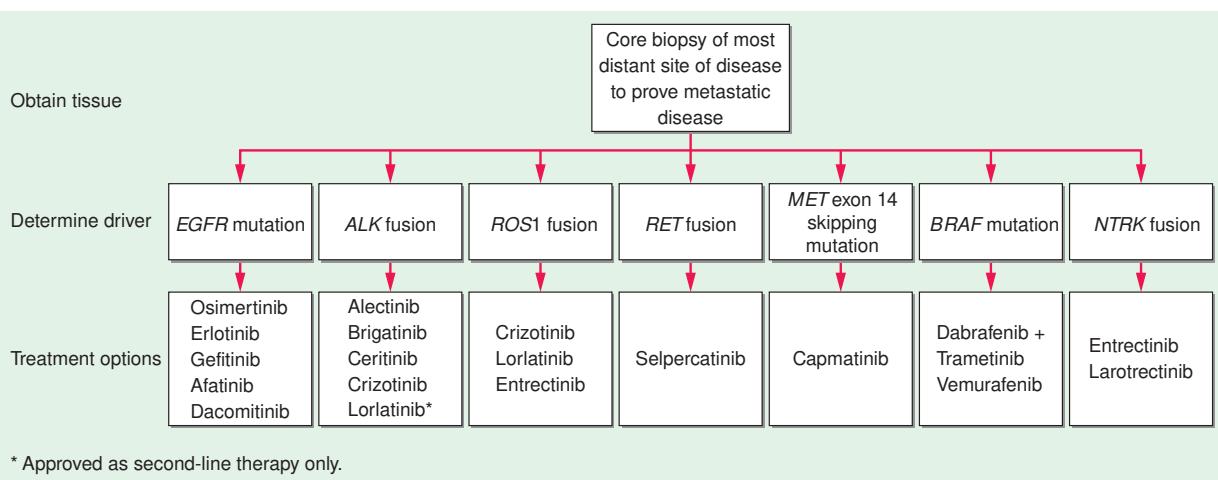


FIGURE 78-7 Approach to targeted therapy in non-small-cell lung cancer (NSCLC).

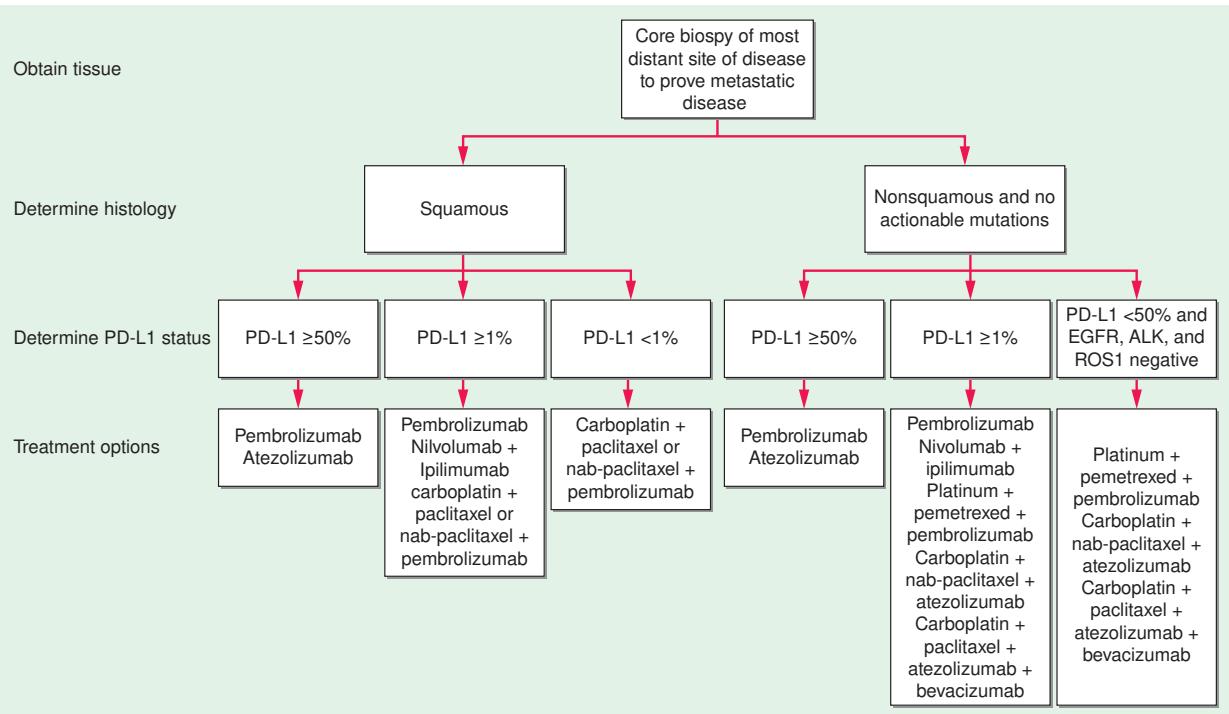


FIGURE 78-8 Approach to first-line therapy in a patient with stage IV, driver mutation-negative non-small-cell lung cancer (NSCLC).

not appear to express the biomarker, and not all PD-L1-positive patients respond to checkpoint inhibition. Importantly patients with driver mutations such as *EGFR* and *ALK* appear to derive greater benefit from targeted therapy than immunotherapy and should be treated with a TKI, even in the presence of high PD-L1 expression. Further evaluation of these agents in the neoadjuvant setting and combined with chemoradiotherapy is ongoing.

Cytotoxic Chemotherapy for Metastatic or Recurrent NSCLC
 Cytotoxic chemotherapy is typically used in combination with immunotherapy as the initial treatment in patients with metastatic or recurrent NSCLC only when there is no contraindication to immunotherapy. Selected chemotherapy agents perform quite differently in squamous carcinomas versus adenocarcinomas. Patients with nonsquamous NSCLC have an improved survival when

TABLE 78-11 Results of Phase 3 Trials Comparing First-Line Immunotherapy with or without Chemotherapy Versus Chemotherapy Alone in Patients with NSCLC

STUDY	THERAPY	NO. OF PATIENTS	OS (MONTHS)	PFS (MONTHS)
Keynote 024 PD-L1 ≥50%	Pembrolizumab	154	30.0	7.9
	Platinum-chemotherapy	151	14.2	3.5
Keynote 042 PD-L1 ≥1%	Pembrolizumab	637	16.7	5.4
	Platinum-chemotherapy	637	12.1	6.5
IMPOWER 110 PD-L1 ≥50% TC or ≥15% IC	Atezolizumab	286	20.2	8.1
	Platinum-chemotherapy	263	13.1	5.0
Keynote 189 Nonsquamous	Pembrolizumab + platinum-chemotherapy	410	NR	8.8
	Platinum-chemotherapy	206	11.3	4.9
Keynote 407 Squamous	Pembrolizumab + platinum-chemotherapy	278	15.9	6.4
	Platinum-chemotherapy	281	11.3	4.8
IMPOWER 150 Nonsquamous	Atezolizumab + platinum-chemotherapy	356	19.2	8.3
	Platinum-chemotherapy	336	14.7	6.8
IMPOWER 130 Nonsquamous	Atezolizumab + platinum-chemotherapy	483	18.6	7.0
	Platinum-chemotherapy	240	13.9	5.5
CheckMate 227	Nivolumab plus ipilimumab	583	17.1	5.1
	Platinum-chemotherapy	583	13.9	5.6
CheckMate-9LA	Nivolumab plus ipilimumab plus two cycles of platinum-chemotherapy	361	14.1	6.8
	Platinum-chemotherapy	358	10.7	5

Abbreviations: IC, immune cells; NR, not reported; OS, overall survival; PFS, progression-free survival; TC, tumor cells.

Note: Platinum-chemotherapy refers to first-line platinum doublet or triplet chemotherapy.

treated with cisplatin and pemetrexed compared to cisplatin and gemcitabine. By contrast, patients with squamous carcinoma have an improved survival when treated with cisplatin and gemcitabine. This survival difference is thought to be related to the differential expression between tumor types of thymidylate synthase (TS). Squamous cancers have a much higher expression of TS compared to adenocarcinomas, accounting for their lower responsiveness to pemetrexed. By contrast, the activity of gemcitabine is not impacted by the levels of TS.

Second-Line Therapy and Beyond Second-line therapy for advanced NSCLC relies on docetaxel; it improves survival compared to supportive care alone. Ramucirumab is a recombinant human IgG1 monoclonal antibody that targets VEGFR-2 and blocks the interaction of VEGF ligands and VEGFR-2. A phase 3 trial demonstrated a significant improvement in progression-free survival and overall survival when ramucirumab was combined with docetaxel as second-line therapy in patients who had progressed on platinum-based chemotherapy. Contrary to bevacizumab, ramucirumab was safe in patients with both squamous and nonsquamous NSCLC and is approved regardless of histology.

Supportive Care No discussion of the treatment strategies for patients with advanced lung cancer would be complete without a mention of supportive care. Coincident with advances in chemotherapy and targeted therapy was a pivotal study that demonstrated that the early integration of palliative care with standard treatment strategies improves both quality of life and overall survival for patients with stage IV NSCLC (Chaps. 12 and 69). Aggressive pain and symptom control are important components of optimal treatment of these patients.

TREATMENT

Small-Cell Lung Cancer

The overall treatment approach to patients with SCLC is shown in Fig. 78-9.

SURGERY FOR LIMITED-DISEASE SMALL-CELL LUNG CANCER

SCLC is a highly aggressive disease characterized by its rapid doubling time, high growth fraction, early development of disseminated disease, and dramatic response to first-line chemotherapy and radiation. In general, surgical resection is *not* routinely recommended for patients because even patients with LD-SCLC still have occult micrometastases. However, the American College of Chest Physicians Evidence-Based Clinical Practice Guidelines recommend surgical resection over nonsurgical treatment in SCLC patients with clinical stage I disease after a thorough evaluation for distant metastases and invasive mediastinal stage evaluation (grade 2C). After resection, these patients should receive platinum-based adjuvant chemotherapy (grade 1C). If the histologic diagnosis of SCLC is made in patients on review of a resected surgical specimen, such patients should receive standard SCLC chemotherapy as well.

CHEMOTHERAPY

In patients with limited-stage SCLC, concurrent chemoradiotherapy with cisplatin-etoposide for four cycles has remained standard of care for over 4 decades. Two randomized phase 3 trials have demonstrated that chemotherapy with either cisplatin or carboplatin plus either etoposide and a PD-L1 inhibitor, atezolizumab (IMPOWER 133) or durvalumab (CASPIAN), provides superior progression-free and overall survival compared to chemotherapy alone, making combination therapy the preferred choice in appropriate patients. Despite response rates to first-line therapy as high as 80%, the median survival ranges from 12 to 20 months for patients with LD and approximately 12 months for patients with ED. Regardless of disease extent, the majority of patients relapse and develop chemotherapy-resistant disease. The prognosis is especially poor for patients who relapse within the first 3 months of therapy; these patients are said to have *chemotherapy-resistant disease*. Patients are said to have *sensitive disease* if they relapse >3 months after their initial therapy and are thought to have a somewhat better overall survival. These patients also are thought to have the greatest potential benefit from second-line chemotherapy. Topotecan and

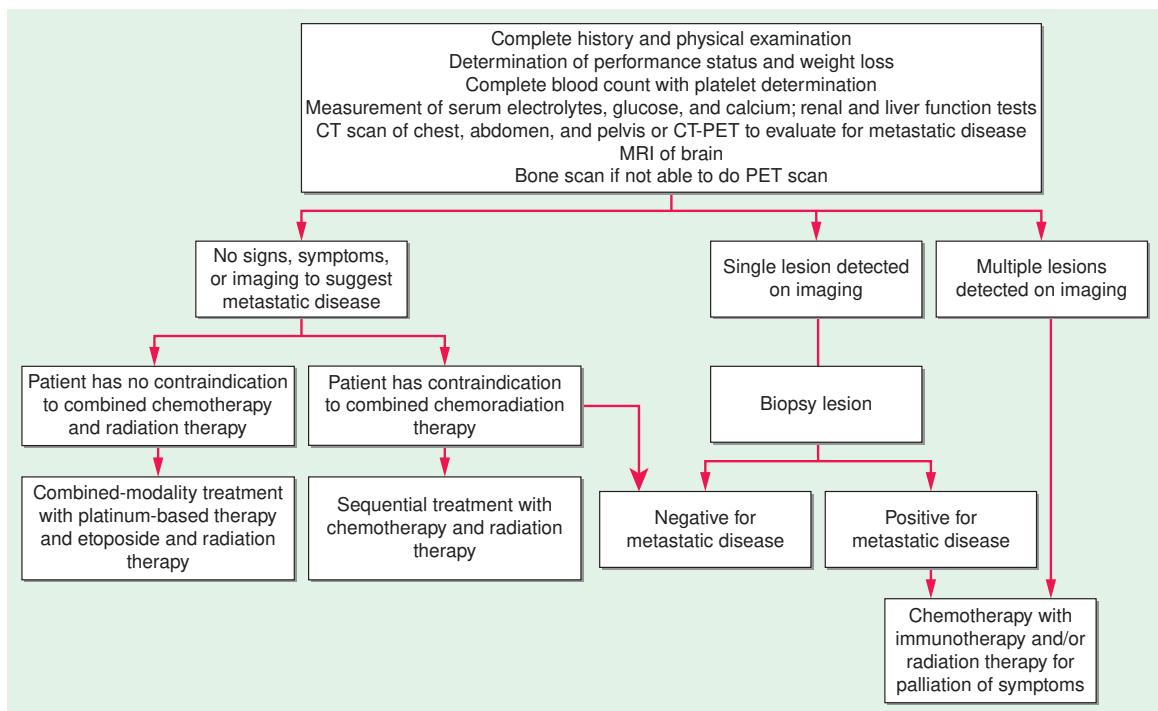


FIGURE 78-9 Algorithm for management of small-cell lung cancer. MRI, magnetic resonance imaging; PET, positron emission tomography.

Lurbinectedin are FDA-approved agents for second-line therapy in patients with SCLC. Topotecan has only modest activity and can be given either intravenously or orally; it appears to have more efficacy in patients with chemotherapy-sensitive disease. Lurbinectedin has a 35% response rate and progression-free survival of 3.5 months, with a greater benefit in patients with chemotherapy-sensitive disease. Other agents with similar low levels of activity in the second-line setting include irinotecan, paclitaxel, docetaxel, vinorelbine, oral etoposide, and gemcitabine.

THORACIC RADIATION THERAPY

Thoracic radiation therapy (TRT) is a standard component of induction therapy for patients with good performance status and limited-stage SCLC. Meta-analyses indicate that chemotherapy combined with chest irradiation improves 3-year survival by 5% as compared with chemotherapy alone. The 5-year survival rate, however, remains disappointingly low at 10–15%. Most commonly, TRT is combined with cisplatin and etoposide chemotherapy due to a superior toxicity profile as compared to anthracycline-containing chemotherapy regimens. As observed in locally advanced NSCLC, *concurrent* chemoradiotherapy is more effective than *sequential* chemoradiation but is associated with significantly more esophagitis and hematologic toxicity. Ideally TRT should be administered with the first two cycles of chemotherapy because later application appears slightly less effective. If for reasons of fitness or availability, this regimen cannot be offered, TRT should follow induction chemotherapy. With respect to fractionation of TRT, twice-daily 1.5-Gy fractionated radiation therapy has been shown to improve survival in LD-SCLC patients but is associated with higher rates of grade 3 esophagitis and pulmonary toxicity. Although it is feasible to deliver once-daily radiation therapy doses up to 70 Gy concurrently with cisplatin-based chemotherapy, there are no data to support equivalency of this approach compared with the 45-Gy twice-daily radiotherapy dose. Therefore, the current standard regimen of a 45-Gy dose administered in 1.5-Gy fractions twice daily for 30 days is being compared with higher-dose regimens in two phase 3 trials, one in the United States and one in Europe. Patients should be carefully selected for concurrent chemoradiation therapy based on good performance status and adequate pulmonary reserve. The role of radiotherapy in ED-SCLC is largely restricted to palliation of tumor-related symptoms such as bone pain and bronchial obstruction.

PROPHYLACTIC CRANIAL IRRADIATION

Prophylactic cranial irradiation (PCI) should be considered in all patients either with LD-SCLC or who have responded well to initial therapy; its role in patients with ED-SCLC is more controversial. A meta-analysis including seven trials and 987 patients with LD-SCLC who had achieved a complete remission after upfront chemotherapy yielded a 5.4% improvement in overall survival for patients treated with PCI. In patients with ED-SCLC who have responded to first-line chemotherapy and had no CNS disease, patients randomized to observation had a higher incidence of brain metastases; however, use of PCI did not improve overall survival. Long-term toxicities, including deficits in cognition, have been reported after PCI but are difficult to sort out from the effects of chemotherapy or normal aging.

THYMIC TUMORS

Thymic tumors are rare malignancies accounting for 0.5–1.5% of all malignancies in the United States with a higher incidence among Asian populations. They are particularly rare among children and young adults with incidence peaking in the fifth decade of life. There is no difference between sexes, and no clear risk factors have been identified.

CLINICAL MANIFESTATIONS

The majority of thymic tumors occur in the anterior mediastinum. Approximately 40% of patients with mediastinal masses will be

asymptomatic with an incidental finding on chest imaging. In patients presenting with an anterior mediastinal mass, if appropriate, serum β -human chorionic gonadotropin (HCG) and α fetoprotein (AFP) should be sent to rule out a germ cell tumor. A patient with a sign or symptom of thymoma or thymic carcinoma may present with chest pain, dyspnea, cough, or superior vena cava syndrome secondary to effects on adjacent organs or a paraneoplastic syndrome, most commonly myasthenia gravis, pure red cell aplasia, or hypogammaglobulinemia. More rare paraneoplastic syndromes include limbic encephalitis, aplastic anemia, hemolytic anemia, and autoimmune disease such as Sjögren's syndrome, polymyositis, rheumatoid arthritis, and ulcerative colitis, among others.

STAGING

Given the rarity of the tumor, patients with suspected thymoma should be evaluated by a multidisciplinary team including a surgeon, medical and radiation oncologist, and pathologist with experience in treating the disease. A CT scan of the chest with contrast is recommended to determine if the mass is resectable based on relationship to surrounding structures. An MRI with contrast may be performed if clinically indicated. A PET scan may be useful in the evaluation of a patient with thymic tumors, although it may be less useful in the staging of thymoma compared to thymic carcinoma. A core needle biopsy is considered standard of care for obtaining a histologic diagnosis of an anterior mediastinal tumor. This may be obtained via CT or ultrasound imaging. However, in some circumstances, a mediastinoscopy or open biopsy may be required.

Thymomas are commonly staged using the Masaoka system or the World Health Organization (WHO) staging system, as described in Table 78-12. WHO types A, AB, and B1 tend to be more well-differentiated, types B2 and B3 are moderately differentiated, and type C is poorly differentiated.

TREATMENT

Surgical resection is the mainstay of treatment for patients with Masaoka type I and II thymic tumors. In patients with type III and IV who have potentially resectable thymic tumors, neoadjuvant chemotherapy may be given to decrease the tumor size and allow for a resection with negative margins. Surgery remains controversial and provides a limited role in the treatment of stage III and IV disease. No

TABLE 78-12 Staging Thymic Tumors

MASAOKA STAGE	DEFINITION
I	Grossly and microscopically encapsulated
IIA	Microscopic transcapsular invasion
IIB	Macroscopic invasion into surrounding tissue excluding pericardium, lung, and great vessels
III	Macroscopic invasion into neighboring organs of the lower neck or upper chest
IVA	Pleural or pericardial dissemination
IVB	Hematogenous or lymphatic dissemination to distal organs

WHO	
A	Tumor with few lymphocytes
AB	Tumor with features of type A and foci rich in lymphocytes
B1	Tumor with features of normal epithelial cells with vesicular nuclei and distinct nucleoli and an abundant population of lymphocytes. Also known as cortical thymoma, lymphocyte-rich thymoma
B2	Thymoma with no or mild atypia with round or polygonal-shaped cells with small component of lymphocytes
B3	Well-differentiated thymic carcinoma with mild atypia
C	Thymic carcinoma with high atypia

additional therapy may be required in patients with type I who have a resection with negative margins. Postoperative radiation therapy may be recommended based on extracapsular extension and the presence of positive margins in patients with type II or III thymic tumors or histologic evaluation WHO B3 and C. Radiation therapy may be beneficial in patients with locally advanced disease (type III or IV) or in patients with symptoms secondary to compression of surrounding structures. Chemotherapy with cisplatin, doxorubicin, and cyclophosphamide (CAP) remains the mainstay of therapy in the neoadjuvant and adjuvant setting as well as first-line therapy in patients with metastatic thymoma, whereas carboplatin and paclitaxel are often employed in patients with thymic carcinoma. Limited additional agents are recommended based on small phase 2 trials as second-line therapy and beyond.

COVID 19 AND LUNG CANCER

COVID-19, a respiratory tract infection caused by SARS-CoV-2, emerged in Wuhan, China, in late 2019. The rapid global spread led the WHO to declare a pandemic in early March 2020. Large retrospective data sets have shown that cancer patients, and particularly patients with lung cancer, are at increased risk of morbidity and mortality from COVID-19. The dilemma of distinguishing COVID-19 symptoms from lung cancer and radiographic diagnosis of pneumonia or pneumonitis from radiation therapy or immunotherapy versus COVID-19 pneumonia has presented a particular challenge to health care providers. Mortality as high as 35% has been reported for patients with lung cancer infected with SARS-CoV-2. Older patients (≥ 65 years old), patients with a worse performance status (Eastern Cooperative Oncology Group performance status ≥ 1), patients on glucocorticoids (≥ 10 mg prednisone equivalent) and anticoagulation, and patients on chemotherapy within 3 months of diagnosis appear to be particularly at risk for mortality if infected. The long-term impact on lung cancer mortality due to delays in screening, diagnosis, and treatment are likely to be felt for years to come.

SUMMARY

The management of SCLC and NSCLC has undergone major change in the past decade, resulting in a reduction in lung cancer mortality. For patients with early-stage disease, advances in radiotherapy and surgical procedures as well as new systemic therapies have greatly improved prognosis in all diseases. For patients with advanced lung cancer, major progress in understanding tumor genetics and tumor immunology has led to the development of rational targets and specific inhibitors, which have documented efficacy in specific subsets of NSCLC. Furthermore, increased understanding of how to activate the immune system to drive antitumor immunity has proven to be a successful therapeutic strategy for a subset of patients with advanced lung cancer. However, only a small subset of patients responds to immune checkpoint inhibitors, and the majority of patients treated with targeted therapies or chemotherapy eventually develop resistance, which provides strong motivation for further research and enrollment of patients onto clinical trials in this rapidly evolving area.

A

David Johnson and Christine Lovly contributed to this chapter in the prior edition, and material from that chapter has been retained here.

FURTHER READING

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79

Breast Cancer

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INTRODUCTION AND BACKGROUND

CONCEPTUAL AND BIOLOGICAL ISSUES OF BREAST CANCER

Breast cancer is a malignant proliferation of epithelial cells lining the ducts or lobules of the breast. In the year 2020, approximately a quarter million cases of invasive and 61,000 cases of *in situ* breast cancer were diagnosed in the United States, with nearly 41,000 deaths. Epithelial malignancies of the breast are the most common cause of cancer in women (excluding skin cancer), accounting for about one-third of all cancer in women. As a result of earlier detection and improved treatments, the mortality rate from breast cancer decreased by more than one-third over the past three decades in high- and middle-income countries. This chapter does not consider rare malignancies presenting in the breast, such as sarcomas and lymphomas, but focuses on the epithelial cancers.

Breast cancer has served as a paradigm for several oncologic principles related to solid tumors. It spans a spectrum of conditions for which different clinical considerations must be made, including risk assessment, prevention, screening, evaluation of breast abnormalities, local and adjuvant systemic treatments, metastatic therapies, and survivorship issues (Fig. 79-1).

The unique biology of breast cancer has rendered it amenable to a variety of therapeutic “targeted” strategies based on the appreciation of differences in subtypes that reflect the need for differences in assessment and therapy. These subtypes include expression of the estrogen receptor (ER) and the human epidermal growth factor receptor type 2 (HER2), as well as germline or somatic mutations in inherited tumor suppressor genes, such as *BRCA1* and *BRCA2*. Identifiable somatic mutations in genes that appear to drive the cancer, including mammalian target of rapamycin (*mTOR*), cyclin-dependent kinase 4 and 6 (*CDK4/6*), and S-phosphatidylinositol-4,5-bisphosphate 3-kinase

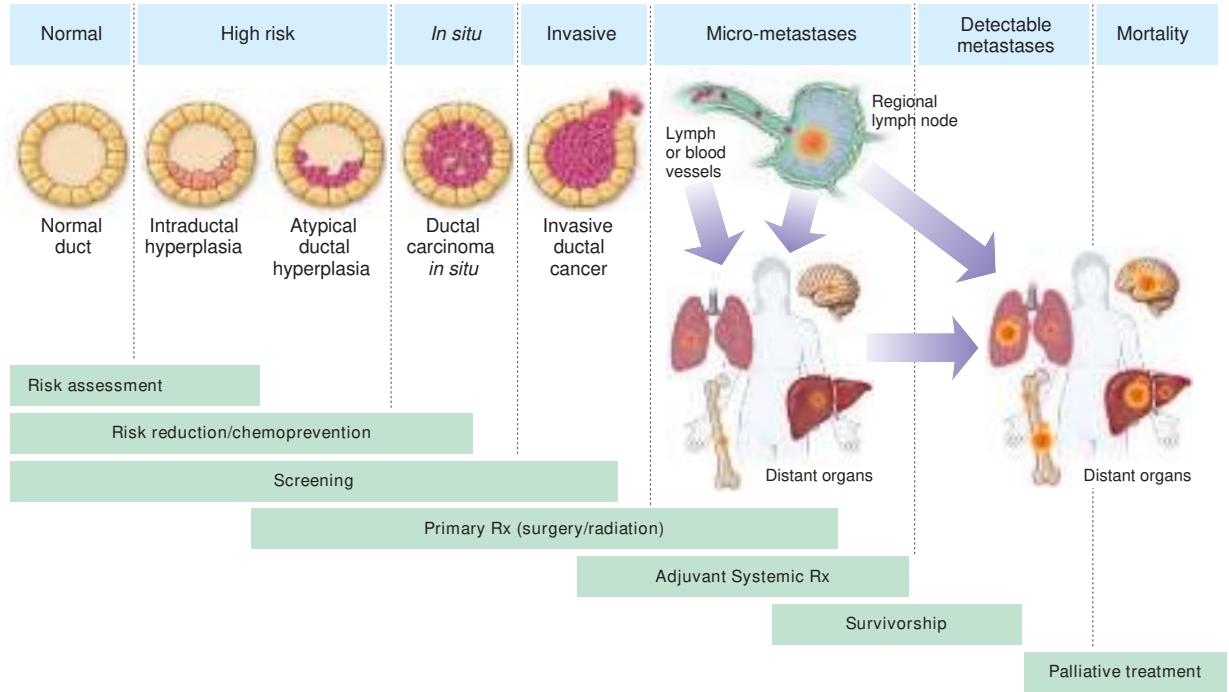


FIGURE 79-1 Breast cancer continuum conceptual model. Most breast cancers begin in epithelial cells within the lobules or ducts. They proceed through a continuum of atypia and hyperplasia to *in situ* malignancy to invasion into surrounding normal tissues followed by intravasation into lymph and blood channels to local lymph nodes and distant organs, culminating in distant metastases. This is a conceptual model. Not all metastatic breast cancers have progressed through these stages, and many lesions do not progress to the next.

catalytic subunit alpha (*PIK3CA*) make it susceptible to specific therapeutic interventions directed against each of these targets (Table 79-1). Furthermore, immune checkpoint inhibition has been applied to specific types of breast cancers.

EPIDEMIOLOGY AND RISK FACTORS

■ CLINICAL, HORMONAL, AND OTHER NONGENETIC RISK FACTORS

Seventy-five percent of all breast cancers occur in women aged >50 years, but breast cancer is not uncommon in women in their forties and can occur in women in their thirties and even twenties, and very rarely in adolescence.

Breast cancer is principally a sex hormone-dependent disease through increased activity of the ER and its ligands, estradiol and estrone (Fig. 79-1, Table 79-1). Indeed, the female-to-male ratio is ~150:1. Relative exposure to both endogenous and exogenous estrogens increases risk of breast cancer. Risk of developing breast cancer is higher in women with early menarche (<12 years) and late first full-term pregnancy (>35 years), and it is increased by exogenous hormone replacement therapy. Women without functioning ovaries, who experience an early menopause, or who never receive combination estrogen/progesterone replacement therapy are much less likely to develop breast cancer than those who have a normal menstrual history. Also, duration of maternal nursing correlates with substantial risk reduction independent of either parity or age at first full-term pregnancy.

Menstrual and reproductive history accounts for 70–80% of the variation in breast cancer frequency in different countries, providing insight into hormonal carcinogenesis. A woman living to age 80 years in North America has a one in nine chance of developing invasive breast cancer. Women who live in agrarian societies, especially in Asia, have traditionally had only 1/5th to 1/10th the risk of breast cancer of

women in North America or Western Europe. However, Asian women who immigrate to North America or European countries during preadolescence or in adolescence have the same risk as women born in these countries. Further, with shifts from agrarian to industrialized economic systems, the incidence of breast cancer has risen dramatically in Asia, approaching that observed in Western nations.

Exogenous use of female hormones also plays a role in breast cancer incidence. The elevated risk related to oral contraceptives is quite modest if present at all. Regardless, this risk is more than balanced by avoidance of an undesired pregnancy and a substantial protective effect against ovarian epithelial and endometrial cancers.

Hormone replacement therapy (HRT) with conjugated equine estrogens plus progestins increases the risk of breast cancer; 6–7 years of HRT nearly doubles the risk of breast cancer and also increases the incidence of adverse cardiovascular events. However, it decreases the risk of bone fractures and colorectal cancer. On balance, more negative than positive events are associated with HRT. Administration of conjugated estrogens is usually combined with companion progesterone to abrogate the increased risk of endometrial cancer with estrogen alone. However, single-agent estrogen replacement therapy in women who have had hysterectomies produces no significant increase in breast cancer incidence and, if anything, reduces the risk. Thus, there are serious concerns about long-term HRT, especially in combination with progestins, in terms of cardiovascular disease and breast cancer. No comparable safety data are available for other less potent forms of estrogen replacement, such as bioequivalent estrogen found in soy, and they should not be routinely used as substitutes. Epidemiologic studies demonstrate a rapid decrease in elevated breast cancer incidence coincident with discontinuation of HRT.

HRT in women previously diagnosed with breast cancer, especially of the subtype that expresses ERs, counteracts much of the effectiveness of antineoplastic endocrine therapies and is contraindicated. Although

TABLE 79-1 Breast Cancer Molecular Features and Associated Targeted Therapies

MOLECULE	GENE THAT ENCODES MOLECULE	ABNORMALITY	CLASS OF TARGETED THERAPIES	SPECIFIC THERAPIES
Estrogen receptor (ER)	<i>ESR1</i>	Overexpression of cellular protein	Endocrine therapies	
			Estrogen ablation (surgical, chemical)	<i>Premenopausal</i> Oophorectomy Luteinizing hormone releasing hormone (LHRH) agonists (goserelin, leuprorelin) or antagonist (triptorelin) <i>Postmenopausal</i> Aromatase inhibitors (AIs) (anastrozole, letrozole, exemestane)
			ER antagonists	Selective estrogen receptor modulators (SERMs) (tamoxifen, toremifene, raloxifene) Selective estrogen receptor downregulators (SERDs) (fulvestrant)
Human epidermal growth factor receptor type 2 (HER2)	<i>c-neu/erbB2</i>	Overexpression of cell surface protein	Antibodies against HER2	Trastuzumab, pertuzumab, margetuximab
			Antibody-drug conjugates against HER2	Ado-trastuzumab emtansine, fam-trastuzumab deruxtecan-nxki
			Tyrosine kinase inhibitors	Lapatinib, neratinib, tucatinib
		Mutations	Tyrosine kinase inhibitors	Neratinib (<i>indication not FDA approved</i>)
Mammalian target of rapamycin (mTOR)	<i>MTOR</i>	Loss of protein suppressor of mTOR pathway, phosphatase and tensin homolog (<i>PTEN</i>)	Tyrosine kinase inhibitor	Everolimus
Cyclin-dependent kinase 4 and 6 (CDK4/6)	<i>CDK4, CDK6</i>	Loss of the protein suppressor of CDK4/6 pathway, retinoblastoma (<i>RB1</i>)	Inhibition of CDK4/6 enzyme activity	Palbociclib, ribociclib, abemaciclib
Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)	<i>PIK3CA</i>	Mutations	Enzyme inhibition of mutated/activated PIK3CA protein	Alpelisib
BRCA1/2	<i>BRCA1, BRCA2</i>	Loss of tumor suppressor activity of BRCA1/2	Inhibition of poly (ADP-ribose) polymerase (PARP) activity and synthetic lethality	PARP inhibitors (olaparib, talazoparib)
TROP-2	<i>TACSTD2</i>	Over expression of TROP-2 cell surface protein	Antibody-drug conjugate against TROP-2	Sacituzumab govitecan
Immune checkpoints	NA	Programmed death-ligand 1 (PD-L1) suppression of immune effector cells	Inhibition of PD-L1/PD-1 suppression of immune effector cells	Atezolizumab

Abbreviations: FDA, U.S. Food and Drug Administration; NA, not applicable.

intravaginal estrogen therapy has been used for atrophic vaginitis associated with antiestrogenic endocrine therapies, it does result in some absorption and systemic estrogenic effects and should generally be avoided.

In addition to sex, age, and hormonal exposure, other risk features for breast cancer have been identified, but none with the kind of relative, attributable, or absolute risks associated with these three factors. Various differences in diets (including Asian agrarian vs modern economic) have been implicated as risk factors, although the role of diet in breast cancer etiology is controversial. Associative links exist between breast cancer risk and total caloric and fat intake, or even specific types of caloric intake, but the exact role of fat in the diet is unproven and may actually intersect with menstrual history and estrogenic exposure.

Central obesity, metabolic syndrome, and type 2 diabetes mellitus are all risk factors for occurrence and recurrence of breast cancer. Moderate alcohol intake also increases the risk by an unknown mechanism. Folic acid supplementation appears to modify risk in women who use alcohol but is not additionally protective in abstainers. Recommendations favoring abstinence from alcohol must be weighed against other social pressures and the possible cardioprotective effect of moderate alcohol intake. Depression is also associated with both occurrence and recurrence of breast cancer.

Certain benign breast pathologic findings, such as atypical hyperplasia and radial scars, are associated with higher risk of subsequent breast cancers. Prior radiation is a risk factor, but principally when delivered in adolescence or early child-bearing ages. Women who have been exposed before age 30 years to radiation in the form of multiple fluoroscopies (200–300 cGy) or treatment for Hodgkin's disease (>3600 cGy) have a substantial increase in risk of breast cancer, whereas radiation exposure after age 30 years appears to have a minimal carcinogenic effect on the breast. Radioactive iodine therapy for thyroid disease is not associated with increased risk of breast cancer, whereas mediastinal radiation in younger women for lymphoma is a powerful risk factor within the radiated field.

■ INHERITED GERMLINE SUSCEPTIBILITY FACTORS

 Family history has long been recognized as a risk factor for breast cancer. A woman with a first-degree history (mother or sister) of breast cancer has an increased relative risk of approximately 30–50% (or one-third to one-half higher) over a woman with no family history. However, family history only accounts for 10–15% of all breast cancers. Most women who develop breast cancer do not have a strong family history. For women without a identifiable inherited genetic

abnormality, it is not clear whether the increased risk associated with family history is due to environmental causes or as yet unidentified genetic abnormalities.

The genetics of breast cancer require an understanding of the distinction between inherited, germline genetic differences among individuals and acquired, somatic genetic changes within cancers. The former are often called mutations but are more properly termed *single nucleotide polymorphisms* (SNPs). Some SNPs are synonymous, meaning they do not change the encoded amino acid in the affected protein product, and therefore are of no clinical significance. Some SNPs are nonsynonymous but may lead to a substituted amino acid that does not change the function of the protein, and they are likewise clinically insignificant. However, if an SNP leads to an amino acid substitution that alters the protein function or results in complete cessation of transcription or translation (a “stop” codon), it is considered deleterious and leads to higher susceptibility to developing cancer. In some cases, the significance of the SNP is unknown, and these are designated *variants of undetermined significance* (VUS).

The genes of interest serve, in the normal cell, to suppress expansion of a potentially malignant clone, either by repairing downstream randomly occurring somatic genetic abnormalities or, if not possible, by inducing programmed cell death, or apoptosis. Somatic genetic changes that are not inherited, including mutations, amplifications, insertions, deletions, translocations, and others, are responsible for the malignant behavior of a cancer, including unrestrained proliferation, as well as extravasation from one site and migration and establishment of metastases into another. As discussed below, some germline and somatic mutations can be exploited therapeutically (Table 79-1).

For most women, the increased risk associated with a family member who has had breast cancer appears to be related to both a weak, and probably multigene, germline susceptibility and similar exposure to environmental/lifestyle risk factors. Only approximately 10% of human breast cancers can be linked directly to a single inherited germline SNP. However, when one is present, the relative and absolute risks for that individual developing breast, and other, cancers in her lifetime are extraordinary.

The *BRCA1* and *BRCA2* genes, located on chromosomal loci 17q21 and 13q12, respectively, are the best characterized breast cancer susceptibility genes and have the greatest clinical importance in assessing genetic risk for breast cancer. Women who inherit mutated alleles of these genes from either parent have at least a 60–80% lifetime chance of developing breast cancer and about a 33% chance of developing ovarian cancer. The cancers that arise within a *BRCA1*-mutated patient are almost exclusively negative for ER, progesterone receptor (PgR), and HER2 (so-called “triple-negative” breast cancers). Men who carry a mutant allele of the gene have an increased incidence of breast and also prostate cancers, although the absolute risk of breast cancer in men with *BRCA2* germline SNPs is far lower than that for women who harbor them.

Overall, <1% of the general population and <5% of all patients with breast cancer harbor deleterious SNPs in *BRCA1* or *BRCA2*. Certain subgroups of women are more likely to have *BRCA1/2* mutations. The incidence is approximately 2% in women of Ashkenazi, Eastern European descent. Approximately 20% of women with triple-negative breast cancers will be positive for deleterious germline *BRCA1* SNPs, and genetic testing is warranted in most patients with triple-negative breast cancer even without a family history.

In contrast to those that arise in *BRCA1* carriers, cancers that arise in *BRCA2* contexts are more likely to be ER positive. The incidence of *BRCA2* mutations is much higher than *BRCA1* in men who develop breast cancer. However, most male breast cancer cases do not occur in *BRCA1/2*-mutated men, and the risk of breast cancer in men who do carry the *BRCA2* mutation is much lower than that in women with this genetic abnormality. Many other inherited germline mutations in known or putative tumor-suppressor genes, such as *p53* (which also accounts in part for the Li-Fraumeni familial cancer syndrome), *PTEN* (which accounts for Cowden’s syndrome), and *PALB1*, have now been identified as important tumor-suppressor genes with clinical implications.

Inherited germline mutations are readily detected in blood tests of normal circulating leukocytes using so-called “panel” assays, which at present provide results from 30–45 different germline genes. However, because the rate of deleterious germline SNPs in these genes in the general population is quite low (well below 1%), germline panel genetic testing of the entire population of women is not recommended. Further, results are confounded by the presence of VUS in known tumor-suppressor genes, such as *BRCA1* and *BRCA2*, or deleterious variants or VUS in genes that are putative, but not proven, tumor-suppressor genes. Such results lead to confusion, anxiety, and inappropriate preventive strategies, such as prophylactic surgery, in individuals who may not actually be at higher risk.

Consensus guidelines on who should be tested include any woman with a family member who has been tested and found to harbor a deleterious SNP in a germline tumor-suppressor gene. Testing is indicated for any breast cancer patient with a triple-negative breast cancer, who is <40 years old, who has synchronous or metachronous contralateral breast cancers, who has a personal history of ovarian cancer, or who has a first-degree relative (mother, father, or sister) with breast or ovarian cancer. All males with breast cancer should be tested. Some guidelines suggest testing any breast cancer patient of Ashkenazi descent. Patients with these mutations should be counseled appropriately.

Some experts have recommended testing any patient diagnosed with breast cancer, both for genetic counseling but also because of the advent of effective therapies directed toward cancers that have deleterious *BRCA1/2* mutations (Table 79-1), although this strategy remains controversial. Regardless, any patient who is found to have inherited germline deleterious SNPs should receive formal genetic testing and counseling about special screening and prevention measures they might take.

PREVENTION OF BREAST CANCER

One major reason to determine risk would be to efficiently apply prevention and/or screening strategies, if either has been shown to be effective for the disease of interest. At present, although diet and exercise are certainly recommended approaches to healthy living, none has been proven to specifically decrease a woman’s risk of breast cancer. Avoidance of combined estrogen/progestin HRT reduces the associated increased risk of breast cancer to that of an average woman not using HRT.

Prophylactic removal of the breasts is an effective, albeit drastic, preventive strategy. Bilateral prophylactic mastectomies reduce the risk of breast cancer incidence and mortality by >95%. Because breasts are not encapsulated organs, some normal breast tissue is always left behind, and therefore, women who elect to have prophylactic mastectomies should be counseled that they still have some risk of developing a new breast cancer. Prophylactic mastectomy is most often chosen by women with germline genetic risk, in whom there is evidence of mortality reduction. For women with average or only mildly elevated risks, such as diagnosis of a unilateral breast cancer, survival is not increased by prophylactic mastectomy, and, because of its obvious adverse effect on sexuality, cosmesis, and breast-feeding, this approach is not considered appropriate.

So-called “chemoprevention” to lower breast cancer risk can be achieved with therapies directed toward the ER/estrogen signaling pathway (Table 79-1). These include the selective estrogen receptor modulators (SERMs) as well as aromatase inhibition. The latter should only be applied in postmenopausal women, since aromatase inhibition can result in a paradoxical increase in circulating estrogen levels in women with functioning ovaries. Chemoprevention with SERMs or aromatase inhibition lowers risk of ER-positive breast cancer by approximately one-half, although it has no effect on the more lethal ER-negative breast cancers. Of interest, prophylactic bilateral oophorectomy and salpingo-oophorectomy, which are often performed in women with high genetic risk (such as those with inherited *BRCA1/2* deleterious SNPs), also reduce breast cancer in addition to ovarian cancer risk.

SCREENING FOR BREAST CANCER FIG. 79 2

Screening mammography results in earlier diagnosis and subsequent local and systemic therapy. Overviews of nine prospective randomized clinical trials demonstrate that screening mammography reduces breast cancer mortality by one-fifth to one-quarter in women aged ≥ 50 years. The relative reduction in breast cancer mortality for women between ages 40 and 50 years is similar, although the absolute numbers of women who benefit in this age group is smaller since the incidence of breast cancer is much lower in younger women. In addition to reducing breast cancer mortality, screening mammography and early detection are more likely to identify tumors at a stage more appropriate for conservative local therapy. Better technology, including digitized mammography, tomosynthesis, routine use of magnified views, and greater skill in interpretation, have all improved the accuracy of mammography. Magnetic resonance spectroscopy has higher sensitivity but lower specificity than mammography. Since none of these newer technologies has been shown to be superior to mammography in terms of mortality reduction, screening of women with standard risk by any technique other than mammography is not recommended.

The issue of screening breast imaging of any sort has been controversial. Although the prospective randomized clinical trials demonstrate a late reduction in breast cancer mortality, they do not demonstrate

improvement in overall survival. Further, many authors have raised concern about diagnosis of cancers that may be biologically insignificant, raising the specter of overdiagnosis and overtreatment. Moreover, the substantial advances in both local and systemic therapies for breast cancer may have reduced the benefit of earlier diagnosis provided by screening. In contrast, in countries that have adopted widespread screening programs, nonrandomized, epidemiologic studies have demonstrated even greater magnitude reductions in breast cancer mortality than those seen in the randomized trials. Taken together, these data have led most guideline bodies to recommend annual screening for women aged 50–70 years. Many have also recommended screening for women in the 40- to 50-year-old range. For older women, caregiver and patient judgment should be used, taking into account comorbidities.

Magnetic resonance imaging (MRI) is recommended for women with particularly dense breasts, women whose first cancer was not detected by mammography, women with an axillary breast cancer presentation but no definable breast mass on physical exam or mammography, and those with high genetic risk, such as *BRCA1* or *BRCA2* carriers or those with Li-Fraumeni, Cowden's, or Bannayan-Riley-Ruvalcaba syndromes. MRI might also be considered for women with a history of radiation therapy to the chest between ages 10 and 30 years. In these women, the positive predictive value of MRI is higher because

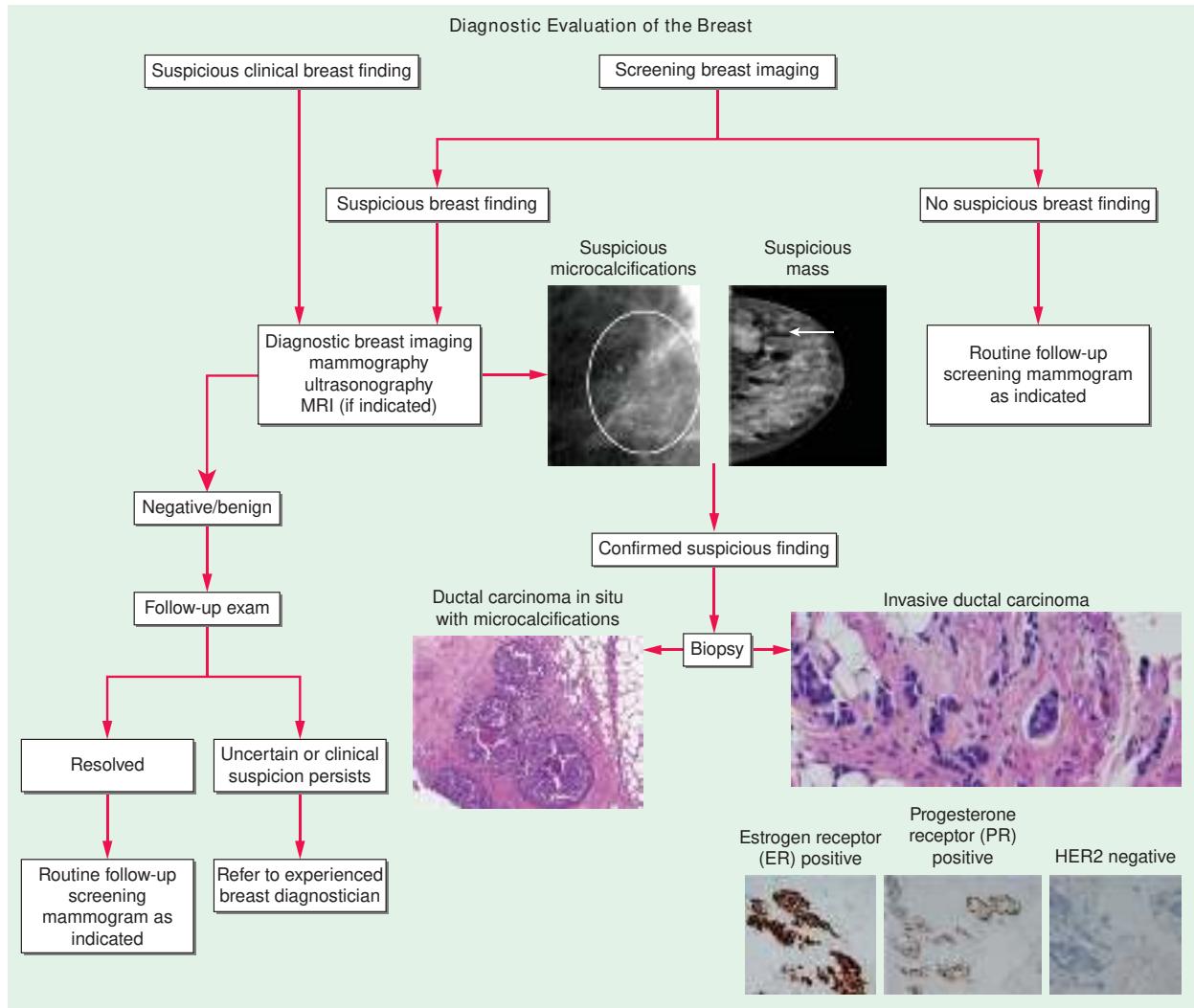


FIGURE 79-2 Evaluation and workup of breast lesions. For more extensive details, see https://www.nccn.org/professionals/physician_gls/pdf/breast-screening.pdf. (Mammographic images courtesy of Drs. Mark Helvie and Colleen Neal, Department of Radiology, Michigan Medicine. Photomicrographs courtesy of Dr. Celina Kleer, Department of Pathology, Michigan Medicine.)

of the higher incidence of cancer, and furthermore, many of them are considering prophylactic mastectomy as an alternative; therefore, the lower specificity and risk of a false-positive finding has been considered more acceptable.

Self-examination or physical breast examinations done by a health professional have poor sensitivity and specificity, and regular breast self-examination is not recommended. Nonetheless, all women should be familiar with how their breasts normally look and feel and report any changes to a health care provider right away. Because the breasts are a common site of potentially fatal malignancy in women, examination of the breast is an important part of a routine physical examination.

Screening breast imaging is not recommended for men, since it is so unusual and easily detected. It is important to note that unilateral lesions should be evaluated in the same manner as in women with an appropriately high index of suspicion.

EVALUATION OF BREAST MASSES

FIG. 79 2

Virtually all breast cancer is diagnosed by biopsy of an abnormality detected either on a mammogram or by palpation. The presence or absence of any risk factors, such as age, family history, or menstrual history cannot be used to exclude more careful workup and, if indicated, a biopsy. Any woman with a persistent breast abnormality should be referred to an experienced breast diagnostician in order to avoid delay in diagnosis and therapy.

■ PALPABLE BREAST MASSES

Proper attention needs to be given to any abnormality either discovered by the patient or appreciated by the health care provider during examination. Most newly diagnosed breast cancers are asymptomatic. Lesions with certain clinical features, including firmness, irregularity, tethering or fixation to the underlying chest wall, and dermal erythema or peau d'orange (skin edema with pockmarking), are very worrisome for breast cancer. In contrast, painful masses and those that are cystic on physical examination are less likely malignant. However, none of these has a high positive or negative predictive value. Likewise, a negative mammogram in the presence of a persistent lump in the breast does not exclude malignancy and, again, deserves careful workup.

In premenopausal women, lesions that are either equivocal or nonsuspicious on physical examination should be reexamined in 2–4 weeks during the follicular phase of the menstrual cycle. Days 5–7 of the cycle are the best time for breast examination. A dominant mass in a postmenopausal woman or a dominant mass that persists through a menstrual cycle in a premenopausal woman requires further evaluation, likely including biopsy if appropriate.

■ ABNORMAL MAMMOGRAM

Diagnostic mammography, which is performed after a palpable abnormality has been detected, should not be confused with *screening mammography*, which is performed in an asymptomatic woman with no previously identified abnormalities.

Abnormalities that are first detected by physical exam and/or screening mammography should be evaluated by diagnostic mammography. Suspicious mammographic abnormalities include clustered, heterogeneous, linear, and branching microcalcifications; densities (especially if spiculated); and new or enlarging architectural distortion. For some lesions, ultrasound may be helpful either to identify cysts or to guide biopsy. If there is no palpable lesion and detailed mammographic studies are unequivocally benign, the patient should have routine follow-up appropriate to the patient's age. If a nonpalpable mammographic lesion has a low index of suspicion, mammographic follow-up in 3–6 months is reasonable. The presence of a breast lump and a negative mammogram does not rule out cancer, and if the physical finding persists or enlarges during follow-up, further evaluation and, if appropriate, a biopsy are indicated.

■ BREAST MASSES IN PREGNANCY OR LACTATION

Breast cancer develops in 1 of 3000–4000 pregnancies. The breast grows during pregnancy under the influence of estrogen, progesterone,

prolactin, and human placental lactogen. After delivery and during lactation, breast tissue continues to be under the influence of unopposed prolactin. Therefore, breast examination during these times can be challenging. Nonetheless, development of a dominant mass during pregnancy or lactation should not be attributed to hormonal changes without appropriate diagnostic evaluation. Stage for stage, breast cancer in pregnant patients is no different from premenopausal breast cancer in nonpregnant patients. However, pregnant women often have more advanced disease because the significance of a breast mass was not fully considered and/or because of endogenous hormone stimulation.

PATHOLOGIC FINDINGS OF THE BREAST

■ BENIGN BREAST HISTOPATHOLOGY

Only ~1 in every 5–10 breast biopsies leads to a diagnosis of cancer, although the rate of positive biopsies varies in different countries and clinical settings due to variable interpretation, medico-legal considerations, and availability of mammograms. The vast majority of benign breast masses are due to fibrocystic changes, a descriptive term for small fluid-filled cysts and modest epithelial cell and fibrous tissue hyperplasia. Women with ductal or lobular cell proliferation (~30% of patients), particularly the small fraction (3%) with atypical hyperplasia, have a fourfold greater risk of developing breast cancer than women who have not had a biopsy, and the risk is even higher if they have an affected first-degree relative. Follow-up breast imaging should be continued, but not on an accelerated or more intense fashion than regularly indicated. Chemoprevention with antiestrogen therapy (SERM or aromatase inhibitor [AI]) should be considered for such patients. Prophylactic mastectomy is not normally indicated. By contrast, patients with a benign biopsy without atypical hyperplasia are at little increased risk and may be followed routinely.

■ NONINVASIVE BREAST NEOPLASMS

Breast cancer develops as a series of molecular changes in the epithelial cells that lead to ever more malignant behavior (Fig. 79-1). These changes range from malignant cells confined within the basement membrane of the lobule or duct, designated "noninvasive" or more commonly "in situ" carcinoma, to cancer cells that have invaded through the basement membrane into the surrounding normal tissue ("invasive" or "infiltrating" cancer). Increased use of mammography has led to more frequent diagnoses of noninvasive breast neoplasms. These lesions fall into two groups: ductal carcinoma *in situ* (DCIS) (Fig. 79-2) and lobular carcinoma *in situ* (LCIS; or lobular neoplasia *in situ* [LNIS]). The management of both entities is controversial.

Ductal Carcinoma *In Situ* Proliferation of cytologically malignant breast epithelial cells within the ducts is termed *ductal carcinoma in situ* (DCIS). Atypical hyperplasia may be difficult to distinguish from DCIS. In many ways, DCIS is really a " premalignant" condition, but probably at least one-third of patients with untreated DCIS develop invasive breast cancer within 5 years. However, many low-grade DCIS lesions do not appear to progress over many years; therefore, many patients are overtreated. Unfortunately, no reliable methods distinguish patients who require treatment from those who may be safely observed.

Mastectomy is nearly 100% effective in preventing a future breast cancer event in that breast and fundamentally can be considered prophylactic surgery, but is often not required for adequate treatment. No prospective randomized studies have directly compared breast-preserving therapy to mastectomy. However, the nearly 100% 10-year survival rates with the former suggest that it is a satisfactory strategy. Breast-preserving therapy refers to excisional surgery alone with or without breast radiation. However, although survival was identical in the two arms of a randomized trial comparing wide excision plus or minus irradiation, the latter caused a substantial reduction in the local recurrence rate as compared with wide excision alone. Addition of tamoxifen or an AI to any DCIS surgical/radiation therapy regimen further improves local control. However, in the largest trial comparing the two in DCIS, anastrozole did not improve distant disease-free or overall survival compared to tamoxifen.

Several prognostic features may help to identify patients at high risk for local recurrence after either lumpectomy alone or lumpectomy with radiation therapy and, therefore, might provide an indication for mastectomy. These include extensive disease within the breast; age <40; and cytologic features such as necrosis, poor nuclear grade, and comedo subtype with overexpression of HER2. In summary, it is reasonable to recommend breast-preserving surgery for patients who have a localized focus of DCIS with clear margins followed by breast irradiation and tamoxifen or anastrozole. Recently, a multifactorial gene expression assay has been shown to predict risk of recurrence in DCIS treated with breast-preserving surgery alone, but it is not clear that the in-breast risk recurrence rate in patients with low recurrence scores is sufficient to avoid radiation. The decision to irradiate such patients depends on the risk aversion to in-breast recurrence balanced against the risk associated with breast irradiation.

For patients with small, unicentric DCIS, axillary lymph node dissection is unnecessary. However, axillary sentinel lymph node (SLN) evaluation, which is discussed in greater detail below, may be indicated for widespread, larger, or poor grade DCIS or if microscopic invasion is identified on a core biopsy. In such cases, subsequent excision or mastectomy may demonstrate invasive disease on the larger specimen. Since SLN mapping is indicated in such patients, doing so at the time of excision or mastectomy avoids a further surgical procedure at a later date.

Lobular Carcinoma (Neoplasia) In Situ The presence of malignant cells within the lobules is termed *lobular carcinoma or neoplasia in situ* (LCIS). LCIS does not usually cause palpable breast masses, nor does it often induce suspicious findings on mammogram. Therefore, it usually is found as an incidental finding during pathologic examination of a breast biopsy performed for some other reason. Unlike DCIS, which is usually confined to a single area in a breast, LCIS is often spread throughout the breast, and it is frequently also found in the contralateral breast.

A diagnosis of LCIS itself does not confer a higher risk of mortality from breast cancer, but it does increase the risk of a subsequent breast cancer. Women with LCIS who do not undergo bilateral prophylactic mastectomy experience a new, invasive cancer in either breast at a rate of approximately 1% per year over at least the next 15–20 years, and probably lifelong. Therefore, LCIS is even more commonly considered a premalignant condition than DCIS, and aggressive local management seems unreasonable. Management options include careful observation with routine mammography and chemoprevention with either a SERM or an AI (for postmenopausal women) for 5 years. Beyond 5 years, such patients should be followed with subsequent annual mammography and semi-annual physical examinations. Bilateral prophylactic mastectomy is an alternative option, although it is no more effective in prolonging survival than the less aggressive approach, and it is associated with substantial cosmetic, and perhaps emotional, morbidity.

■ INVASIVE BREAST CANCERS

Invasive breast cancers are of more concern than *in situ* lesions because they harbor the capacity to metastasize and cause substantial morbidity and mortality (Fig. 79-1). Eighty-five percent of invasive breast cancers are ductal in origin (Fig. 79-2), 10% are lobular or mixed ductal/lobular, and the other 5% are made up of so-called “special types” including mucinous or colloid (2.4%), tubular (1.5%), medullary (1.2%), and papillary (1%). Although not universally true, prognosis for the special types tends to be better than standard ductal or lobular cancers.

STAGING AND DIAGNOSTIC CONSIDERATIONS

Cancer staging has been traditionally based on the size of the tumor (T) and the presence or absence of regional nodal (N) and distant metastases (M). More recently, tumor grade and biological characteristics, such as expression of ER and HER2, have been incorporated into staging, making the system quite complex. Staging can be performed clinically or pathologically, before or after adjuvant systemic therapy. These are designated as a prefix before the stage as cTNM or pTNM

if determined before or yTNM if determined after systemic (neoadjuvant) therapy. Although staging is an important part of the surgical evaluation and pathology reporting system, the specific elements that inform the clinician of both prognosis and likelihood of response to specific therapies have become more critical determinants of patient care than a simple stage designation. Importantly, imaging for detection of distant metastases is not needed in a patient with no signs or symptoms of widespread disease and who has a T3 or smaller tumor and fewer than four involved axillary lymph nodes, since the odds of finding distant metastases in such patients are low and the risk of false positives outweighs true-positive findings. Although finding bone marrow micrometastases or circulating tumor cells (cMO(i+)) has been associated with worse prognosis, how to integrate these into routine clinical care has not been determined, and their assessment is not recommended in patients with early-stage disease.

TREATMENT

Early-Stage Breast Cancer

GENERAL CONSIDERATIONS

Goals of Therapy The goal of therapy for breast cancer in patients who do not have obvious evidence of distant metastases (meaning outside the breast, chest wall, and regional lymph nodes) is cure, or at least substantial survival prolongation. For these patients, treatment strategies are divided into primary and systemic considerations. Primary therapies consist of surgical and radiation treatments directed toward the breast and locoregional lymph nodes. These approaches are designed to minimize the odds of locoregional recurrence while maintaining quality of life and cosmesis as much as possible by excising the cancer and sterilizing unaffected breast tissue as appropriate. Adjuvant systemic treatments, consisting of endocrine, anti-HER2, and/or chemotherapies, are given to treat micrometastases that may have already escaped to distant sites but are not yet detectable.

Prognostic and Predictive Factors All treatments for breast cancer are based on prognostic and predictive factors. Prognostic factors provide an indication of how likely a cancer will recur either locally or in distant organs in the future if a patient is not treated with the respective treatments. Predictive factors are used to determine if a given treatment is likely to work or not, assuming the patient's prognosis justifies treatment (or further treatment assuming the patient has been treated in some manner already).

Anatomic prognostic features include visual and physical examination findings of locally advanced breast cancer (T4 lesions: skin erythema [“inflammatory”] or edema [“peau d'orange”], nodules, or ulceration or tumor fixation to the chest wall). In patients without any of these findings, the most important prognostic features remain tumor size (T) and lymph node (N) status.

Biologic features, such as histologic tumor grade as well as ER, PgR, and HER2 status, are also prognostic. Indeed, gene expression patterns, or “signatures,” have demonstrated that breast cancer is actually many different diseases and can be divided into a series of intrinsic subtypes. These subtypes are driven principally, although not exclusively, by expression of ER and HER2 and their respective associated pathways, as well as measures of cellular proliferation and other less important but still contributory biologic features. These intrinsic subtypes are important clinically, both in influencing natural history as well as in prognosis and therapeutic decision making. Four different intrinsic subtypes are recognized: luminal, HER2-like, basal, and claudin-low. Some, if not all, have been further divided into subgroups.

Luminal breast cancers are almost always positive for ER and negative for HER2 amplification. **Luminal A** tumors have the highest levels of ER and downstream related genes, are almost universally negative or low in HER2, are usually low grade, have low proliferative thrust, and have a generally favorable prognosis. They are most likely to respond to endocrine therapy and may appear

to be less responsive to chemotherapy. *Luminal B* breast cancers tend to be PgR negative, may express HER2 but at low levels, are usually higher grade, and have higher proliferative activity than luminal A tumors. Prognosis is somewhat worse than for luminal A cancers, and although not yet proven, they may be more sensitive to chemotherapy.

HER2-amplified breast cancers exhibit co-amplification and overexpression of other genes adjacent to *HER2*. Historically, the clinical prognosis of such tumors was poor, but it has markedly improved with the introduction of targeted anti-*HER2* therapies.

Basal breast cancers are mostly negative for expression of ER/PgR and HER2. Tumors of this type are often called “triple-negative” malignancies, although this is a general term, and such cancers have been further subgrouped based on other genetic abnormalities. They tend to be high grade and express cytokeratins 5/6 and 17 as well as vimentin, p63, CD10, α -smooth muscle actin, and epidermal growth factor receptor (EGFR). Patients with germline *BRCA1* mutations also usually fall within this molecular subtype.

Normal breast-like and *claudin-low* cancers have also been distinguished, but at present, these designations have failed to have clinical significance.

Over the past decade, several multiparameter tests based on gene expression have been developed to determine prognosis in patients who have node-negative, ER-positive, and HER2-negative disease. These assays have been principally used to guide decisions regarding use of adjuvant chemotherapy, as discussed below. Predictive features are usually used to guide targeted systemic therapies. These include ER for endocrine treatments and HER2 for anti-*HER2* therapies, such as trastuzumab, and more recently *BRCA1/2* and *PIK3CA* mutations for poly (ADP ribose) polymerase (PARP) inhibitors and PIK3CA inhibitors, respectively.

LOCAL (PRIMARY) TREATMENTS

In the 1980s, the Halsted radical mastectomy was replaced with the less disfiguring modified radical mastectomy, in which chest wall muscles are preserved and only a sampling of axillary lymph nodes are removed. Subsequently, breast-conserving treatments, consisting of surgical excision of the primary tumor (lumpectomy, quadrantectomy, or partial mastectomy) often followed by locoregional radiation, were introduced and shown to have equal if not slightly superior outcomes to those associated with mastectomy. For women undergoing breast conservation, postlumpectomy radiation is usually indicated, although it may be less necessary in older women with ER-positive, node-negative breast cancer, since their risk of subsequent in-breast recurrence is quite low with surgery and endocrine therapy only. When lumpectomy with negative tumor margins is achieved and radiation is delivered appropriately, breast conservation is associated with a recurrence rate in the breast of $\leq 5\%$.

Not all patients are candidates for breast-conserving therapy. Contraindications include large tumor to breast ratio, inability to achieve clear margins with adequate cosmesis after extensive surgery, multifocal cancers, extensive four-quadrant DCIS, and inability to receive radiation. The latter issue arises in women with dermal autoimmune disease (such as lupus erythematosus), prior radiation to the site, and/or lack of available radiation treatment facilities. Further, although not contraindicated, breast-conserving therapy may be less cosmetically acceptable than mastectomy with reconstruction if the nipple-areolar complex is involved with cancer and must be sacrificed. This is a personal choice, and some women prefer mastectomy, especially those with high genetic risks for second breast cancers.

Enigmatically, in spite of the supporting data, only approximately one-third of women in the United States are managed by lumpectomy. It appears that many women still undergo mastectomy who could safely avoid this procedure and probably would if appropriately counseled. Most patients should consult with an experienced breast surgeon and radiation oncologist before making a final decision concerning local therapy. Indeed, a multimodality clinic in which the surgeon, radiation oncologist, medical oncologist, and

other caregivers cooperate to evaluate the patient and develop a treatment plan is usually considered a major advantage by patients.

For patients who do undergo mastectomy, nipple-areolar-sparing mastectomy preserves the dermis and epidermis of the nipple but removes the major ducts from within the nipple lumen and often provides more acceptable cosmesis when combined with reconstruction. This approach is often a preferable option for patients who are having prophylactic surgery or those with cancer who are candidates for immediate reconstruction. Nipple-sparing mastectomy is contraindicated in the presence of inflammatory breast cancer, clinical involvement of the nipple-areolar complex, nipple retraction, Paget disease, bloody nipple discharge, or multifocality. The safety of nipple-sparing mastectomy is based on retrospective, nonrandomized cohort series. In a meta-analysis of 20 studies (5594 patients), overall and disease-free survival and locoregional recurrence rates appeared similar to those of patients undergoing modified radical mastectomy.

After mastectomy, breast reconstruction is an acceptable option. Breast reconstruction can be achieved by either placement of an exogenous implant (usually silicon) or by transferring autologous tissue from another site, such as the abdomen, latissimus dorsi, or gluteal areas, to the breast. Of note, patients should be aware that a reconstructed breast is usually insensate. Risks of reconstruction include surgical complications such as infection and hemorrhage. Reconstruction does not hinder detection of future recurrences, nor is silicone implant reconstruction associated with non-cancer-related syndromes, although on occasion, these can rupture and removal is required. Breast implant-associated anaplastic large cell lymphoma is an extraordinarily rare complication of textured silicone implants. Although occasionally associated with metastatic lymphoma, it is usually confined locally and highly curable. The optimal choice of implant reconstruction should be made with an experienced breast plastic surgeon.

Postmastectomy chest wall and regional nodal radiation reduces locoregional recurrence and improves survival. It is indicated for patients with high risk of locoregional recurrence, such as those with tumors ≥ 5 cm, four or more positive axillary lymph nodes, or postoperative positive margins. Postmastectomy radiation is not indicated in women with cancers < 2 cm, negative lymph nodes, and negative margins. It is considered for women who fall into the areas between these (2–5 cm, one to three positive nodes, or close margins) and is usually recommended if a patient has one to three involved axillary lymph nodes. Many radiation oncologists and plastic surgeons prefer postmastectomy radiation before reconstruction.

The survival of patients who have recurrence in the breast after proper treatment (adequate surgery and radiation if indicated) is somewhat worse than that of women who do not have in-breast recurrences, but it is better than those who suffer locoregional recurrence after mastectomy. Thus, locoregional recurrence is a negative prognostic variable for long-term survival but not the cause of distant metastasis.

Evaluation and Treatment of the Axillary Lymph Nodes SLN mapping and biopsy (SLNB) is generally the standard of care for women with localized breast cancer and clinically negative axilla. This procedure involves injecting a dye or radioactive tracer into the involved breast and, a few hours (4–24) later, undergoing resection of the axillary node containing the dye or tracer. If that lymph node is negative for tumor, more extensive axillary surgery is not required, avoiding much of the risk of postdissection lymphedema. Even in the presence of sentinel lymph node involvement, further axillary surgery may not be required for selected patients, such as older women and those with ER-positive cancers.

ADJUVANT SYSTEMIC THERAPIES

The use of adjuvant systemic therapy is based on the concept that with increasing generations of cellular replication, genetic abnormalities accumulate. These mutations occur randomly and may lead to sensitivity or resistance to therapies, but of course, the latter

is of greater concern. Almost all patients with metastatic breast cancer are destined to die with, if not of, their cancer. However, treatment with the same therapies administered earlier, in the setting of micrometastatic disease only, is more effective than waiting until symptomatic, documented metastases occur and substantially improves survival. More than half of the women who would otherwise die of metastatic breast cancer remain disease-free and experience considerable survival advantage when treated with the appropriate adjuvant systemic regimen.

Prognostic and Predictive Variables Adjuvant systemic therapies are of three types: (1) chemotherapy; (2) endocrine therapy; and (3) anti-HER2 therapies. The decision of whether to apply adjuvant systemic therapy, and which type, depends on prognostic and predictive features as well as the combined judgment of the patient and caregiver.

Prognostic Factors As noted, prognostic factors help define who most likely needs, or perhaps more importantly does not need, adjuvant systemic therapy. In contrast, predictive factors help identify which therapies are likely to work, independent of prognosis (Table 79-1). The most important prognostic variables are provided by *tumor staging: tumor size (T), lymph node status (N), and detectable distant metastases (M)* (Table 79-2). *Histologic grading* is also important. Tumors with a poor nuclear grade (grade 3) have a higher risk of recurrence than tumors with a good nuclear grade (grade 1). Infiltrating lobular cancer, which is almost always ER positive, has roughly the same prognosis as ER-positive infiltrating ductal cancer, although the lobular subtype may be slightly worse. Lobular cancers are harder to detect on mammography and within axillary lymph nodes than ductal cancers, and when they do metastasize, they often spread to unusual sites, such as mesothelial surfaces, the ovaries, and gastrointestinal organs. Among the special types of breast cancer, pure tubular and mucinous cancers are associated with very favorable prognoses. Medullary cancers are often triple negative with poor nuclear grade, but paradoxically, they have a heavy infiltrating lymphocyte component, and they also have a favorable prognosis. However, before treatment is directed toward these types of cancers, their histology should be confirmed by an experienced breast pathologist.

Adjuvant systemic therapy may not be needed at all for patients with very small (<1 cm) tumors and negative lymph nodes. However, every patient with invasive breast cancer has some risk of subsequent distant metastases. Most patients are more likely to accept endocrine therapy for a very small potential benefit than they would accept chemotherapy for the same calculated advantage because the former is much less often associated with either life-threatening or permanently life-changing toxicities.

The greatest controversy concerns the recommendation for adjuvant *chemotherapy*. Since no established factor predicts sensitivity or resistance for this class of treatments, the decision must be made on prognosis alone. Overall, chemotherapy reduces the risk of recurrence over the 10 years subsequent to primary diagnosis by approximately one-third. For patients with T4 cancers or many positive lymph nodes, the risk of distant recurrence (and thus not

being cured) in the subsequent decade is 50% or higher. Therefore, a one-third reduction of a 50% risk of recurrence means that at least 15–20% (one-third × 50%) of women will be cured who would not have been cured in the absence of adjuvant chemotherapy. The life-threatening or permanently life-changing toxicities of adjuvant chemotherapy are ~1–2%, and therefore, almost all medical oncologists would recommend adjuvant chemotherapy in this setting.

In contrast, adjuvant chemotherapy is rarely justified in most women with tumors <1 cm in size whose axillary lymph nodes are negative. However, this decision is very much influenced by the expression of ER and HER2. For example, the risk of recurrence of a patient with a small, node-negative but triple-negative breast cancer over the succeeding 10 years without any adjuvant therapy is 15%. If chemotherapy reduces this risk by approximately one-third or more, then approximately 5% or more of patients will be cured who would otherwise have died of their disease. Likewise, a patient with ER- and PgR-negative but HER2-positive disease has a slightly worse prognosis, with a risk of recurrence over 10 years of approximately 20%. She will benefit not only from the adjuvant chemotherapy but also from anti-HER2 therapy, so that her potential absolute benefit is even higher. Many, but not all, clinicians would recommend adjuvant chemotherapy for such patients.

On the other hand, patients with ER-positive disease have a better prognosis than those with ER-negative breast cancer, and adjuvant endocrine therapy will further reduce the odds of recurrence by approximately one-half. Therefore, the same patient in the example above (<1 cm, node negative) but who has an ER-positive and HER2-negative cancer has a lower initial risk of recurrence (~10% over 10 years). She is very likely to accept adjuvant endocrine therapy, further lowering her estimated risk of recurrence to ~5%. Even if chemotherapy reduces this residual risk by approximately one-third, no more than 1–2% (one-third × 5%) of patients will benefit. This potential benefit is approximately the same as the number of patients who will suffer life-threatening or permanently life-changing toxicities from chemotherapy. Thus, in this case, most clinicians would recommend adjuvant endocrine therapy but not chemotherapy.

Multiparameter gene expression assays have refined prognostic determination, particularly in node-negative, ER-positive, and HER2-negative breast cancers. These tests include the 21-gene Oncotype DX, the 12-gene Endopredict, the 58-gene ProSigna, and the 2-gene Breast Cancer Index. Furthermore, several investigators have reported that analysis of ER, PgR, HER2, and Ki67 by immunohistochemistry (IHC4) also provides prognostic information in this group, but the analytical validity of this assay is quite variable among different pathologists. Assuming adequate adjuvant endocrine therapy, the prognosis of such patients whose tumors have low recurrence scores, which usually identifies luminal A type cancers, with one of these assays is so good they can safely forego adjuvant chemotherapy. Indeed, the same is true for such patients with intermediate Oncotype DX recurrence scores. In contrast, those with high recurrence scores (>25) appear to have luminal B cancers, and the benefits of adjuvant chemotherapy clearly outweigh the risks.

The largest data set for directing care has been generated using the 21-gene recurrence score. However, only one of these tests should be ordered for a single patient, since they do not always give the same results, and there are no data to determine which, in the case of discordance, might be “correct.” Use of these assays to determine prognosis in patients with higher anatomic stage, such as T3b/T4 lesions, or multiple positive lymph nodes, especially if more than three, is still under investigation.

Several measures of tumor growth rate correlate with early relapse, but their use is problematic due to analytical variability. Of these, assessment using immunohistochemical (IHC) assays for the proliferation marker Ki67 is the most widespread. However, substantial lab-to-lab variability and disagreement regarding optimal cut points exist. At present, in standard practice outside of a highly skilled laboratory, Ki67 expression is not used to make clinical decisions.

TABLE 79-2 5-Year Survival Rate for Breast Cancer by Stage

STAGE	5-YEAR SURVIVAL, %
0	99
I	92
IIA	82
IIB	65
IIIA	47
IIIB	44
IV	14

Source: Modified from data of the National Cancer Institute: Surveillance, Epidemiology, and End Results (SEER).

Predictive Factors The two most important predictive factors in breast cancer are ER and HER2 expression, and they should be performed on all primary or metastatic cancer biopsy specimens (Table 79-1). Adjuvant endocrine therapy reduces the risk of recurrence by one-half or more in patients with ER-rich cancers, whereas no detectable benefit is noted in patients with ER-poor or -negative cancers. ER is expressed as the percentage of positive cells within the cancer after IHC staining. Endocrine therapy is recommended for any patient with $\geq 10\%$ positive cells, but not for those whose cancers only have 0–1% staining. The evidence supporting benefit in cases with 1–9% expression is weak, but given the potential benefit and relatively low toxicities of endocrine therapy, it is recommended for such patients with a low threshold for discontinuation if side effects are intolerable.

The HER2 protein is the target for anti-HER2-directed therapies. Adjuvant trastuzumab therapy reduces the risk of distant recurrence and death in patients with HER2-positive breast cancer by one-third or more but has no discernable effect on HER2-negative cancers. HER2 status is determined using either IHC staining for protein overexpression or fluorescent *in situ* hybridization (FISH) for gene amplification. IHC staining of 3+ (on a scale of 0–3+) is considered positive, whereas 0–1+ is considered negative. For cases with 2+ staining, reflex FISH analysis is recommended. FISH can either be used as the initial evaluation or for additional evaluation in IHC 2+ cases. HER2 is considered amplified if the ratio of HER2 to centromere signal on chromosome 17 is ≥ 2.0 . FISH is unnecessary if IHC is 3+ or 0–1+, nor is there reason for IHC testing if FISH is ≥ 0 .

No reliable predictive factors exist for chemotherapy in general or for specific types of chemotherapies. It has been hypothesized that chemotherapy may be more active in ER-negative and/or HER2-positive cancers. Luminal B cancers may be more chemotherapy sensitive, whereas luminal A cancers are perceived to be relatively chemotherapy resistant. At present, none of the tests for intrinsic subtype should be used to determine not to give chemotherapy to patients with poor anatomic *prognosis*, such as those with T4 or multiple positive nodes, based on *prediction* of resistance. Attempts to identify reliable predictive factors for individual classes of chemotherapeutic agents (such as anthracyclines, alkylating agents, or taxanes) have been unsuccessful. The platin salts (carboplatin, cisplatin) may have higher activity in patients with triple-negative breast cancer and perhaps in patients with HER2-positive disease. The PARP inhibitors may be more active in patients whose tumors have defects in homologous recombination DNA repair, a group that includes those with *BRCA* mutations.

Adjuvant Regimens • **Endocrine Therapy** Adjuvant endocrine therapy is indicated for nearly all patients with a diagnosis of ER-positive breast cancer and never for those with ER-negative disease. Two adjuvant endocrine therapy strategies are proven: the SERM tamoxifen or estrogen ablation. In addition to being effective in preventing new cancers and reducing the risk of locoregional recurrences in patients with DCIS, tamoxifen reduces the risk of distant recurrence and death due to invasive breast cancer by ~40% over the decade following diagnosis. It is equally effective in pre- and postmenopausal women, although it may be slightly less effective in very young (<40 years) patients. Because tamoxifen is a SERM, it has mixed ER antagonism (in the breast and brain) and agonism (in the bone, liver, and uterus). Therefore, it is active against breast cancer in the prevention, adjuvant, and metastatic settings.

Side effects of tamoxifen are predictable based on ER antagonism, including frequent hot flashes as well as vaginal discomfort/sexual dysfunction and myalgias and arthralgias. The agonistic effect results in reduction of osteopenia/osteoporosis, especially in postmenopausal women, but it increases thrombosis risk and endometrial cancers due to this effect in the liver and uterus, respectively.

Estrogen depletion can be achieved surgically in premenopausal women by oophorectomy or ovarian suppression with a gonadotropin-releasing hormone (GnRH) superagonist, such as goserelin or leuprolide, which invoke a tachyphylactic response, or a GnRH antagonist, such as triptorelin. However, women with nonfunctioning ovaries, whether induced or by natural menopause, still produce small amounts of estrogen by adrenal synthesis of estrogen precursors (testosterone, dehydroepiandrosterone [DHEA]). These are converted to estradiol and estrone by aromatase activity in peripheral fat and possibly cancer cells. In postmenopausal women, circulating estrogen can be reduced to nearly imperceptible levels with the use of oral AIs: anastrozole, letrozole, and exemestane. The three AIs are not significantly different in activity or toxicity. All are slightly more effective than tamoxifen.

Toxicities of the AIs are predictable based on very low estrogen levels. These include hot flashes, musculoskeletal symptoms, and atrophic vaginitis/sexual dysfunction. They also induce or worsen osteoporosis and fractures, although this effect can be abrogated with bone-modifying agents, such as bisphosphonates or rank ligand antagonists (denosumab).

For both tamoxifen and the AIs, musculoskeletal symptoms mimicking osteoarthritis and arthralgias can be treated with physical therapy and nonsteroidal anti-inflammatory drugs. After a brief period of washout after discontinuation, switching from one AI to another relieves this symptom in approximately a third of patients. These symptoms can also be reduced with either acupuncture or the antidepressant duloxetine. If AIs cannot be tolerated, tamoxifen is a reasonable therapy, assuming no contraindications, such as a past history of thrombosis or high risk of cerebrovascular disease. Hot flashes from either class of drugs are alleviated in approximately one-half of patients with use of one of several different antidepressant drugs.

For premenopausal women, optimal endocrine therapy depends on prognosis and patient choice. Complete estrogen depletion is slightly more effective than tamoxifen alone, but it may also be associated with more bothersome side effects, such as hot flashes, vaginal dryness, and sexual dysfunction. Complete estrogen depletion, consisting of either oophorectomy or chemical suppression of gonadotropins coupled with an AI, is indicated for women with worse prognosis, in particular node positivity. For those with more favorable prognosis, tamoxifen alone or with ovarian suppression is adequate and produces better quality of life. The AIs should not be administered to women with functioning, or dormant, ovaries, since the negative hypothalamic-pituitary feedback can result in a rebound overproduction of ovarian estrogens.

The duration of adjuvant endocrine treatment is unclear. Formerly, the standard recommendation was at least 5 years of therapy, which clearly reduces the risk of recurrence during that time and for a few years after discontinuation. However, the annual risk of distant recurrence during the subsequent 15 years is 0.5–3%, depending on the initial T and N status. Extended adjuvant endocrine therapy with either tamoxifen or an AI for at least 5 more years continues to reduce this late risk of relapse. The decision of whether to continue adjuvant endocrine therapy or not after 5 years must therefore take into consideration initial risk (T, N, grade), current side effects and potential cumulative toxicities, and the patient's perception of the relative and absolute benefits and risks.

Chemotherapy Multiple-agent adjuvant chemotherapy is more effective than single-agent chemotherapy. Although chemotherapeutic agents are usually delivered in combination, sequential single-agent chemotherapy is as effective, and may be slightly less toxic, although it requires longer total duration to deliver. Administration of four to six cycles of chemotherapy appears to be optimal; one cycle is less effective than six, but more than six cycles have generally increased toxicity without further efficacy. Importantly, although chemotherapy is combined with anti-HER2 therapy in patients with HER2-positive cancers, concurrent endocrine therapy, in particular tamoxifen, is antagonistic with chemotherapy.

Therefore, they are administered sequentially, starting the endocrine therapy after completion of chemotherapy.

Several chemotherapeutic agents have activity in the adjuvant setting. These include alkylating agents (principally cyclophosphamide), anthracyclines (doxorubicin, epirubicin), antimetabolites (5-fluorouracil [5-FU], capecitabine, methotrexate), the taxanes (paclitaxel, docetaxel), and the platinum salts (cisplatin, carboplatin). Within classes, randomized trials have failed to demonstrate superiority of one agent versus another (e.g., doxorubicin vs epirubicin, or paclitaxel vs docetaxel). Escalation above an optimal dose is not more effective. The antineoplastic advantage of more frequent scheduling for most individual agents has been demonstrated in a well-done meta-analysis. Weekly or every-other-week paclitaxel is superior to every-3-week infusion, whereas, enigmatically, the opposite is true for docetaxel. Taken together, the data support giving adjuvant chemotherapy in a dose-dense fashion.

The oldest combination regimen consists of cyclophosphamide, methotrexate, and 5-FU (CMF). Addition of an anthracycline or substitution of an anthracycline for the antimetabolites improves outcomes slightly, albeit with slightly increased risk of heart failure and secondary leukemia. Addition of a taxane to an anthracycline-based regimen further modestly reduces the chances of distant recurrence and death. Likewise, addition of an anthracycline to a taxane-based regimen is also modestly more effective than a taxane plus cyclophosphamide alone.

Which regimen is appropriate for a patient must be individualized based on prognosis, comorbid conditions, and the perspective of the patient. For example, the modest relative improvement of giving an anthracycline, cyclophosphamide, and a taxane (AC-T) may not translate to a sufficiently large absolute improvement in survival in a patient with a relatively small (T2) tumor and negative nodes, whereas that same relative reduction in death may translate to a sufficiently large absolute benefit in a patient with a worse prognosis. Therefore, the former patient might best be served with a taxane/cyclophosphamide (TC) regimen alone, while the latter might wish to accept the added risk of congestive heart failure and leukemia associated with the anthracyclines.

Neoadjuvant Chemotherapy Preoperative, or “neoadjuvant,” treatment involves the administration of adjuvant systemic therapy, most commonly chemotherapy, before definitive surgery and radiation therapy. Neoadjuvant endocrine therapy for patients with ER-positive disease is usually given preoperatively for 4–6 months. However, it is generally reserved for patients for whom a reason for surgical delay exists, such as comorbid conditions.

The objective partial and complete response rates of patients with breast cancer to neoadjuvant chemotherapy range from 10 to 75% depending on the intrinsic subtype of the cancer and the regimen used. Thus, many patients will be “downstaged” by neoadjuvant chemotherapy. In this circumstance, patients with locally advanced, inoperable cancers may become candidates for surgery, and approximately 15% of patients who are not considered eligible for breast-conserving surgery may become so due to shrinkage of their cancer. However, overall survival has not been improved using this approach as compared with the same drugs given postoperatively.

Patients who achieve a pathologic complete remission (pCR) after neoadjuvant chemotherapy have a substantially improved survival compared to those who do not. It is unknown if this observation implies that the latter group did not benefit or just had a worse initial prognosis, yet still gained some benefit. Delivering more therapy to patients who do not have a pCR is appealing. However, it is possible that these patients have chemotherapy-resistant disease, and therefore, more chemotherapy may not be of value. Clearly nonchemotherapeutic strategies, such as adjuvant endocrine therapy if they have an ER-positive breast cancer and adjuvant anti-HER2 therapy if their cancer is HER2 positive, are warranted.

Adding or changing systemic therapies may benefit selected groups of patients who do not have a pCR. Approximately 6 months

of a postsurgical oral fluoropyrimidine, capecitabine, reduces distant metastases in patients with triple-negative breast cancer who have residual disease after non-fluoropyrimidine-containing neoadjuvant chemotherapy. Similarly, postoperative therapy with an antibody-drug conjugate consisting of trastuzumab and the antitubulin emtansine (ado-trastuzumab emtansine) is superior to continuing unconjugated trastuzumab in patients with HER2-positive breast cancer who did not achieve pCR with preoperative chemotherapy and trastuzumab.

Chemotherapy Toxicities Chemotherapy is associated with nausea, vomiting, and alopecia in nearly 100% of patients. Nausea and vomiting are usually well controlled with modern antiemetics. Small but convincing studies have suggested that the strategy of constricting blood flow to the scalp with various means of cooling is commonly effective in sparing hair loss, without evidence of increased scalp metastases.

More importantly, chemotherapy causes potential life-threatening or life-changing toxicities in 2–3% of all treated patients. These include neutropenia and fever, with a risk of infection of ~1%, which can be prevented with appropriate use of the growth factor filgrastim. Secondary myelodysplasia and leukemia occur in ~0.5–1% of patients treated with anthracyclines as well as with high cumulative doses of cyclophosphamide, usually occurring within 2–5 years of treatment. The anthracyclines cause cumulative dose-related congestive heart failure, which occurs in ~1% of patients treated with standard four to five cycles at 60 mg/m². Peripheral neuropathy is the major dose-limiting and life-changing toxicity of the taxanes. Neuropathy occurs during treatment in ~15–20% of patients, and permanent, chronic neuropathy persists in 3–5%. Many patients complain of cognitive dysfunction, so-called “chemo-brain.” Although occasional cases of apparent organic chemotherapeutic toxic effects on cognitive function are noted, much of this syndrome may be due to anxiety, depression, and fatigue caused by the diagnosis itself or the treatment for it. Although not always, cognitive functioning usually returns to age-adjusted baseline several months after discontinuation of therapy.

Anti-HER2 Therapy The humanized anti-HER2 monoclonal antibody trastuzumab decreases both risk of recurrence and mortality in early-stage breast cancer. Trastuzumab is optimally delivered concurrently with chemotherapy, particularly in association with a taxane. Concurrent treatment with an anthracycline is generally avoided, since the main toxicity of trastuzumab is cardiac dysfunction, which appears more often when the agent is delivered simultaneously with doxorubicin. In patients with reasonably favorable prognosis (T1 or T2, node negative), single-agent paclitaxel plus trastuzumab is an adequate regimen. The addition of a second anti-HER2 monoclonal antibody, pertuzumab, in combination with trastuzumab is modestly superior to trastuzumab alone. When given in the neoadjuvant setting, this combination results in higher pCR rates than single-agent trastuzumab. At least in patients with poor prognostic features, such as positive axillary lymph nodes, the combination significantly reduces distant metastases and perhaps mortality. As noted, neoadjuvant studies have demonstrated that postoperative ado-trastuzumab emtansine is superior to trastuzumab in patients who do not achieve a pCR.

Trastuzumab is administered intravenously weekly or every 3 weeks. Twelve months of trastuzumab therapy are optimal with no additional benefit beyond 12 months. Treatment for 6 months is more effective than no trastuzumab therapy but is inferior to 12 months. A preparation of trastuzumab for subcutaneous injection has been approved by the U.S. Food and Drug Administration.

Selected anti-HER2 tyrosine kinase inhibitors have activity against HER2-positive breast cancer, but their benefit in the adjuvant setting is limited. Lapatinib does not add to trastuzumab therapy, and single-agent adjuvant lapatinib is inferior to single-agent trastuzumab. Another anti-HER2 tyrosine kinase inhibitor, neratinib, is modestly superior to no anti-HER2 therapy. Neratinib has

not been compared to trastuzumab either as a single agent or in combination.

Toxicities of Anti-HER2 Adjuvant Therapies In general, the anti-HER2 therapies are safe and effective. Occasionally patients experience allergic reactions to an initial cycle of trastuzumab, but these usually do not recur. Trastuzumab can cause cardiac muscle dysfunction, although it is rare to observe symptomatic congestive heart failure from adjuvant trastuzumab. Baseline and serial echocardiographic monitoring is indicated. Patients with a past history of cardiac abnormalities should not receive trastuzumab or should be followed and treated by a cardiologist with experience in this condition. Pertuzumab is associated with loose stools and diarrhea, which can usually be managed with antidiarrheal therapy, such as loperamide. The chemotherapy payload of ado-trastuzumab emtansine can cause thrombocytopenia and peripheral neuropathy.

Skeletal Strengthening Agents Bone-strengthening agents that are commonly used to treat osteoporosis, specifically the bisphosphonates, have some limited activity in preventing recurrent breast cancer to bone, particularly in postmenopausal women. In addition, bisphosphonate therapy also reduced breast cancer mortality in this subgroup. The benefit is not significantly associated with any specific bisphosphonate class, treatment schedule, ER status, nodal status, tumor grade, or concomitant chemotherapy. As expected, bone fractures are reduced (relative risk [RR] 0.85; 95% confidence interval [CI] 0.75–0.97; $2 p = .02$). Joint guidelines from the American Society of Clinical Oncology and Cancer Care Ontario recommend “that, if available, zoledronic acid (4 mg intravenously every 6 months) or clodronate (1,600 mg/d orally) be considered as adjuvant therapy for postmenopausal patients with breast cancer who are deemed candidates for adjuvant systemic therapy. Further research comparing different bone-modifying agents, doses, dosing intervals, and durations is required.” The rank-ligand inhibitor denosumab does not prevent relapse in bone or other sites, nor does it reduce mortality.

Novel Adjuvant Systemic Agents Other exciting adjuvant strategies are being tested (Table 79-1). These include PARP inhibitors (olaparib, talazoparib) in patients with known germline *BRCA1* or *BRCA2* mutations or those with triple-negative cancers that share similar defects in DNA repair in their etiology. Likewise, the mTOR inhibitor everolimus and the CDK4/6 inhibitors (palbociclib, ribociclib, abemaciclib) are being tested in the adjuvant setting in combination with antiestrogen therapy. The remarkable results of immune checkpoint inhibitors in other cancers have led to studies of this approach in both metastatic and post-neoadjuvant chemotherapy settings. Their activity with chemotherapy in triple-negative disease appears promising.

STAGE III BREAST CANCER

Ten to 25% of patients present with so-called locally advanced or stage III breast cancer at diagnosis. Many of these cancers are technically operable (T3), whereas others, particularly cancers with chest wall involvement, inflammatory breast cancers, or cancers with large matted axillary lymph nodes (T4 or N2-3), cannot be managed with surgery initially. Neoadjuvant downstaging facilitates local therapy. Radiotherapy either to the chest wall after mastectomy or to the breast after tumor excision is almost always recommended, as is regional lymph node treatment. Adjuvant anti-HER2 and endocrine therapies are also used as appropriate. These patients should be managed in multimodality clinics to coordinate local and systemic therapies. Such approaches produce long-term disease-free survival in ~30–50% of patients.

SIMULTANEOUS NEW PRIMARY WITH DETECTABLE METASTASES

In the screening era, only a small fraction of patients (~5%) present with a new primary lesion and simultaneous metastases, detected either due to symptoms of distant disease or because they had staging scans due to locally advanced disease. Several retrospective single-institutional experiences have suggested that neoadjuvant systemic therapy followed by local therapy (breast surgery and

radiation) is associated with prolonged survival. However, two prospective randomized trials have failed to demonstrate any survival benefit. Currently, local therapy for such patients is considered on a case-by-case basis depending on the response to systemic therapy and the patient's overall performance status and desires.

BREAST CANCER SURVIVORSHIP ISSUES

The odds of surviving breast cancer have increased dramatically over the past 35 years due to a combination of early detection and more effective therapies. Without these advances, >60,000 American women would have suffered breast cancer mortality in 2020, and over one-quarter million women are alive who would not have been otherwise. Coupled with the women who would have been cured even before the impressive advances of the past three decades, millions are breast cancer survivors. Thus, all clinicians, not just oncologists, need to be aware of survivorship issues in patients with previously diagnosed and treated breast cancer.

At present, no special follow-up procedures, such as serial circulating tumor biomarkers or systemic radiographic/scintigraphic imaging, are indicated in an asymptomatic patient with no physical findings of recurrence. Although randomized trials have demonstrated slightly higher incidence of detection of metastases with lead times of 3–12 months by surveillance of asymptomatic patients compared to no special follow-up, no evidence suggests that earlier detection improves overall survival. If anything, such surveillance may worsen quality of life due to higher anxiety levels associated with the testing and toxicities associated with earlier treatment in patients who were otherwise doing well at that time.

However, the risk of late metastases in breast cancer survivors is small but real, especially those who had ER-positive disease. These patients remain at risk for distant recurrence for at least 20 years after initial diagnosis, and probably lifelong. The annual risk is relentless, ranging from 0.5% per year for patients with initially negative lymph nodes and grade 1 tumors <1 cm to as high as 3% per year for those who initially had multiple positive lymph nodes. Therefore, especially in patients with prior ER-positive cancers, the physician must carefully assess and evaluate new symptoms, considering whether they might be due to the cancer, the treatment, or an unassociated condition. Judgment needs to be used to decide if blood tests or imaging are required in order to avoid missing a lesion for which appropriate treatment would improve the patient's quality of life but also to diminish overtesting, with associated inconvenience, anxieties, false-positive results, and cost. Serial echocardiography should be performed every 3 months for patients on adjuvant trastuzumab, but not after it is discontinued.

Several observations suggest that perhaps the recommendations not to do intensive surveillance in patients without signs or symptoms of recurrence might need to be reconsidered. First, the unrelenting annual incidence of long-term distant recurrence for patients with ER-positive disease demonstrates that none of these patients can ever be considered free of risk of metastases. Second, available diagnostic tests have become substantially more sophisticated in the past decade. These include the advent of liquid biopsies beyond just circulating protein markers, such as circulating tumor DNA and circulating tumor cells, as well as more sensitive and specific scintigraphic and imaging techniques, such as positron emission tomography. Finally, the identification of several highly effective targeted therapies, including new endocrine, anti-HER2, and other therapies, provides opportunities to deliver more beneficial and less toxic therapies than the few chemotherapeutic and endocrine agents that were available at the time the older randomized trials were performed (Table 79-1). Ongoing trials are addressing whether incorporating these new technologies and treatments might improve survival as opposed to waiting for emerging symptoms to initiate additional treatment strategies. At present, no clear answers are apparent.

Likewise, serial monitoring for long-term, life-threatening toxicities associated with chemotherapy, such as myelodysplastic syndromes or congestive heart failure, is not warranted since these are quite uncommon and likely to cause obvious symptoms requiring proper evaluation if they occur.

For patients on endocrine therapy, quality-of-life issues may be critical, including hot flashes, sexual difficulties, musculoskeletal complaints, and risk of osteoporosis. Although estrogen therapy, given orally, transdermally, or transvaginally, effectively reduces these side effects, careful consideration should be given for estrogen replacement therapy to these patients because it may counteract the efficacy of the endocrine therapy. Locally administered therapies are often very effective and likely less risky. Nonhormonal treatments, such as selected antidepressants for hot flashes and musculoskeletal symptoms, and counseling and water-based lubricants for sexual issues can be quite helpful. It is important to screen bone density in patients on an AI more frequently than is recommended for the average postmenopausal woman, since total estrogen depletion results in enhanced risk of osteoporosis and risk of fracture. All women should be counseled to take daily calcium and vitamin D replacement, and if osteoporosis is present or osteopenia is worsening, bone-strengthening agents should be administered.

METASTATIC DISEASE

Diagnostic Considerations (Fig. 79-3) About 15–20% of patients treated for localized breast cancer develop metastatic disease in the subsequent decade after diagnosis. Soft tissue, bone, and visceral (lung and liver) metastases each account for approximately one-third of sites of initial relapses. However, by the time of death, most patients will have bone involvement. Recurrences can appear at any time after primary therapy, but at least half occur >5 years after initial therapy, especially in patients with ER-positive disease. A variety of host factors can influence recurrence rates, including depression and central obesity, and these diseases should be managed as aggressively as possible.

For patients with no prior history of metastases, a biopsy of suspicious physical or radiographic lesions should be performed for confirmation that the lesion does represent recurrent cancer. One should not assume that an apparent abnormality is a breast cancer metastasis. Many benign conditions, such as tuberculosis, gallstones, sarcoidosis,

hyperparathyroidism, or other nonmalignant diseases, can mimic a recurrent breast cancer and are of course treated much differently. Moreover, if biopsy is positive for metastases, re-evaluation of ER and HER2 is indicated, since these can differ between the primary and metastatic lesions in up to 15% of cases. Analysis for PIK3CA mutations should be performed if the cancer is ER positive. Predictors of immune checkpoint inhibitor susceptibility, such as PD-L1 expression, should be determined in triple-negative metastatic breast cancers (Table 79-1). Many experts are also recommending some form of next-generation sequencing of all metastatic cancers from any site, although this recommendation is controversial.

Once a recurrence/metastasis is established, some form of body imaging should be performed—either a scintigraphic bone scan and chest and abdomen CT scan or a positron emission tomography (PET)/CT scan, depending on caregiver's preference. Brain scanning (CT or MRI) is not indicated in the absence of any cognitive or neurologic signs or symptoms in most patients. However, because of increased risk of brain metastases in HER2-positive breast cancer, some experts do recommend central nervous system (CNS) imaging in such patients even in the absence of clinical indications. Regardless, body scans provide a perspective of extent of disease, which may guide therapeutic decisions, as well as the need for ancillary treatments, such as bone-modifying agents if skeletal metastases are present.

Considerations Regarding Goals of Therapy Although treatable, metastatic disease is rarely if ever cured. The median survival for all patients diagnosed with metastatic breast cancer is <3 years, but with remarkable variability depending on intrinsic subtype and treatment effects. Patients with triple-negative metastatic breast cancer have the shortest expected survival, whereas those with ER-positive disease can expect to live the longest. HER2 positivity was initially found to be a very poor prognostic factor in metastatic breast cancer, but the availability of several effective targeted treatments has improved the expected survival rates to at least those of ER-positive patients, if not better.

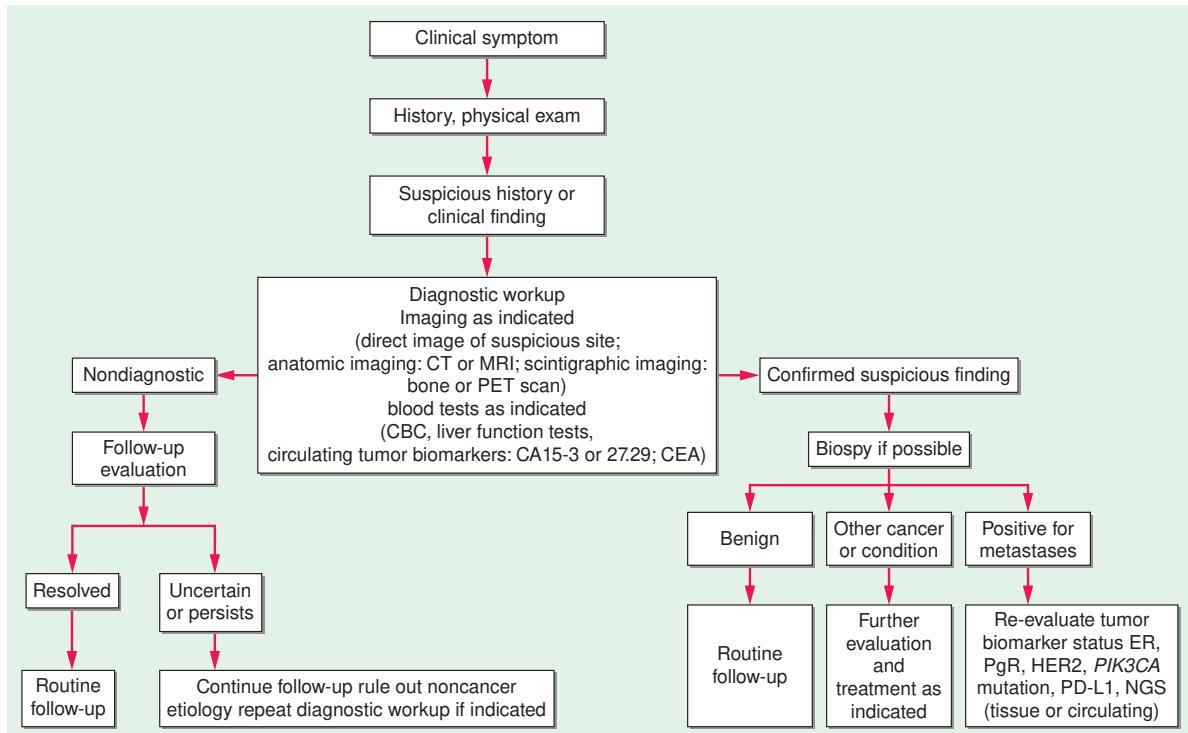


FIGURE 79-3 Evaluation of new signs or symptoms in a patient with prior history of early-stage breast cancer. See text for details. CBC, complete blood count; CEA, carcinoembryonic antigen; ER, estrogen receptor; NGS, next-generation sequencing; PET, positron emission tomography; PgR, progesterone receptor.

The overall goal of treatment of metastatic disease is palliation or, put simply, to “keep the patient feeling as well as she can for as long as she can.” A secondary goal is improved survival. Overall survival has not been improved by advocating more aggressive or toxic therapies, such as high-dose or combination chemotherapy, but rather by using more selective and biologically based therapy, including endocrine or anti-HER2 therapies in patients with ER- or HER2-positive breast cancers, respectively.

Generally, a new treatment is continued until either progression or unacceptable toxicities are evident. These are both evaluated by serial history and physical examinations and periodic serologic evaluation for hematologic or hepatic abnormalities, as well as circulating tumor biomarker tests (assays for MUC1 [CA15-3 or CA27.29] and for carcinoembryonic antigen [CEA] and occasionally CA125). If all these evaluations fail to suggest progression, it is unlikely that imaging will contribute. However, if one or more of these suggest progression, whole-body imaging with whichever modality(ies) was used at baseline is indicated.

The choice of therapy requires consideration of local therapy needs, specifically surgical approaches to particularly worrisome long-bone lytic lesions or isolated CNS metastases. New back pain in patients with breast cancer should be explored aggressively on an emergent basis, usually with a spine MRI; to wait for neurologic symptoms is a potentially catastrophic error. Metastatic involvement of endocrine organs can occasionally cause profound dysfunction, including adrenal insufficiency and hypopituitarism. Similarly, obstruction of the biliary tree or other impaired organ function may be better managed with a local therapy than with a systemic approach. Radiation as an adjunct to or instead of surgery is an important consideration for particularly symptomatic disease in long or vertebral bones, locoregional recurrences, and CNS metastases. In many cases, systemic therapy can be withheld while the patient is managed with appropriate local therapy.

Aggressive local treatment, such as excision, radiation, radiofrequency ablation, or cryotherapy of metastases to the lung, liver, or other distant sites, does not improve survival. Although appealing, these strategies are associated with increased toxicity and cost and should be reserved for palliation.

Locoregional recurrence on the chest wall or surrounding lymph nodes is an exception to this principle. Some of these lesions may well represent new primary cancers, even in the case of prior mastectomy, since some residual at-risk normal tissue can remain. Regardless, rendering the patient disease-free by surgery and radiation, if appropriate, followed by adjuvant systemic therapy, such as endocrine therapy if the cancer is ER positive, or chemotherapy if ER negative, is indicated. Anti-HER therapy is also appropriate if the cancer is HER2 positive.

Selection of the systemic therapy strategy depends on the overall medical condition of the patient, the hormone receptor and HER2 status of the tumor, and clinical judgment. Because therapy of systemic disease is palliative, the potential toxicities of therapies should be balanced against expected response rates. Several variables influence the response to systemic therapy. For example, the presence of ER and PgR is a strong indication for endocrine therapy, even for patients with limited visceral (lung/liver) disease. On the other hand, patients with short disease-free intervals or rapidly progressive visceral disease (liver and lung) with end-organ dysfunction, such as lymphangitic pulmonary disease, are unlikely to respond to endocrine therapy.

Many patients with bone-only or bone-dominant disease have a relatively indolent course. Because the goal of therapy is to maintain well-being for as long as possible, emphasis should be placed on avoiding the most hazardous complications of metastatic disease, including pathologic fracture of the axial skeleton and spinal cord compression. Under such circumstances, systemic chemotherapy has a modest effect, whereas radiation therapy may be effective for long periods. Patients with bone involvement should receive concurrent bone-strengthening agents, such as bisphosphonates or the humanized monoclonal anti-RANK ligand antibody denosumab.

These therapies have been proven to reduce bone pain, fractures, and hypercalcemia of malignancy.

Many patients are inappropriately treated with toxic regimens into their last days of life. Often, oncologists are unwilling to have the difficult conversations that are required with patients nearing the end of life, and not uncommonly, patients and families can pressure physicians into treatments with very little survival value. Although systemic therapy is designed to deliver palliation, formal palliative care consultation and realistic assessment of treatment expectations need to be reviewed with patients and families. We urge consideration of formal palliative care consultations for patients who have received at least two lines of therapy for metastatic disease.

SYSTEMIC TREATMENTS FOR METASTATIC BREAST CANCER

Endocrine Therapy (Table 79-1) Approximately 30–70% of patients with ER-positive breast cancer will benefit from endocrine therapy. Potential endocrine therapies are summarized in Table 79-1. Available strategies include SERMs (tamoxifen, toremifene), the AIs (anastrozole, letrozole, exemestane), and the selective estrogen receptor downregulator (SERD) fulvestrant. Additive endocrine therapies, including treatment with progestins and androgens and, enigmatically, pharmacologic doses of estrogens, are all active, but they may be associated with unacceptable side effects in many women and are rarely used. Tamoxifen withdrawal (as well as withdrawal of pharmacologic doses of estrogens) induces responses in ~15% of patients, but with the advent of so many other therapies for metastatic disease, this strategy is also rarely used in modern oncology.

The sequence of endocrine therapy is variable. Patients who respond to one endocrine therapy have at least a 50% chance of responding to a second endocrine therapy. It is not uncommon for patients to respond to two or three sequential endocrine therapies. Many, but not all, women with ER-positive breast cancer who suffer a recurrence will do so either while still taking or after recently discontinuing a prior adjuvant endocrine therapy (either tamoxifen or an AI). In most postmenopausal patients, if they have never received an AI or discontinued adjuvant AI many years before recurrence, the initial endocrine therapy should be an AI rather than tamoxifen. As noted, AIs are not used in women with functioning ovaries because their hypothalamus can respond to estrogen deprivation by producing gonadotropins that promote ovarian estrogen synthesis. Fulvestrant is usually used in sequence after AI therapy. Compared to single-agent therapy, combination endocrine therapies increase the chances of response, but they do not appear to increase the ultimate time to chemotherapy use or overall survival. Combinations of chemotherapy with endocrine therapy are not useful.

Over the past decade, several different targeted agents have been shown to enhance outcomes of patients with ER-positive metastatic breast cancer when combined with endocrine therapy (Table 79-1). Addition of an inhibitor of mTOR, everolimus, to endocrine therapy improves time to progression. Everolimus is commonly associated with mucositis, which can be prevented or alleviated by use of dexamethasone-containing mouthwash. Diarrhea is also a common side effect and can be lessened with antidiarrheal medications such as loperamide.

Inhibitors of CDK4/6 (palbociclib, ribociclib, abemaciclib) also substantially improve progression-free survival, and even overall survival when combined either with an AI or fulvestrant (Table 79-1). Most experts now recommend a CDK4/6 inhibitor with endocrine therapy as first-line therapy for ER-positive metastatic disease. They can cause dangerous neutropenia, although rarely to the extent seen with chemotherapy. Nonetheless, absolute neutrophil counts need to be monitored closely with appropriate adjustments in dose and schedule. Fatigue is also an occasional side effect, and abemaciclib frequently causes diarrhea. Similarly, an inhibitor of PIK3CA protein, alpelisib, prolongs progression-free survival in patients whose cancers harbor activating mutations of this gene. Like everolimus, it too causes mouth sores and diarrhea.

These targeted agents should not be given simultaneously but rather in sequence as appropriate.

Chemotherapy Unlike many other epithelial malignancies, breast cancer responds to multiple chemotherapeutic agents, including anthracyclines, alkylating agents, taxanes, and antimetabolites. Multiple combinations of these agents have been found to improve response rates somewhat, but they have had little effect on duration of response or survival. Unless patients have rapidly progressive visceral (lung, liver) metastases with end-organ dysfunction, single-agent chemotherapy is preferable, used in sequence as one drug fails going on to the next. Given the significant toxicity of most drugs, the use of a single-agent therapy will minimize toxicity by sparing the patient exposure to drugs that would be of little value. No method to select the drugs most efficacious for a given patient has been demonstrated to be useful.

Most oncologists use capecitabine, an anthracycline, or a taxane for first-line chemotherapy, either in a patient with ER-positive disease that is refractory to endocrine therapy or for a patient with ER-negative breast cancer. Within these general classes, one particular agent is no more preferable than another (such as doxorubicin vs epirubicin or paclitaxel vs docetaxel), and the choice has to be balanced with individual needs. Objective responses in previously treated patients may also be seen with gemcitabine, vinorelbine, and oral etoposide, as well as a newer class of agents, epothilones. Platinum-based agents have become far more widely used in both the adjuvant and advanced disease settings for some breast cancers, particularly those of the triple-negative subtype.

Anti-HER2 Therapy (Table 79-1) Initial use of a trastuzumab, either alone or with chemotherapy, improves response rate, progression-free survival, and even overall survival for women with HER2-positive disease. Indeed, anecdotal reports suggest that, on occasion, a few patients with HER2-positive metastatic breast cancer may be cured. Addition of pertuzumab to trastuzumab is more effective than trastuzumab alone. The antibody-drug conjugate, ado-trastuzumab emtansine, is effective after progression on trastuzumab. Another antibody-drug conjugate, fam-trastuzumab-deruxtecan-nxki, has been shown to be active even in patients who have progressed on multiple other anti-HER2 therapies, including ado-trastuzumab emtansine. A monoclonal antibody, margetuximab, has been engineered to specifically enhance antibody-dependent cell-mediated cytotoxicity against tumor cells overexpressing HER2. In a phase 3 trial, margetuximab plus chemotherapy improved overall survival by 1.8 months compared with trastuzumab plus chemotherapy.

Inhibitors of the HER2 tyrosine kinase domain also have activity against HER2-positive breast cancers. Lapatinib is effective when added to chemotherapy after patients progressed on trastuzumab. In addition, even after progression on trastuzumab, combination trastuzumab and lapatinib is superior to lapatinib alone. When added to oral capecitabine, neratinib is more effective than lapatinib in patients who received two or more prior anti-HER2-based regimens. In patients with heavily pretreated HER2-positive disease, including those with brain metastases, adding tucatinib to trastuzumab and capecitabine resulted in better progression-free survival and overall survival outcomes than adding placebo. Of interest, 2–3% of breast cancers that do not amplify or overexpress HER2 contain activating mutations in the gene encoding for it. Preclinical models and preliminary trials suggest that neratinib is particularly active against this mutation.

PARP Inhibitors (Table 79-1) PARP inhibitors induce synthetic lethality of cancer cells with inactive *BRCA1/2* or cancers that have *BRCA*-like biology by virtue of an ineffective homologous recombination DNA repair mechanism. Both olaparib and taloparib have been approved for patients whose cancers have developed in the context of germline *BRCA1/2* mutations. Both agents, given as a single agent, are as effective as standard chemotherapy, but in general, they are less toxic. Unfortunately, responses are relatively short lived. PARP inhibitors are now being investigated in combination with

chemotherapy and with immune checkpoint inhibitors. PARP inhibitors can cause mild nausea and occasional vomiting as well as fatigue.

Sacituzumab Govitecan (Table 79-1) An antibody-drug conjugate, sacituzumab govitecan, showed activity in a nonrandomized trial of patients with triple-negative metastatic breast cancer. Sacituzumab govitecan combines a humanized immunoglobulin G antibody targeted against TROP-2SN-38 with the active metabolite of irinotecan. In a single-arm, phase 2 trial, this agent elicited responses in one-third of such patients.

Immune Checkpoint Inhibitors (Table 79-1) These therapies permit immune effector cells to recognize and eliminate host cancer cells based on their recognition of neoantigen expression in tumor cells due to chromosomal instability and accumulated mutations. The excitement over immune checkpoint inhibitors has spread to metastatic breast cancer, especially of the triple-negative subtype. Atezolizumab in combination with nab-paclitaxel improves progression-free and perhaps overall survival, although exclusively against cancers with infiltrating immune cells that express PD-L1 (Table 79-1). The side effects of these agents can be life threatening, consisting of induction of inflammatory autoimmune responses in nearly every organ imaginable. These include thyroiditis, pneumonitis, myo- and pericarditis, esophagitis, gastritis and colitis, hepatitis, pancreatitis, hypophysitis, and dermatitis. The endocrine toxicities tend to be irreversible. Careful management should be handled by an experienced team.

Bone-Modifying Agents Bone-modifying agents, such as bisphosphonates or the anti-RANK antibody denosumab, are recommended for all patients with bone metastases. These agents substantially reduce the incidence of cancer-related skeletal events, such as bone pain, fracture, and hypercalcemia of malignancy. The bisphosphonates may cause myalgias and skeletal pain lasting a few hours to days after infusion. Both strategies have been associated with osteonecrosis of the jaw. The incidence of this complication is reduced by ensuring adequate dentition before treatment and by delivering treatment every 3 months, instead of monthly. The former has been shown to have equal efficacy.

BREAST CANCER IN PREGNANCY

As noted, breast cancer is unusual during pregnancy but does occur. Because of pregnancy-related physical breast changes, diagnosis is frequently delayed. Workup is the same as for non-pregnancy-related breast cancers, except radiographic staging should be limited or avoided, especially of the abdomen. Prognosis is similar, stage for stage, as that for age-matched women who are not pregnant. Pregnancy termination is usually not required. However, it is strongly advised that the patient be referred to a high-risk pregnancy program.

Remarkably, adjuvant chemotherapy including doxorubicin and cyclophosphamide can be safely given beyond the first trimester. Taxanes and the platinum salts may be administered safely. In contrast, anti-HER2 antibody therapies have resulted in unacceptable fetal malformations and pregnancy complications and should be avoided. Likewise, endocrine therapies should be delayed until after delivery. In general, a reasonable strategy is to deliver combination neoadjuvant chemotherapy with either concurrent or sequential single agents to permit sufficient embryogenesis followed by delivery and then breast primary therapy (surgery/radiation). Further adjuvant therapies, including additional chemotherapy and/or anti-HER2 and endocrine therapies can be delivered postoperatively. Breast feeding is discouraged, since these agents may cross into milk.

MALE BREAST CANCER

Breast cancer is ~1/150th as frequent in men as in women; ~2000 men developed breast cancer annually in the United States. Risk factors include inherited deleterious SNPs in *BRCA2*, as well as increased exposure to endogenous or exogenous estrogen. Men with Klinefelter's syndrome have two or more copies of the X chromosome and have higher levels of estrogen. Other conditions of hyperestrogenism, such as in hepatic failure and with exogenous

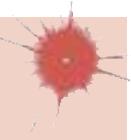
estrogen use in transgender situations, are also associated with higher risk of male breast cancers. However, the vast majority of men who present with breast cancer have none of these conditions.

Breast cancer usually presents in men as a unilateral lump in the breast and is frequently not diagnosed promptly. Given the small amount of soft tissue and the unexpected nature of the problem, locally advanced presentations are somewhat more common. Although gynecomastia may initially be unilateral or asymmetric, any unilateral mass in a man >40 years old should be biopsied. On the other hand, bilateral symmetric breast development rarely represents breast cancer and is almost invariably due to endocrine disease or a drug effect. Nevertheless, the risk of cancer is much greater in men with gynecomastia; in such men, gross asymmetry of the breasts should arouse suspicion of cancer.

Approximately 90% of male breast cancers contain ERs, and the disease behaves similarly to that in a postmenopausal woman. When matched to female breast cancer by age and stage, its overall prognosis is identical. Male breast cancer is best managed by mastectomy and axillary lymph node dissection or SLNB, although some men prefer breast-conserving therapy. Patients with locally advanced disease or positive nodes should also be treated with irradiation. No randomized studies have evaluated adjuvant therapy for male breast cancer, but extrapolation from treatment with women suggests it is indicated. If the cancer is ER positive, which is often the case, tamoxifen is usually the agent of choice. AIs are also effective in men. Anecdotal evidence supports use of gonadotropin-releasing hormones, such as leuprolide, in combination with an AI, since testosterone is a substrate for the aromatase enzyme. The sites of relapse and spectrum of response to chemotherapeutic drugs are virtually identical for breast cancers in either sex.

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Cancers of the upper gastrointestinal tract include malignancies of the esophagus, stomach, and small bowel. Esophageal, gastroesophageal junction, and gastric cancers are among the most common of human malignancies, with 1.5 million global new cases diagnosed in 2018. In the United States, a lower risk area, it is estimated that in 2020, esophageal cancer will be diagnosed in 18,440 people and cause 16,170 deaths; for gastric cancer, 27,600 new cases will be diagnosed and 11,010 deaths will occur. Small intestine cancers are rare.

ESOPHAGEAL CANCER

INCIDENCE AND CAUSATIVE FACTORS

Two distinct forms of cancer with different epidemiologies, causative factors, and genomic profiles arise within the esophagus: squamous cell cancers, which occur more frequent in the upper and mid esophagus; and adenocarcinomas, which are almost always located in the lower esophagus and at the gastroesophageal junction. The incidence of esophageal cancer varies up to 20-fold based on geographic distribution: it is relatively uncommon in North America, but has a high incidence in Asia (especially China), the Normandy coast of France, and Middle Eastern countries such as Iran. This marked global variation is likely due to different causative factors in the development of the malignancy, leading to two different cancer types within the same tissue: squamous cell cancers are more common in high-incidence areas, usually with lower Human Development Index (HDI) scores (a measure of economic development that includes standard of living, health, and education). Overall, approximately 572,000 new cases of esophageal cancer were diagnosed globally in 2018; esophageal cancer was the seventh most common cause of malignancy and the third most common cause of cancer-related mortality, with an estimated 508,000 deaths.

The clearest high-risk factors for the squamous cell cancer subtype in Western countries are alcohol and tobacco abuse; concurrent alcohol and tobacco abuse further increases the risk. Ingestion of extremely hot substances (such as tea in Iran and mate [maté] in South America) has been proposed as a risk factor; in India, chewing the areca (betel) nut increases the risk of esophageal squamous cell cancers. Less common risk factors include chronic achalasia, radiation therapy (such as is delivered for treatment of Hodgkin's lymphoma or breast cancer), lye ingestion, and Plummer-Vinson (Patterson-Kelly) syndrome (iron deficiency anemia, glossitis, cheilosis, and the development of esophageal webs) (**Table 80-1**). Adenocarcinoma of the lower esophagus and gastroesophageal junction has been the predominant histologic subtype in the United States and Western Europe for several decades, now making up >75% of all incident cases. Risk factors for adenocarcinoma (**Table 80-2**) include chronic reflux esophagitis leading to inflammation and the development of Barrett's esophagus (the finding of glandular gastric type mucosa extending into the esophagus). Although obesity increases the risk of reflux esophagitis, a substantial number of patients with newly diagnosed adenocarcinoma of the esophagus and gastroesophageal junction are younger and fit; Barrett's esophagus may still be found in these patients. In patients with adenocarcinoma of the lower esophagus in which Barrett's esophagus is not present, the disease may arise without Barrett's esophagus, or an extensive tumor found at diagnosis may obliterate previous areas of Barrett's. Genomic alterations may be identified even before the development of frank adenocarcinoma in patients with dysplasia associated with Barrett's esophagus. These include mutations of *TP53*, a gene critical in regulating uncontrolled cell division, and aneuploidy in dysplastic regions. Risk of progression of Barrett's esophagus to cancer is about 0.4–0.5% per year. Management of Barrett's esophagus is discussed in **Chap. 323**.

TABLE 80-1 Some Etiologic Factors Associated with Squamous Cell Cancer of the Esophagus

Excess alcohol consumption
Cigarette smoking
Other ingested carcinogens
Nitrates (converted to nitrites)
Smoked opiates
Fungal toxins in pickled vegetables
Mucosal damage from physical agents
Hot tea
Lye ingestion
Radiation-induced strictures
Chronic achalasia
Host susceptibility
Esophageal web with glossitis and iron deficiency (i.e., Plummer-Vinson or Paterson-Kelly syndrome)
Congenital hyperkeratosis and pitting of the palms and soles (i.e., tylosis palmaris et plantaris)
? Dietary deficiencies of selenium, molybdenum, zinc, and vitamin A

As opposed to other gastrointestinal malignancies, such as colorectal cancer, inherited cancer susceptibility genes are rarely associated with esophagus and gastroesophageal junction cancers. An exception is the rare inherited cancer susceptibility gene driving tylosis palmaris and plantaris; a mutation in the *RHBDF2* gene is associated with an increased risk for squamous cell cancers of the esophagus. Lynch syndrome modestly increases the risk of gastric and potentially gastroesophageal junction adenocarcinomas.

■ SCREENING AND SURVEILLANCE OF HIGHER RISK GROUPS

Because of its low incidence in North America and the absence of proven blood-based biomarker for esophageal cancer assays, screening of the asymptomatic general population using, e.g., upper endoscopy is not currently recommended in the United States. Periodic endoscopy is used for surveillance of higher risk patients, such as those with Barrett's esophagus and especially with dysplasia, based on expert opinion guidelines.

■ GENOMIC ALTERATIONS

Within a tissue, subtyping has revealed substantial genomic differences between adenocarcinomas and squamous cell cancers of the esophagus. An integrated analysis involving several different genomic platforms performed by The Cancer Genome Atlas (TCGA) Research Network investigators demonstrated that esophageal squamous cell cancers more closely resembled squamous cell carcinomas of other primary sites, such as the head and neck, than adenocarcinomas arising in the esophagus. Three molecular subclasses of squamous cell cancer were identified (of note, as opposed to squamous cell cancer of the head and neck, human papillomavirus was not identified in any of the three subgroups). Among other differences, the spectrum of genomic amplifications in squamous cell cancers are substantially different than that of adenocarcinomas. In adenocarcinomas, *ERBB2* (*HER2*) was frequently amplified, as were *VEGFA* and *GATA4/6*. The genomic profile for esophageal and gastroesophageal junction adenocarcinomas was very similar to the chromosomally unstable variant of gastric

adenocarcinoma, suggesting that proximal gastric and gastroesophageal junction tumors may have a similar driving factor (see below). Other studies comparing transcriptomes of adenocarcinomas and squamous cell cancers across tissues (i.e., the same tumor histology arising in different organs, such as squamous cell cancers and adenocarcinomas from the esophagus, lung, and uterine cervix) found that histologies among the different organs showed more similarity than between the different histologies within the same organ. In addition to implications regarding driving factors in the initiation and progression of cancer, these genomic alterations are important for therapeutic decisions involving systemic agents given in the neoadjuvant or postoperative adjuvant setting or for advanced metastatic disease. For esophageal cancer, genomic abnormalities that should be considered in prescribing drug-based therapy include analysis for *HER2* amplification, PD-L1 expression, and hypermutated tumors/microsatellite instability (see below).

CLINICAL FEATURES

■ PRESENTING SYMPTOMS

The most common symptoms leading to suspicion of esophageal cancer are dysphagia or odynophagia and, less frequently, hematemesis or melena. More subtle symptoms include anorexia and weight loss, and fatigue and shortness of breath if anemia from gastrointestinal bleeding is present. Because the symptoms of dysphagia or odynophagia are usually not perceived by the patient until substantial obstruction of the esophageal lumen has occurred, the large majority of patients with esophageal cancer are found with locally advanced if not metastatic disease. Patients with symptoms of dysphagia and/or odynophagia should undergo upper endoscopy (rather than a barium contrast study) to determine the presence or absence of malignancy; biopsy should be performed at the same setting to determine histology. Depending on the tumor stage, molecular diagnostic or next-generation sequencing (NGS) analysis to assist in determine potential therapies would be performed. These studies should be done on all patients with metastatic disease as it will guide therapy. NGS requires adequate tumor cellularity, which is sometimes difficult to achieve from endoscopic biopsy. Some high-volume U.S. centers routinely perform NGS on all specimens, including from the primary tumor for patients without metastatic disease.

■ STAGING

Therapeutic strategy is based on the stage of the disease using a system such as the eighth edition of the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging system. The T stage is based on the size of the tumor and depth of penetration through the esophageal wall (which for most of its course is not covered by serosa so that invasion through the muscle layer leads directly into periesophageal tissues) (Fig. 80-1). Patients with regional lymph node metastases are still potentially curable. Metastatic disease is generally treated with palliative intent with rare exceptions. Because neoadjuvant (preoperative) therapy is widely employed for esophageal cancer in an effort to improve subsequent surgical outcomes, the AJCC TNM staging system includes clinical, pathologic (for patients undergoing initial surgery as first treatment), and ypTNM staging assessment for those treated with preoperative therapy. See Table 80-3 for the TNM staging classification for gastric cancer, which is similar to esophageal cancer.

Determining tumor extent includes careful physical examination, which may reveal palpable lymphadenopathy or hepatomegaly; imaging studies including computed tomography (CT) and fluorodeoxyglucose (FDG) positron emission tomography (PET)/CT scan are used to assess for metastatic disease. If no metastatic disease is identified, endoscopic ultrasonography (EUS) is commonly performed to more definitively determine depth of penetration of the primary tumor (T) and regional lymph node involvement. For tumors of the mid and upper esophagus (5% of esophageal cancers are in the upper third of the esophagus, 20% in the middle third, and 75% in the lower third), bronchoscopy may be performed to rule out invasion of the tracheobronchial tree.

TABLE 80-2 Some Etiologic Factors Associated with Adenocarcinoma of the Esophagus

Chronic gastroesophageal reflux
Obesity
Barrett's esophagus
Male sex
Cigarette smoking

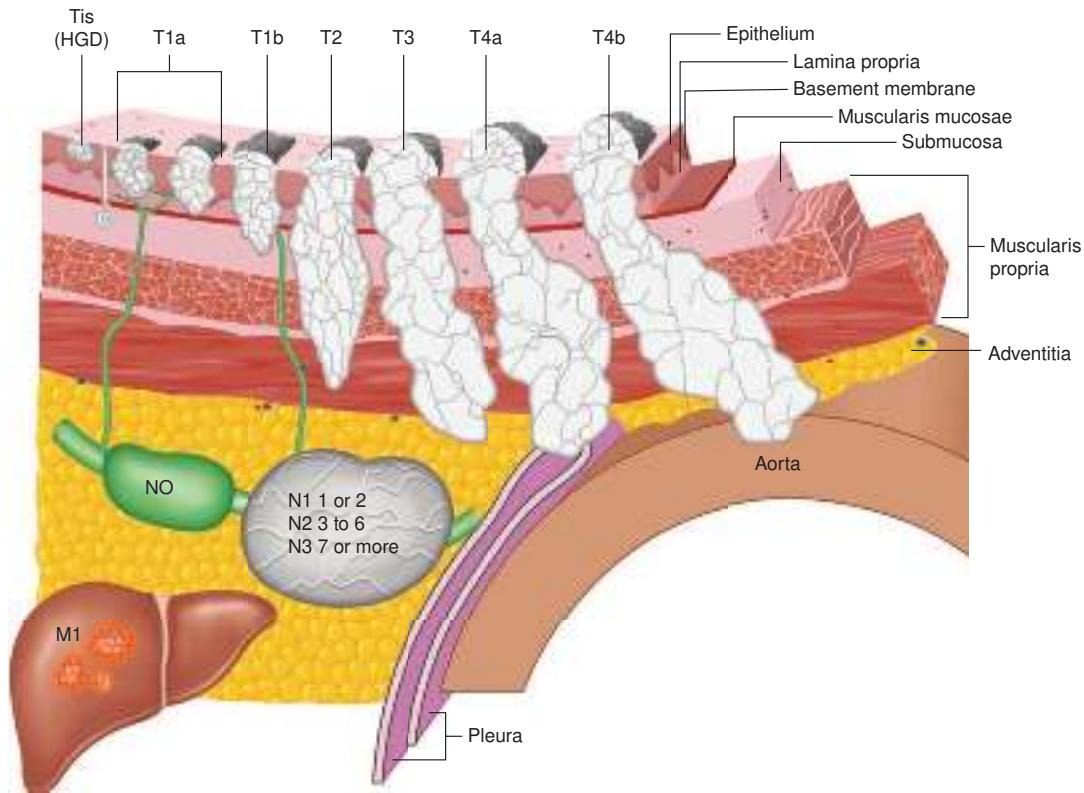


FIGURE 80-1 Patterns of spread of esophageal cancer and the basis for anatomic staging. HGD, high-grade dysplasia. (Reproduced with permissions from TW Rice et al: Cancer of the esophagus and esophagogastric junction: An eighth edition staging primer. *J Thorac Oncol* 12:36, 2017.)

TABLE 80-3 AJCC Prognostic Stage Groups for Esophageal Cancer Using cTNM (Pretreatment)

TNM	CLINICAL STAGE	PRESENTING AT THIS STAGE ^{a,b}	5-YEAR SURVIVAL RATE	
			SQUAMOUS	ADENOCARCINOMA
cTis, N0, M0	0	1.2%	75%	82%
cT1-2, N0, M0	I	17%	75%	78%
cT1-2, N1-3, M0	IIA	7%	53%	50%
cT3-4a, N0, M0	IIB	13%	40%	40%
cT3-4a, N1-3, M0 ^c	III	31%	25%	25%
cT4b, any N, M0	IVA		17%	21%
cAny T, any N, M1	IVB	5%	10%	18%

Survival by ypTNM Staging After Neoadjuvant Chemotherapy

TNM	yp STAGE	ESTIMATED 5-YEAR SURVIVAL RATE	
		SQUAMOUS	ADENOCARCINOMA
T1-2, N0, M0	I	46%	52%
T1, N1, M0			
T3, N0-1, M0	II	34%	38%
T2, N1-2 M0			
T1, N2-3, M0			
T4a, N0, M0			
T4a, N1-3 M0	III	22%	27%
T4b, any N, M0			
T3, N2-3, M0			
T2, N3, M0			
Any T, any N, M1	IV	10%	12%

^aSquamous cell and adenocarcinoma histologies combined. ^bSurgical series; underestimates incidence of M1 disease at presentation. ^cIncidence includes cT4b and cAnyN M0.

Sources: Adapted from TW Rice et al: CA Cancer J Clin 67:304, 2017; TW Rice et al: Dis Esophagus 29:707, 2016; and TW Rice et al: personal communication.

The finding of invasion of the trachea or bronchus rules out surgical intervention with curative intent. Regional lymph nodes may be biopsied under EUS guidance. If metastatic disease is suspected, biopsy to confirm tumor staging and to obtain adequate tissue for molecular and genomic alterations analysis should be performed. If systemic therapy is indicated as a portion of the treatment (for metastatic disease or for preoperative therapy for locally advanced cancers), serial FDG-PET/CT scans, using decrease in FDG avidity as a surrogate measure of effectiveness, are increasingly being used to guide whether the initial therapy should be continued or changed.

TREATMENT

Esophageal Cancer

Although the prognosis for patients with esophageal cancer (all stages) is still poor, a slow but steady improvement in 5-year survival has been noted. Because no effective early detection methods exist, the number of patients found to have very-early-stage cancers at the time of diagnosis has not markedly increased; the modest improvement in survival is probably a combination of somewhat improved systemic therapy as well as decreased operative morbidity and mortality when surgery is performed by high-volume surgeons at high-volume centers, as well as improvements in the delivery of external-beam radiation therapy.

For patients without evidence of metastatic disease, the goal of therapy is cure, usually by employing combined-modality therapies. Except for patients with early-stage esophageal cancer, which might be treated by surgery alone (or for very-early-stage lesions, by endomucosal resection alone), systemic drug therapy plus external-beam radiation therapy is a standard of care option for esophageal cancers. For selected patients with gastroesophageal cancers, systemic therapy alone may be given before definitive surgical resection. For patients with squamous cell cancers of the upper and mid esophagus, combined chemotherapy plus concurrent radiation therapy is a standard of care option, with surgery reserved for patients not achieving a complete radiographic and endoscopic response. Chemotherapy plus concurrent radiation was superior to radiation therapy alone in several clinical trials. Increasingly, all systemic therapy given with curative intent is given before operation, although if surgery is the initial therapy and the patient is found to have more locally advanced cancer at pathology (e.g., regional lymph node metastasis), postoperative systemic therapy is used in the adjuvant setting. Adjuvant chemotherapy is more frequently indicated in patients with adenocarcinoma than squamous cell cancers.

For patients with metastatic disease, the goal of therapy is symptom palliation and life extension. No randomized trials of supportive care only versus systemic therapy plus best supportive care have been reported in patients with esophageal cancers. For gastric cancer (a similar histology as distal esophageal and gastroesophageal junction tumors as discussed above), clinical trials performed in the 1980s and 1990s indicated a modest improvement in 1- and 2-year survival when systemic therapy was initiated versus best supportive care only. While the cytotoxic chemotherapy regimens used for palliation have not changed dramatically over the past 10 years, subgroups of patients have been identified who benefit from therapies targeting specific genomic alterations. Approximately 20–25% of patients with adenocarcinoma of the esophagus or gastroesophageal junction are found to have amplified or overexpressed *HER2*; trastuzumab plus chemotherapy results in higher response rates and longer progression-free and overall survival compared to chemotherapy alone. Immune modulation therapy using PD-1 inhibitors is second-line palliative therapy for patients who have esophageal cancers expressing PD-L1 or having hypermutated or microsatellite-unstable genotype. Molecular diagnostic or genomic alteration analysis assays to identify these biomarkers should be performed routinely in patients with metastatic esophageal cancer to help guide therapy.

Supportive measures to improve nutrition and quality of life include placement of an endoluminal stent in the setting of high-grade obstruction; use of enteral nutrition can also be performed using a percutaneous gastrostomy. Photodynamic therapy and endoscopic laser therapy have been used to treat endoluminal obstruction.

TUMORS OF THE STOMACH

■ ADENOCARCINOMA OF THE STOMACH

Incidence and Causative Factors A century ago, gastric adenocarcinomas were among the most common of malignancies in the United States. Since the 1920s, the incidence of gastric cancer has steadily decreased; while the reason for this has not been definitively identified, it coincided with widespread use of refrigeration and a decreased need for food preservatives. In 2020, it is estimated that there will be 27,600 new cases of gastric cancer diagnosed in the United States; while now seen much less frequently, it remains a lethal disease, with 11,010 deaths. Globally, gastric cancer is still very common, with an overall global incidence of 1.03 million new cases per year and 780,000 deaths, making gastric cancer the third most common cause of cancer mortality. High-incidence areas, as is the case for esophageal cancers, include large Asian countries such as China, Korea, and Japan; South American countries such as Chile; and Eastern European countries.

While the number of new cases of body and distal gastric cancers has decreased in Western, high-HDI countries, the incidence of adenocarcinomas of the gastroesophageal junction has markedly increased in the same areas over the past several decades. The ingestion of high concentrations of nitrates found in dried, smoked, and salted foods may be a contributing factor. Bacteria such as *Helicobacter pylori* and ingestion of partially decayed bacterially contaminated food may lead to the generation of carcinogenic nitrites from nitrates (Table 80-4). A causative factor is suspected to be chronic inflammation due to reflux of gastric contents into the esophagus, particularly in obese people. Obesity alone is not the cause, as a substantial number of these patients are fit and not overweight. Early-onset gastric cancers (gastric cancer occurring in patients under the age of 50), primarily proximal or gastroesophageal junction cancers, have also increased. A second cause of chronic inflammation, *H. pylori* infection, is a known driver in many cases of gastric cancer. While *H. pylori* is extremely common, occurring in approximately half of all humans, gastric cancer occurs in only a small subset of those infected. Higher cancer risk has been associated with certain strains of *H. pylori*; a specific human genetic predisposition has not yet been identified. Supportive evidence that *H. pylori* infection is a causative factor in the development of gastric cancer includes prospective studies demonstrating that treatment of *H. pylori* infection decreases the overall risk of gastric cancer. For example, patients with *H. pylori* infection who had at least one first-degree relative with a history of gastric cancer (increasing their own risk of stomach cancer) were randomly assigned to placebo or treatment for *H. pylori*. The group receiving *H. pylori* eradication showed a significant decrease in the incidence of gastric cancer (especially for

TABLE 80-4 Nitrate-Converting Bacteria as a Factor in the Causation of Gastric Carcinoma^a

Exogenous sources of nitrate-converting bacteria:

Bacterially contaminated food (common in lower socioeconomic classes, who have a higher incidence of the disease; diminished by improved food preservation and refrigeration)

Helicobacter pylori infection

Endogenous factors favoring growth of nitrate-converting bacteria in the stomach:

Decreased gastric acidity

Prior gastric surgery (antrectomy) (15- to 20-year latency period)

Atrophic gastritis and/or pernicious anemia

? Prolonged exposure to histamine H₂-receptor antagonists

^aHypothesis: Dietary nitrates are converted to carcinogenic nitrites by bacteria.

those in whom *H. pylori* was successfully eradicated) compared to the control group. Earlier studies had demonstrated that treatment of *H. pylori* in Korean patients who had a prior very-early-stage gastric cancer decreased the incidence of a second gastric cancer. These data suggest that treatment of asymptomatic *H. pylori* gastric infection should be considered for patients who have a first-degree relative who has had gastric cancer or who themselves have a prior history of an early-stage gastric cancer.

In addition to chronic inflammatory conditions, inherited cancer susceptibility genes increase the risk of gastric cancer. These include mutations of *CDH1*, which encodes for the cell cohesion gene e-cadherin; germline *CDH1* mutations markedly increase the risk for the diffuse cell (signet cell) gastric cancer subtype (see below for discussion of histologic subtypes). Patients with an inherited deleterious *CDH1* mutation are considered for prophylactic gastrectomy. *CDH1* mutations also increase the risk for lobular breast cancer. Germline mutations in the mismatch repair pathway (Lynch syndrome) slightly increase the risk for gastric cancer. Other inherited cancer susceptibility genetic syndromes that increase the risk of gastric cancer include familial adenomatous polyposis, juvenile polyposis, and Peutz-Jeghers syndrome. Inherited cancer susceptibility genes such as *BRCA* mutations may not significantly increase risk for gastric cancer. Surveillance programs for the higher risk germline cancer susceptibility genes should be employed.

Gastric cancer stem cells originating in the bone marrow may play an important role in the development of gastric cancer. *H. pylori* may be an inciting factor for recruitment of such bone marrow gastric stem cells. If this hypothesis is confirmed, it may have important implications for therapy of gastric cancers.

Clinical Features • SURVEILLANCE STRATEGIES As is the case for esophageal cancer, the overwhelming majority of Western patients with gastric cancer are symptomatic at the time of diagnosis. Early detection programs, in Japan and Korea, where gastric cancer has been among the most common of malignancies (although its incidence has been decreasing), include upper endoscopy and, in Japan, upper endoscopy and serum pepsinogen; these programs have increased the number of patients found with early gastric cancer and decreased mortality rates. This strategy has not been cost effective in populations in which the incidence of gastric cancer is much lower, such as in the United States. In high-incidence areas, treatment of symptomatic *H. pylori* is a preventive measure. Exceptions to routine surveillance in the United States are asymptomatic patients with *CDH1* (and other cancer susceptibility gene) mutations who may be part of early detection programs and in whom prophylactic gastrectomy is a management option.

PRESENTING SYMPTOMS Presenting symptoms include vague upper abdominal discomfort, hematemesis or melena, anorexia and early satiety, and unexplained weight loss. For patients with esophagogastric junction cancers, dysphagia or odynophagia may be the presenting symptom. Anemia may be found due to occult bleeding. These symptoms and signs lead to upper (and if site of bleeding is uncertain, lower) endoscopy and biopsy (endoscopy has long replaced barium contrast radiography as an initial diagnostic step). Occasionally, imaging using CT performed to evaluate abdominal symptoms may identify gastric thickening or a gastric mass leading to upper endoscopy. Physical examination can reveal left supraclavicular adenopathy (Virchow's node), a perumbilical mass (Sister

Mary Joseph nodule), a pelvic mass on rectal exam (Blumer's shelf), ascites, or an ovarian mass (Krukenberg tumor). More commonly, physical examination is unrevealing.

Upper endoscopy may reveal an ulcer or ulcerated mass, biopsy of which shows adenocarcinoma. For the diffuse subtype of gastric cancer, a mass or ulceration may not be seen, but rather, thickened gastric rugae may be noted. Initial biopsy may not reveal diffuse gastric cancer, which may track below the mucosal surface. In these patients, EUS may guide biopsy.

Histopathology Classification of Primary Gastric Adenocarcinomas The large majority (~85%) of gastric malignancies are adenocarcinomas or subtypes of adenocarcinoma. Other malignancies, discussed below, include neuroendocrine tumors (carcinoid tumors), primary gastric lymphomas, gastrointestinal stromal tumors (GISTs), and other rare malignancies. Using the Lauren classification, pathologists classify adenocarcinomas on the basis of histopathology as intestinal (more common) or diffuse subtype (~20%). As noted above, the diffuse subtype is associated with inherited *CDH1* mutations; in addition, in the TCGA genomic analysis of gastric cancer, approximately a third of diffuse subtype cases had somatic *CDH1* mutations. The intestinal subtype is associated with *H. pylori* infection and atopic gastritis. Histologic grade also influences the clinical course.

Genomic analysis performed by several groups has resulted in molecular classifications of gastric cancer that may, in the future, inform staging systems, provide a better understanding of the driving factors in the development of gastric cancer, and provide important information on treatment options (Fig. 80-2). For example, the TCGA group reported the results of a multiplatform analysis of 295 patients with previously untreated gastric cancer; both Western and Asian patients were included in the analysis. Four subtypes of gastric cancer were identified: high Epstein-Barr virus burden, microsatellite unstable with hypermutation, genetically stable (associated with the diffuse subtype), and chromosomal unstable. The Asian Cancer Research Group (ACRG), studying primary tumors from 300 Korean patients, analyzed gene expression profiles and found four subtypes: mesenchymal, microsatellite unstable, microsatellite stable with *TP53* expressed, and microsatellite unstable with *TP53* mutated. Clinical outcome was correlated with genomic subtype in both studies, with microsatellite

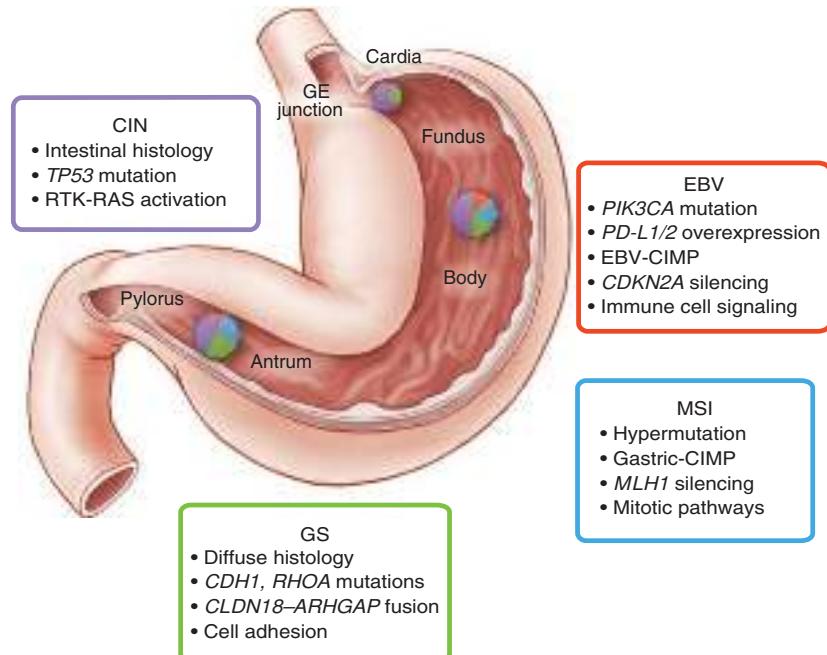


FIGURE 80-2 Molecular/genomic characterization of subtypes of gastric carcinomas. CIMP, CpG-island methylator phenotype; CIN, chromosomally unstable; EBV, Epstein-Barr virus-associated; GE, gastroesophageal; GS, genetically stable; MSI, microsatellite instability-associated.

unstable tumors having the best outcome and genetically stable (TCGA) and mesenchymal (ACRG) types the worst.

In addition to histopathology, molecular diagnostics (including NGS) are an important part of the pathology workup. The molecular subtypes have therapeutic implications; for example, ~20% of gastric cancer or gastroesophageal junction cancer patients' tumors have overexpression or amplification of *HER2*, which would lead to the addition of agents such as trastuzumab as part of systemic treatment for metastatic disease. Immune modulation therapy may be used in patients with hypermutated tumors, found by NGS or by polymerase chain reaction (PCR) for microsatellite instability (MSI). An evaluation for overexpression or amplification of *HER2*, quantification of PD-L1 by immunohistochemistry, and assessment of MSI by PCR or deficient mismatch repair protein (dMMRP) expression should be a routine part of the pathology workup of patients with metastatic gastric cancer.

More controversial is whether these assays should also be routinely performed in patients with potentially operable gastric cancer because the addition of trastuzumab to neoadjuvant chemotherapy has not yet been shown to change outcome. In large-volume centers, NGS is routinely performed on pretreatment biopsies. Currently, the finding on pathologic assays of positive tumor Epstein-Barr virus (identified in 8–10% of gastric cancer patients) does not change therapeutic options.

Staging Once a diagnosis of a primary gastric adenocarcinoma is made, algorithms for clinical evaluation of stage include physical examination and imaging studies (Fig. 80-3; Table 80-5). Tumor-related biomarkers such as carcinoembryonic antigen (CEA) or CA19-9 may be elevated but are nonspecific (may be elevated in a number of other gastrointestinal and other site cancers). Diagnostic CT scan of the chest, abdomen, and pelvis should be performed. If metastatic

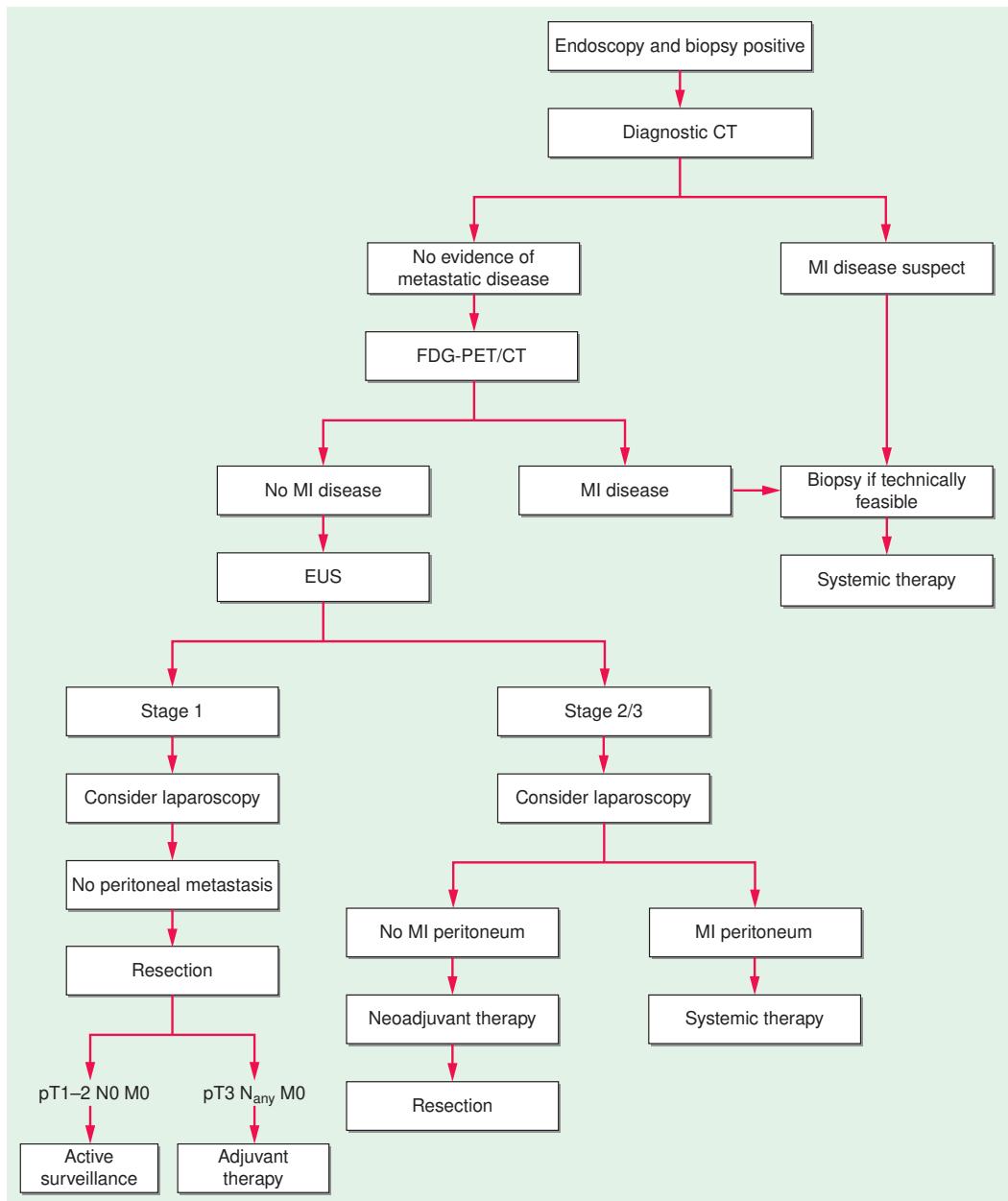


FIGURE 80-3 Staging for gastric adenocarcinoma. CT, computed tomography; EUS, endoscopic ultrasound; FDG-PET, fluorodeoxyglucose positron emission tomography.

TABLE 80-5 Staging System for Gastric Carcinoma

STAGE	TNM	FEATURES	DATA FROM ACS IN THE UNITED STATES	
			NO. OF CASES, %	5-YEAR SURVIVAL, %
0	TisN0M0	Node negative; limited to mucosa	1	90
IA	T1N0M0	Node negative; invasion of lamina propria or submucosa	7	59
IB	T2N0M0 T1N1M0	Node negative; invasion of muscularis propria	10	44
II	T1N2M0 T2N1M0	Node positive; invasion beyond mucosa but within wall <i>or</i> Node negative; extension through wall	17	29
	T3N0M0			
IIIA	T2N2M0 T3N1-2M0	Node positive; invasion of muscularis propria or through wall	21	15
IIIB	T4N0-1M0	Node negative; adherence to surrounding tissue	14	9
IIIC	T4N2-3M0	>3 nodes positive; invasion of serosa or adjacent structures		
	T3N3M0	7 or more positive nodes; penetrates wall without invading serosa or adjacent structures		
IV	T4N2M0 T1-4N0-2M1	Node positive; adherence to surrounding tissue <i>or</i> Distant metastases	30	3

Abbreviations: ACS, American Cancer Society; TNM, tumor-node-metastasis.

disease is suspected on imaging, a biopsy of a metastatic site should be strongly considered to confirm stage IV disease, which changes the goals of care from potentially curative to palliative treatment. As is the case for esophageal cancer, FDG-PET/CT, which is more sensitive than diagnostic CT scan in identifying sites of metastatic disease, should be performed if the anatomic CT is negative for metastatic disease. Note, however, that FDG-PET may be noninformative (the primary tumor may not be FDG-avid, particularly in diffuse-type gastric cancer). If imaging does not reveal metastatic disease, EUS should be strongly considered to determine depth of penetration of the primary tumor and the presence or absence of regional lymphadenopathy suspicious for metastasis. Lymph node biopsy and, on occasion, biopsy of left hepatic parenchymal lesions found on EUS can be performed at the same setting. Endoscopic biopsies usually provide enough tumor tissue for molecular diagnostic pathology testing for HER2, MSI/MMRP, and PD-L1 assessment; it may not provide enough tissue for genomic alteration analysis. If neoadjuvant therapy is planned, laparoscopy should be considered to allow evaluation of the peritoneal cavity, with peritoneal washing for cytology if no peritoneal metastases are visible. The peritoneal cavity is a common site of metastases, especially from diffuse-type gastric cancer. The finding of peritoneal involvement either visibly or by positive cytology is staged as metastatic disease and diminishes the chance for curative resection.

The staging classification for gastric cancer is summarized in Table 80-5. Three staging classifications are provided: cTNM clinical staging (before any therapy has been given), pTNM pathologic staging (for patients not undergoing preoperative therapy), and a post-neoadjuvant therapy classification staging (ypTNM). The three components take

into account current standard of care options for therapy in which the AJCC prognostic stage groups from clinical staging guide therapeutic decisions. For example, after clinical evaluation, a large percentage of newly diagnosed patients will be found to have higher-stage primary cancers (penetrating through the gastric wall [T3 or T4] or lymph node-positive tumors), in which case perioperative (neoadjuvant) systemic therapy may be chosen. Pathologic examination of the resected specimen for prognostic stage classification must take into account exposure to preoperative therapies that may lead to downstaging (thus, ypTNM staging). Nomograms have been developed for predicting outcome in patients undergoing surgery as initial treatment.

TREATMENT

Gastric Cancer

POTENTIALLY CURABLE GASTRIC CANCER: SURGERY

Surgical removal of the primary tumor with negative microscopic margins (an R0 resection) and with resection of regional lymph nodes is currently the only curative therapy; with surgery alone, 5-year survival rates are approximately 25%. If tumor cells are found at the margin of resection (R1) or if visible cancer is left at the time of surgical removal of the primary tumor (R2), surgery is palliative rather than curative. For patients with early-stage tumors (mostly clinical stage I), surgery without perioperative systemic therapy may be performed. For patients with more locally advanced tumors (clinical stages II, IIIB, III), who compose approximately 70% of newly diagnosed operable patients, multimodality therapy (surgery and systemic chemotherapy) improves overall survival. Both neoadjuvant (preoperative) and postoperative systemic therapy are accepted approaches. If staging studies demonstrate a locally advanced cancer (T3/4 or node positive), preoperative treatment is recommended in a medically fit patient. If surgery is performed first and a locally advanced cancer is found, postoperative chemotherapy or chemotherapy plus chemoradiation is recommended. For selected very-early-stage gastric cancers (primary tumors that are \leq cm in diameter, are well to moderately differentiated, do not invade the deep submucosa, and do not show lymphovascular invasion or lymph node metastasis), which are not commonly found in the United States, endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) may be performed by experienced gastroenterologists in place of surgical resection, with favorable results in studies in high-incidence areas such as Japan.

For patients in whom the primary tumor is in the distal stomach, a subtotal gastrectomy is the preferred surgical procedure. For tumors of the proximal stomach, the options for resection include total gastrectomy or, alternatively, proximal gastrectomy. Esophagogastrectomy is performed for tumors involving the gastroesophageal junction. In selected patients, a jejunostomy feeding tube may be placed if postoperative radiation therapy is part of the treatment plan.

As noted above, laparoscopy is commonly performed at high-volume centers before a final decision regarding the role of surgery. If staging has already demonstrated clinically suspicious lymph nodes or an advanced T stage tumor, but laparoscopy does not demonstrate peritoneal metastasis, perioperative chemotherapy is given before surgical resection.

Palliative resection of the primary tumor is usually performed only if symptoms such as uncontrollable bleeding or obstruction are present that cannot be relieved by other means.

As is the case for colorectal cancer, a correlation exists between the number of lymph nodes removed and sampled and outcome. Sentinel lymph node biopsy is not performed in gastric cancer outside of a research study setting. The goal is to examine at least 15 lymph nodes from the resected specimen; it is more controversial whether more extensive lymph node resection itself affects outcome; the extent of lymphadenopathy can be classified using a D0-D3 system with a higher number meaning more extensive lymphadenopathy. In the United States, a modified D2 (D1+)

resection preserving the spleen and avoiding pancreatectomy is recommended but should be performed by experienced surgeons at high-volume centers. Japanese investigators and others have used very extensive lymph node dissections, but studies have not demonstrated an advantage for a D3 resection. Both resection of the primary tumor and its regional lymph nodes can be performed laparoscopically in appropriate patients.

In the hands of experienced surgeons, operative mortality would be anticipated to be $\leq 2\%$.

NEOADJUVANT AND POSTOPERATIVE ADJUVANT THERAPY FOR RESECTABLE GASTRIC CANCER

The large majority of potentially resectable Western gastric cancer patients have locally advanced tumors (cTNM stage IIA/B or III). Multimodality therapy using systemic chemotherapy improves 5-year survival rates by 10–15% compared to surgery alone. A widely cited study, the MAGIC clinical trial, randomly assigned patients with potentially resectable disease to receive perioperative chemotherapy or to proceed directly to surgery. Five-year overall survival for patients undergoing surgery alone was 23%; for those receiving pre- and postoperative chemotherapy, it was 36%. On the basis of this and other clinical trials, for most medically fit patients with stage cTNM II and III resectable gastric cancers, preoperative systemic chemotherapy followed by resection and, if tolerable, postoperative chemotherapy is a standard of care. Chemoradiation as given for esophageal cancers is usually used for gastroesophageal junction tumors. Preoperative chemoradiation or preoperative chemotherapy followed by chemoradiation for gastric cancer, as opposed to esophageal or gastroesophageal junction cancers, has been studied but is still an investigational approach. For patients being treated with multimodality therapy, close interactions among the surgeon, medical oncologist, and radiation oncologist are essential.

Clinical trials have compared different cytotoxic chemotherapy regimens, most of which include a platinum compound—either cisplatin or oxaliplatin. Currently, a platinum compound plus a fluorinated pyrimidine, such as fluorouracil or capecitabine, given for three to four cycles before surgery is a standard of care option. Drug combinations are favored; an example is the FOLFOX regimen, which includes fluorouracil, leucovorin, and oxaliplatin. For very fit patients, a combination of fluorouracil, oxaliplatin, and docetaxel (FLOT) may be chosen. Addition of trastuzumab to chemotherapy has not improved outcomes for patients with HER2-positive cancers. Careful monitoring of chemotherapy-related toxicities with appropriate dose modifications is important. Several clinical trials have included both preoperative and postoperative systemic therapy; a substantial number of patients will have a slow postoperative recovery and not receive postoperative treatment. Maximizing the ability to give preoperative chemotherapy is an important consideration. For patients receiving preoperative systemic chemotherapy and undergoing a D2/D1+ dissection, postoperative chemoradiation therapy has not improved outcome.

For patients in whom the primary tumor has been resected and who did not receive preoperative chemotherapy, who are found to have stage II or III cancers, or who have <15 lymph nodes found in the resected specimen, postoperative chemoradiation is a treatment option. Chemoradiation therapy may also be given for unresectable cancers in selected patients.

PALLIATIVE THERAPY FOR INCURABLE GASTRIC CANCER

Patients with clinical stage IV gastric cancers with an adequate performance status should be offered systemic drug therapies. Small clinical trials performed in the 1980s and 1990s showed a survival benefit for systemic therapy compared to best supportive care only. The cytotoxic chemotherapy regimens most commonly employed are still based on a platinum compound and a fluorinated pyrimidine (e.g., FOLFOX, as is used in the perioperative setting). However, two subgroups of gastric cancer patients have been identified who benefit from the addition of noncytotoxic

agents. Those whose tumors have overexpressed or amplified *HER2* should receive *HER2*-targeted agents such as trastuzumab plus cytotoxic chemotherapy because a modest survival advantage has been demonstrated. Additional *HER2*-targeted therapy using trastuzumab-deruxtecan, a monoclonal antibody-drug conjugate, has encouraging activity in patients whose tumors were refractory to trastuzumab. For patients with MSI/dMMRP gastric cancers, PD-1 inhibitors such as pembrolizumab should be used (currently approved in the second-line setting). Immune modulation therapy using PD-1 inhibitors such as pembrolizumab or nivolumab is also approved in gastric cancer with tumor specimens having $\geq 1\%$ PD-L1 expression, with modest response rates. The development of more effective immune therapies and their combination with cytotoxic chemotherapy (and in combination with trastuzumab plus chemotherapy in *HER2*-positive patients) as initial treatment are areas of active investigation.

When disease progresses after first-line treatment, other therapies include the combination of a VEGF receptor-targeted agent, ramucirumab, either alone or in combination with paclitaxel. As noted above, immune modulation therapy may cause remissions in patients whose tumors have at least 1% PD-L1 expression. PD-1 inhibitors are the preferred option for patients whose tumors are microsatellite unstable. Several other cytotoxic agents have activity in the palliative setting including irinotecan and trifluridine-tipiracil. Best results from clinical trials indicate overall survival for treated patients with stage IV disease is still only 12–15 months.

Radiation therapy using shorter regimens may be employed to palliate bleeding. For patients with advanced incurable disease, other supportive measures include placement of a duodenal stent to relieve gastric outlet obstruction; in selected patients, surgical procedures for gastric outlet obstruction may be performed. Radiation therapy might be used if not previously given. Enteral feeding using a jejunostomy tube may support nutritional needs.

GASTRIC LYMPHOMAS

Lymphomas of the stomach are an uncommon (~3%) but important subgroup of gastric malignancies. They are extranodal non-Hodgkin's lymphomas (NHL). The gastrointestinal tract is the most common site for extranodal NHL, and the stomach is the most common site within the gastrointestinal tract. The presenting symptoms are similar to those of the much more common adenocarcinoma of the stomach described above, including pain, anorexia, and bleeding. Symptoms of fever and night sweats occur in 10–15% of patients with gastric NHL. Because the treatment options are so different, obtaining adequate tissue for definitive pathologic examination is crucial in diagnosing gastric lymphomas. On occasion, this may be challenging because, similar to diffuse subtype adenocarcinoma, lymphomas may track below the mucosal surface. Multiple deep biopsies and mucosal resection may be needed to provide enough tissue for definitive pathologic assessment.

Potential driving forces in the development of gastric lymphomas include active or prior *H. pylori* infection, which is associated with mucosa-associated lymphoid tissue (MALT) subtype gastric lymphomas. MALT lymphomas may develop in nearly any organ, but the stomach is the most frequent primary site, accounting for ~35% of all MALT lymphomas. Antibacterial therapy directed against *H. pylori* can be a highly effective treatment in these patients. Other forms of NHL may involve the stomach either as primary gastric lymphoma or as a secondary site of disease, including both B-cell (e.g., mantle cell lymphoma, Burkitt's lymphoma, and follicular lymphoma) and T-cell lymphomas (e.g., enteropathy-associated T-cell lymphoma, anaplastic large-cell lymphoma, and peripheral T-cell lymphoma).

Staging is performed in a fashion similar to gastric adenocarcinoma, but the staging classification is different (see below). In addition to a contrast-enhanced diagnostic-quality CT scan of the chest, abdomen, and pelvis, an FDG-PET/CT scan may be helpful. EUS may be used to determine depth of invasion in patients in whom no evidence of metastatic disease is noted. Examination of the peripheral blood and bone marrow aspirate should be considered as part of the workup.

In all patients with gastric lymphoma, *H. pylori* infection status should be evaluated. If *H. pylori* testing is negative by histopathology, noninvasive testing by either stool antigen test or urea breath test should be used. If rituximab will be part of the treatment plan (see below), hepatitis B testing should be performed.

■ STAGING

The TNM staging system is not employed for gastric lymphomas. The Lugano staging system for gastrointestinal lymphomas (a modification of the Ann Arbor staging system) divides patient groups into stages I, II, and IV. Stage I tumors are limited to the gastric wall; stage II tumors have regional lymph node involvement or invasion of local structures; stage IV tumors have either more extensive lymph node involvement or have distant metastasis, including to the bone marrow or other extranodal sites.

■ PATHOLOGIC CLASSIFICATION

The two most common histologic subtypes of gastric lymphoma are marginal zone B-cell lymphomas (gastric marginal zone B-cell lymphomas or MALT; ~40% of newly diagnosed patients) and diffuse large B-cell lymphomas (DLBCLs; ~55%). The distinction is critical because therapeutic options are different. As part of the pathologic evaluation, for patients with *H. pylori*-positive gastric lymphomas, the finding of a t(11;18) translocation identifies a subgroup less likely to respond to *H. pylori* eradication. This translocation may be detected using PCR or fluorescent in situ hybridization (FISH); it creates a chimeric protein composed of the amino terminal of *APII* (apoptosis inhibitor) and the carboxy terminus of *MALT1*, leading to activation of nuclear factor- κ B signaling.

TREATMENT

Gastric Lymphoma

Unlike adenocarcinoma of the stomach, surgical resection has no role in the treatment of primary gastric lymphoma in the absence of complications of therapy such as perforation or uncontrollable bleeding. Resection of gastric lymphoma does not improve outcome.

For patients with MALT lymphoma, eradication of *H. pylori* with antibiotics is highly effective therapy. If tests for *H. pylori* are positive and t(11;18) translocation assay is negative, one of the currently accepted antibiotic regimens for treating *H. pylori* should be the initial therapy. *H. pylori* eradication is associated with high response rates including complete remissions in the majority of patients. The time to remission may be prolonged (in some studies averaging 15–16 months); therefore, careful monitoring is important before determining that a MALT tumor is not responding to anti-*H. pylori* therapy. For patients in whom the t(11;18) translocation assay is positive, options for therapy include anti-*H. pylori* antibiotic therapy plus involved-field radiation therapy or, if radiation is contraindicated, the use of single-agent rituximab, a monoclonal antibody targeting CD20. For patients who are *H. pylori* negative, moderate-dose (24–30 Gy) involved-site radiation therapy or single-agent rituximab is a treatment option. For selected, more advanced, stage IV MALT patients who have not responded to or who have progressed after receiving anti-*H. pylori* antibiotic therapy and/or rituximab, cytotoxic chemotherapy regimens such as R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) or rituximab and lenalidomide may be considered.

DLBL may be a result of transformation from more indolent MALT lymphoma or may arise de novo. De novo tumors are more likely to be BCL2 and CD10 positive. MALT lymphomas that have transformed to DLBCLs are more frequently BCL2 and CD10 negative.

For patients with DLBCL, earlier stage tumors may be treated by combination chemotherapy alone or chemotherapy plus involved-field radiation therapy. For more advanced gastric DLBCL tumors, chemotherapy using R-CHOP or R-EPOCH (rituximab, etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin)

regimens is standard of care. Some reports have suggested that eradication of *H. pylori* is effective treatment for early-stage DLBCL when the patient also has *H. pylori*.

UNCOMMON TUMORS OF THE ESOPHAGUS AND STOMACH

■ NEUROENDOCRINE TUMORS

Neuroendocrine tumors (NETs) of the esophagus are rare, accounting for <1% of gastrointestinal NETs. They may present with dysphagia and odynophagia similar to that of more common squamous cell or adenocarcinoma of the distal esophagus and gastroesophageal junction or with more nonspecific symptoms such as substernal discomfort or burning consistent with reflux esophagitis. A potential driving factor of higher grade NET is smoking. The initial diagnostic evaluation includes upper endoscopy and biopsy. Pathology may reveal a well-differentiated grade 1 or grade 2 NET with a low metastatic potential; at the other end of the spectrum are small-cell or large-cell NETs, which are fully malignant and frequently metastasize. EUS to assess depth of penetration and presence or absence of regional lymph node metastasis is usually performed. Imaging studies include CT or FDG-PET/CT scan to assess for metastatic disease, particularly in higher grade tumors. For lower grade tumors, somatostatin analog imaging studies such as gallium-68 DOTATATE may be performed if metastatic disease is suspected. For lower grade tumors, endoscopic resection including EMR or ESD may be performed. Small-cell or large-cell NETs that are not metastatic are usually treated with chemotherapy plus external-beam radiation therapy using chemotherapy regimens similar to those employed for small- and large-cell neuroendocrine cancers of the lung (Chap. 84). Systemic therapy for metastatic small- and large-cell esophagogastric NETs is also modeled on therapy for small- and large-cell thoracic NETs.

Gastric NETs (also called gastric carcinoid tumors) represent 7–9% of gastrointestinal NETs but <1% of gastric neoplasms. They are divided into three types (Chap. 84). For all gastric NETs, initial evaluation includes upper endoscopy and biopsy. EUS may be helpful in assessing depth of invasion for larger tumors and for assessing regional lymph node metastases in type 3 tumors. Somatostatin analog imaging using gallium-68 DOTATATE PET/CT scanning may be performed if metastatic disease is suspected. The finding of unresectable metastatic disease that is gallium-68 DOTATATE avid not only provides staging information but also guides potential therapy using somatostatin receptor-targeted therapy.

TREATMENT

Gastric Neuroendocrine Tumors

Type 1 tumors are usually treated endoscopically with polypectomy or endomucosal resection. For larger tumors (>2 cm) or tumors invading through the muscularis or to regional lymph nodes, surgical resection is recommended. Type 2 tumors have a higher risk for regional lymph node metastasis and are usually treated surgically, although selected patients may have a combination of endoscopic resection and limited surgical resection. Type 3 tumors are not associated with elevated gastrin levels and have a higher propensity for regional lymph node metastasis and distant metastasis. Surgery is the treatment of choice for localized type 3 tumors, although EMR has been used in selected patients. Adenocarcinoma of the stomach may be found in 5–10% of type 3 tumors.

■ GASTROINTESTINAL STROMAL TUMORS

Gastrointestinal stromal tumors (GISTs) are rare tumors of the gastrointestinal tract associated with somatic mutations in the *cKIT* (the majority) or *PDGFRA* genes, which are both driver mutations and targets for systemic therapy for metastatic disease; in a minority of cases, neither gene is mutated. GISTs arise from Cajal cells, which bridge between the autonomic nerves to the muscle layer of the bowel. The stomach is the most frequent primary site (~50%), followed by

the small bowel in about a third of cases. As endoscopy for other indications has become more widely used, otherwise asymptomatic and probably clinically insignificant small GISTs have been identified more frequently; it is not clear that the actual incidence has substantially increased. Symptoms associated with GISTs include acute gastrointestinal bleeding leading to melena and/or hematemesis. Anemia may be reflected in generalized weakness. With larger tumors, abdominal distention and pain may be presenting symptoms. At endoscopy, a non-specific smooth bulging mass covered by normal mucosa is the most frequent finding. Initial biopsy may not reveal an epithelial neoplasm. EUS both to assess the extent of the neoplasm and to guide biopsy in order to obtain adequate tissue for histology and molecular pathology may be helpful.

Histologically, a spindle cell neoplasm is the most common subtype (~70%), with epithelioid cells making up 20%; 10% of cases are mixed histology. Immunohistochemical stains for the presence of c-kit or CD34 positivity and mutational analysis of *cKIT* and *PDGFRA* should be performed in all patients. These help to distinguish between GISTs and leiomyoma neoplasms. For nonmetastatic primary GISTs, laparoscopic surgical resection, if feasible, is the treatment of choice; because lymph node metastases are unusual, wedge or segmental resection is preferred. Endoscopic resection has been used in selected cases. Neither histology nor the presence of a *cKIT* or *PDGFRA* mutation distinguishes GISTs with clinically malignant phenotype from those that have a benign course. Higher risk tumors (larger size, higher mitotic index) may present with or develop metastatic or locally unresectable disease. For these patients, use of a c-kit tyrosine kinase inhibitor such as imatinib for tumors with *cKIT* mutations offers effective palliation. Avapritinib is used for tumors with certain *PDGFRA* mutations. However, resistance almost invariably develops, and the development of newer agents effective in tumors with secondary mutations is a high priority. For high-risk GISTs, adjuvant therapy with imatinib for 3 years improves relapse-free and overall survival.

■ SMALL BOWEL NEOPLASMS

Although the number of new cases of small-bowel neoplasms is substantially less than that of gastric neoplasms (in 2020, there were an estimated 11,110 new cases in the United States, representing 3–4% of gastrointestinal malignancies), the spectrum of malignant tumors of the small bowel is similar and includes NETs (carcinoid), adenocarcinomas, lymphomas, and GISTs. Neuroendocrine cancers are slightly more frequent (40–45%) than adenocarcinomas (30–40%), with the remainder mostly lymphomas and ~8% GISTs. The duodenum is the most common portion of the small bowel in which malignancies develop (~50%), with ~30% occurring in the jejunum and 20% in the ileum. NETs are the most common benign and malignant tumors of the ileum. Risk factors for the development of adenocarcinoma include inflammatory bowel disease (Crohn's disease) and inherited germline mutation syndromes such as Lynch syndrome, familial adenomatous polyposis (FAP), and Peutz-Jeghers syndrome. Celiac disease is associated with a higher incidence of both small-bowel adenocarcinomas and T-cell lymphomas.

While an asymptomatic small-bowel primary adenocarcinoma might be found during surveillance in patients at high risk (such as someone with FAP), symptoms due to obstruction or bleeding (which may be occult) lead to the diagnosis of a small-bowel tumor in a substantial number of patients. Both adenocarcinoma and lymphomas might present with perforation. The development of anemia or obstructive symptoms in a patient with a germline cancer susceptibility gene mutation should lead to a high degree of suspicion for developing malignancy. Evaluation by diagnostic CT imaging may reveal an obstructing lesion. CT and/or MRI enterography can be helpful if the diagnostic CT is not informative. Endoscopy using techniques such as double balloon enteroscopy or video capsule endoscopy allows (for the former) tissue diagnosis as well as localization. Video capsule endoscopy is contraindicated in the setting of obstruction. For NETs, a gallium-68 DOTATATE scan may identify both the primary site as well as metastatic disease. Blood tumor biomarkers are nonspecific for the primary site (CEA or CA19-9 for adenocarcinoma or serum

chromogranin for NET); these assays are better used to monitor response or progression of disease rather than for diagnosis.

Adenocarcinoma of the small bowel appears to be increasing in incidence. The median age for sporadic small-bowel tumors is in the seventh or eighth decade of life, but genetically predisposed patients and those with inflammatory bowel disease may be diagnosed at a much earlier age. African Americans have a higher incidence of small-bowel cancer than whites. While systemic therapies are usually modeled on agents used to treat colorectal cancer, genomic analyses have indicated that small-bowel adenocarcinoma has distinct genomic alterations compared to either colorectal or gastric cancers. Genomic alterations less frequent in small-bowel than in colorectal cancers include *TP53*, *BRAF* V600E, and *APC* mutations, whereas the rate of *KRAS* mutations is similar to colorectal cancer. Within small-bowel sites, the most striking difference is the higher rate of *ERBB2* alterations (of which a minority are amplifications) in duodenal cancers. Not surprisingly, because Lynch syndrome increases the risk of small-bowel adenocarcinoma, 15–20% of these tumors are MSI high or mismatch repair deficient; small-bowel adenocarcinoma associated with celiac disease also may have an increased rate of MSI-high tumors. MSI/MMRP status should be assessed in all patients with small-bowel adenocarcinoma. Somatic tumor genomic analysis may suggest a germline mutation, but appropriate genetic testing for a germline driver mutation should be performed in all patients with small-bowel adenocarcinoma.

Small-bowel adenocarcinoma has its own staging classification in the AJCC eighth edition.

TREATMENT

Small-Bowel Adenocarcinomas

Surgical resection with negative microscopic margins (R0), as is the case for other gastrointestinal tumors, is the best chance for cure. For duodenal adenocarcinomas, a Whipple procedure may be needed; for more distal duodenal cancers and jejunal adenocarcinomas, a segmental resection with adequate margins should be performed. Distal ileal tumors may require right hemicolectomy.

Small-bowel cancers are frequently found with locally advanced disease at the time of diagnosis. If the tumor is resectable, postoperative adjuvant systemic therapy is currently recommended for lymph node-positive patients, using regimens such as capecitabine-oxaliplatin. Benefit from adjuvant therapy has not yet been proven. Small-bowel cancers developing in patients with Lynch syndrome probably have a better prognosis; if colorectal cancer is a model, postoperative adjuvant therapy for patients with Lynch syndrome should be combination chemotherapy, not single-agent fluorinated pyrimidines. The value of immune modulation therapy is not established. For duodenal cancers, chemoradiation is considered if the resection margins are still positive.

For patients with advanced metastatic disease, in the absence of Lynch syndrome or a hypermutated tumor, similar cytotoxic regimens as deployed for gastric or colon cancers have been widely used. For tumors that are MSI high or dMMPR, immunotherapy is indicated; for tumors that are *HER2* amplified or *BRAF* mutated, targeted therapy may be useful.

■ SMALL BOWEL GASTROINTESTINAL STROMAL TUMORS

Like small-bowel adenocarcinomas, small-bowel GISTs may present with obstruction or bleeding. Diagnostic techniques are those employed for other small-bowel neoplasms. While the pathological criteria for malignant potential are somewhat different than those used for gastric GISTs, postoperative management and treatment of metastatic disease are the same as those described above for gastric GIST.

■ CARCINOID NEUROENDOCRINE TUMORS OF THE SMALL BOWEL

Carcinoid tumors are the most common small-bowel neoplasms. For not yet identified reasons, the incidence of small-bowel carcinoid

tumors has markedly increased over the past several decades. Although known risk factors include inherited genetic cancer susceptibility genes such as *MEN1* and neurofibromatosis 1 (*NFI*), these are unlikely to be the cause of the increase (Chap. 84). However, although the incidence has increased, small-bowel carcinoids are still uncommon, with an estimated incidence of approximately nine cases per million in the United States; the disease is more common in African Americans than whites.

Anatomically, the ileum is the most common part of the small bowel affected (~49%), followed by the duodenum and the jejunum. As is the case for gastric carcinoid tumors, grade is based on mitotic rate and/or Ki-67 immunohistochemistry. Histologic differentiation also uses the same criteria as in gastric carcinoid tumors.

In the absence of metastatic disease to the liver in the subgroup of patients whose tumors are functional (i.e., produce a hormone, usually serotonin; a duodenal NET may be a gastrinoma), presenting symptoms may be vague abdominal discomfort until or unless small-bowel obstruction occurs. Carcinoid syndrome may be found in patients whose tumors are diagnosed with already established hepatic metastasis. Because the liver is very efficient at clearing serotonin on first pass, carcinoid syndrome as a result of small-bowel carcinoid tumors usually does not occur in the absence of hepatic metastasis.

Clinical evaluation includes a diagnostic-quality CT or MRI of the abdomen and pelvis. For duodenal carcinoid tumors, upper endoscopy with EUS is also performed, and for jejunal or ileal carcinoid tumors, colonoscopy is performed. Small-bowel imaging may be performed by CT enterography; if an obstructing tumor is suspected, capsule endoscopy should be avoided. The diagnosis of a metastatic NET may be suspected from the radiographic appearance on CT imaging. Somatostatin analog imaging using gallium-68 DOTATATE is helpful in assessing extent of disease in patients whose tumors are somatostatin analog avid, as well as in identifying patients who may benefit from therapy targeting the somatostatin receptor. The AJCC eighth edition cancer staging manual has a specific small-bowel NET TNM stage classification.

The majority of patients present with locoregional disease, with approximately 40% having identified lymph node metastasis. Ten to 15% of patients have metastatic disease (usually to the liver) at the time of initial diagnosis.

Initial management should be surgical resection with curative intent; for patients with extensive adenopathy involving the root of the mesentery, vascular reconstruction may be required. Since small-bowel NETs may involve multiple tumors (15–30% of patients), the entire small bowel should be carefully examined. For patients with functional carcinoid tumors, somatostatin analog therapy using agents such as octreotide should be given before the induction of anesthesia to avoid a carcinoid crisis. For patients with hepatic metastasis, resection or regional therapy including ablation or hepatic artery embolization for functional tumors may provide effective palliation. Carcinoid syndrome may also be palliated by somatostatin receptor targeted therapy in the patients in whom gallium-68 DOTATATE scanning is positive (the majority of patients with carcinoid syndrome), including agents such as octreotide or lanreotide, or by peptide-directed radiation therapy using lutetium-177. Everolimus, an mTOR kinase inhibitor, has modest activity in metastatic small-bowel carcinoid tumors.

BENIGN NEOPLASMS OF THE SMALL BOWEL

As is the case for malignant small-bowel tumors, benign neoplasms of the small bowel are rare. In addition to cancers, the precursor lesion adenomas or hamartomas (from which cancers develop) may be driven by inherited cancer susceptibility genes (FAP, Lynch syndrome, Peutz-Jeghers syndrome, among others.). Other benign neoplasms include lipomas, leiomyomas, neurofibromas, and benign lymphoid nodular hyperplasia.

Patients with benign small-bowel neoplasms not associated with an inherited cancer susceptibility gene (in which case a benign tumor may be found during surveillance) are usually asymptomatic. A mass may be noted on an imaging study (usually a CT) ordered for another reason. Workup for occult or overt bleeding or intussusception of the bowel may lead to the discovery of a benign small-bowel neoplasm.

Diagnostic evaluation may include video capsule endoscopy and double balloon or push enteroscopy. In general, benign neoplasms, if found during surveillance, are removed endoscopically, if technically feasible, to decrease the risk of intussusception in Peutz-Jeghers syndrome. Mucosectomy may be used to treat bleeding hemangiomas.

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81

Lower Gastrointestinal Cancers

Robert J. Mayer



Lower gastrointestinal cancers include malignant tumors of the colon, rectum, and anus.

COLORECTAL CANCER

INCIDENCE

Cancer of the large bowel is second only to lung cancer as a cause of cancer death in the United States: 149,500 new cases were identified in 2021, and 52,980 deaths were due to colorectal cancer. The incidence rate has decreased significantly during the past 30 years in individuals 50 years of age or older, likely due in large part to enhanced and more compliantly followed screening practices. Similarly, mortality rates in the United States in this age group have decreased by more than 30%, resulting largely from early detection and improved treatment. During the same period of time, however, the incidence rate for colorectal cancer in men and women <50 years of age, having no enhanced genetic risk factor or family history for the disease, has risen by 2% each year with the presence of symptoms in this age group often being initially attributed to other causes, resulting in a more advanced disease stage at the time of diagnosis being frequently observed. A corresponding increase in mortality rates from colorectal cancer in this young adult population is now evident while, simultaneously, the trend for a decreased death rate in older individuals has continued. No distinguishing etiologic or molecular factor or clinical characteristic to account for the rising incidence of colorectal cancer in younger men and women has yet been identified with lifestyle patterns, such as diet and obesity beginning at an earlier age, having been proposed.

POLYPS AND MOLECULAR PATHOGENESIS

Most colorectal cancers, regardless of etiology, arise from adenomatous polyps. A polyp is a grossly visible protrusion from the mucosal surface and may be classified pathologically as a nonneoplastic hamartoma (e.g., *juvenile polyp*), a hyperplastic mucosal proliferation (*hyperplastic polyp*), or an adenomatous polyp. Only adenomas are clearly premalignant, and only a minority of adenomatous polyps evolve into cancer. Adenomatous polyps may be found in the colons of ~30% of middle-aged and ~50% of elderly people; however, <1% of polyps ever become malignant. Most polyps produce no symptoms and remain clinically undetected. Occult blood in the stool is found in <5% of patients with polyps.

A number of molecular changes are noted in adenomatous polyps and colorectal cancers that are thought to reflect a multistep process in the evolution of normal colonic mucosa to life-threatening invasive carcinoma. These developmental steps toward carcinogenesis include, but are not restricted to, point mutations in the *K-ras* proto-oncogene; hypomethylation of DNA, leading to gene activation; loss of DNA (*allelic loss*) at the site of a tumor-suppressor gene (the adenomatous polyposis coli [*APC*] gene) on the long arm of chromosome 5 (5q21); allelic loss at the site of a tumor-suppressor gene located on chromosome 18q (the deleted in colorectal cancer [*DCC*] gene); and allelic loss at chromosome 17p, associated with mutations in the *p53* tumor-suppressor gene (see Fig. 71-2). Thus, the altered proliferative pattern of the colonic mucosa, which results in progression to a polyp and then to carcinoma, may involve the mutational activation of an oncogene followed by and coupled with the loss of genes that normally suppress tumorigenesis. It remains uncertain whether the genetic aberrations always occur in a defined order. Based on this model, however, cancer is believed to develop only in those polyps in which most (if not all) of these mutational events take place.

Clinically, the probability of an adenomatous polyp becoming a cancer depends on the gross appearance of the lesion, its histologic features, and its size. Polyps may be pedunculated (stalked) or sessile (flat-based), adenomatous or serrated. Invasive cancers develop more frequently in sessile, serrated (i.e., "flat") polyps. Histologically, adenomatous polyps may be tubular, villous (i.e., papillary), or tubulovillous. Villous adenomas, most of which are sessile, become malignant more than three times as often as tubular adenomas. The likelihood that any polypoid lesion in the large bowel contains invasive cancer is related to the size of the polyp, being negligible (<2%) in lesions <1.5 cm, intermediate (2–10%) in lesions 1.5–2.5 cm, and substantial (10%) in lesions >2.5 cm in size.

Following the detection of an adenomatous polyp, the entire large bowel should be visualized endoscopically because synchronous lesions are noted in about one-third of cases. Colonoscopy should then be repeated periodically, even in the absence of a previously documented malignancy, because such patients have a 30–50% probability of developing another adenoma and are at a higher-than-average risk for developing a colorectal carcinoma. Adenomatous polyps are thought to require >5 years of growth before becoming clinically significant; colonoscopy need not be carried out more frequently than every 3 years for the vast majority of patients.

ETIOLOGY AND RISK FACTORS

Risk factors for the development of colorectal cancer are listed in Table 81-1.

TABLE 81-1 Risk Factors for the Development of Colorectal Cancer

Diet: Animal fat, obesity

Hereditary syndromes

 Polyposis coli

 MEN-associated polyposis

 Nonpolyposis syndrome (Lynch's syndrome)

Inflammatory bowel disease

Streptococcus bovis bacteremia

Tobacco use

Diet The etiology for most cases of large-bowel cancer appears to be related to environmental factors. The disease occurs more often in upper socioeconomic populations who live in urban areas. Mortality from colorectal cancer is directly correlated with per capita consumption of calories, meat protein, and dietary fat and oil as well as elevations in the serum cholesterol concentration and mortality from coronary artery disease. Geographic variations in incidence largely are unrelated to genetic differences because migrant groups tend to assume the large-bowel cancer incidence rates of their adopted countries. Furthermore, population groups such as Mormons and Seventh Day Adventists, whose lifestyle and dietary habits differ somewhat from those of their neighbors, have significantly lower-than-expected incidence and mortality rates for colorectal cancer. The incidence of colorectal cancer has increased in Japan since that nation has adopted a more "Western" diet. At least three hypotheses have been proposed to explain the relationship to diet, none of which is fully satisfactory.

ANIMAL FATS One hypothesis is that the ingestion of animal fats found in red meats and processed meat leads to an increased proportion of anaerobes in the gut microflora (the "microbiome"), resulting in the conversion of normal bile acids into carcinogens. This provocative hypothesis is supported by several reports of increased amounts of fecal anaerobes (*Fusobacterium nucleatum*, *Bacteroides fragilis*) in the stools of patients with colorectal cancer. Diets high in animal (but not vegetable) fats are also associated with high serum cholesterol, which is also associated with enhanced risk for the development of colorectal adenomas and carcinomas.

INSULIN RESISTANCE The large number of calories in Western diets coupled with physical inactivity has been associated with a higher prevalence of obesity. Obese persons develop insulin resistance with increased circulating levels of insulin, leading to higher circulating concentrations of insulin-like growth factor type I (IGF-I). This growth factor appears to stimulate proliferation of the intestinal mucosa.

FIBER Contrary to prior beliefs, the results of randomized trials and case-controlled studies have failed to show any value for dietary fiber or diets high in fruits and vegetables in preventing the recurrence of colorectal adenomas or the development of colorectal cancer.

The weight of epidemiologic evidence, however, implicates diet as being the major etiologic factor for colorectal cancer, particularly diets high in animal fat and in calories.

HEREDITARY FACTORS AND SYNDROMES

Up to 25% of patients with colorectal cancer have a family history of the disease, suggesting a hereditary predisposition. Inherited large-bowel cancers can be divided into two main groups: the well-studied but uncommon polyposis syndromes and the more common nonpolyposis syndromes (Table 81-2).

Polyposis Coli Polyposis coli (familial polyposis of the colon) is a rare condition characterized by the appearance of thousands of adenomatous polyps throughout the large bowel. It is transmitted as an autosomal dominant trait; the occasional patient with no family history probably developed the condition due to a spontaneous mutation. Polyposis coli is associated with a deletion in the long arm of chromosome 5 (including the *APC* gene) in both neoplastic (somatic mutation) and normal (germline mutation) cells. The loss of this genetic material (i.e., allelic loss) results in the absence of tumor-suppressor genes whose protein products would normally inhibit neoplastic growth. The presence of soft tissue and bony tumors, congenital hypertrophy of the retinal pigment epithelium, mesenteric desmoid tumors, and ampullary cancers in addition to the colonic polyps characterizes a subset of polyposis coli known as *Gardner's syndrome*. The appearance of malignant tumors of the central nervous system accompanying polyposis coli defines *Turcot's syndrome*. The colonic polyps in all these conditions are rarely present before puberty but are generally evident in affected individuals by age 25 years. If the polyposis is not treated surgically, colorectal cancer will develop in almost all patients aged <40 years. Polyposis coli results from a defect in the colonic mucosa, leading to an abnormal proliferative pattern and impaired DNA repair mechanisms.

TABLE 81-2 Heritable (Autosomal Dominant) Gastrointestinal Neoplasia Syndromes

SYNDROME	DISTRIBUTION OF POLYPS	HISTOLOGIC TYPE	MALIGNANT POTENTIAL	ASSOCIATED LESIONS
Familial adenomatous polyposis	Large intestine	Adenoma	Common	None
Gardner's syndrome	Large and small intestines	Adenoma	Common	Osteomas, fibromas, lipomas, epidermoid cysts, ampullary cancers, congenital hypertrophy of retinal pigment epithelium
Turcot's syndrome	Large intestine	Adenoma	Common	Brain tumors
MYH-associated polyposis	Large intestine	Adenoma	Common	None
Lynch syndrome (nonpolyposis syndrome)	Large intestine (often proximal)	Adenoma	Common	Endometrial and ovarian tumors (most frequently), gastric, genitourinary, pancreatic, biliary cancers (less frequently)
Peutz-Jeghers syndrome	Small and large intestines, stomach	Hamartoma	Rare	Mucocutaneous pigmentation; tumors of the ovary, breast, pancreas, endometrium
Juvenile polyposis	Large and small intestines, stomach	Hamartoma, rarely progressing to adenoma	Rare	Various congenital abnormalities

Once the multiple polyps are detected, patients should undergo a total colectomy. Medical therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) such as sulindac and selective cyclooxygenase-2 inhibitors such as celecoxib can decrease the number and size of polyps in patients with polyposis coli; however, this effect on polyps is only temporary, and the use of NSAIDs has not been shown to reduce the risk of cancer. Colectomy remains the primary therapy/prevention. The offspring of patients with polyposis coli, who often are prepubertal when the diagnosis is made in the parent, have a 50% risk for developing this premalignant disorder and should be carefully screened by annual flexible sigmoidoscopy until age 35 years. Proctosigmoidoscopy is a sufficient screening procedure because polyps tend to be evenly distributed from cecum to anus, making more invasive and expensive techniques such as colonoscopy or barium enema unnecessary. Testing for occult blood in the stool is an inadequate screening maneuver. If a causative germline *APC* mutation has been identified in an affected family member, an alternative method for identifying carriers is testing DNA from peripheral blood mononuclear cells for the presence of the specific *APC* mutation. The detection of such a germline mutation can lead to a definitive diagnosis before the development of polyps.

MYH-Associated Polyposis MYH-associated polyposis (MAP) is a rare autosomal recessive syndrome caused by a biallelic mutation in the *MUT4H* gene. This hereditary condition may have a variable clinical presentation, resembling polyposis coli or colorectal cancer occurring in younger individuals without polyposis. Screening and colectomy guidelines for this syndrome are less clear than for polyposis coli, but annual to biennial colonoscopic surveillance is generally recommended starting at age 25–30 years.

Lynch Syndrome Lynch syndrome, previously known as hereditary nonpolyposis colon cancer, is another autosomal dominant trait. It is characterized by the presence of three or more relatives with histologically documented colorectal cancer, one of whom is a first-degree relative of the other two; one or more cases of colorectal cancer diagnosed before age 50 in the family; and colorectal cancer involving at least two generations. In contrast to polyposis coli, Lynch syndrome is associated with an unusually high frequency of cancer arising in the proximal large bowel. The median age for the appearance of an adenocarcinoma is <50 years, 10–15 years younger than the median age for the general population. Despite having a poorly differentiated, mucinous histologic appearance, the proximal colon tumors that characterize Lynch syndrome have a better prognosis than sporadic tumors from patients of similar age. Families with Lynch syndrome often include individuals with multiple primary cancers; the association of colorectal cancer with either ovarian or endometrial carcinomas is especially strong in women, and an increased appearance of gastric, small-bowel, genitourinary, pancreaticobiliary, and sebaceous skin tumors has been

reported as well. It has been recommended that members of such families undergo annual or biennial colonoscopy beginning at age 25 years, with intermittent pelvic ultrasonography and endometrial biopsy for afflicted women; such a screening strategy has not yet been validated. Lynch syndrome is associated with germline mutations of several genes, particularly *hMSH2* on chromosome 2 and *hMLH1* on chromosome 3. These mutations lead to errors in DNA replication and are thought to result in DNA instability because of defective repair of DNA mismatches resulting in abnormal cell growth and tumor development. Testing tumor cells through molecular analysis of DNA for “microsatellite instability” or immunohistochemical staining for deficiency in mismatch repair proteins in patients with colorectal cancer and a positive family history for colorectal or endometrial cancer may identify probands with Lynch syndrome.

■ INFLAMMATORY BOWEL DISEASE

(Chap. 326) Large-bowel cancer is increased in incidence in patients with long-standing inflammatory bowel disease (IBD). Cancers develop more commonly in patients with ulcerative colitis than in those with granulomatous (i.e., Crohn's) colitis, but this impression may result in part from the occasional difficulty of differentiating these two conditions. The risk of colorectal cancer in a patient with IBD is relatively small during the initial 10 years of the disease, but then appears to increase at a rate of ~0.5–1% per year. Cancer may develop in 8–30% of patients after 25 years. The risk is higher in younger patients with pancolitis.

Cancer surveillance strategies in patients with IBD are unsatisfactory. Symptoms such as bloody diarrhea, abdominal cramping, and obstruction, which may signal the appearance of a tumor, are similar to the complaints caused by a flare-up of the underlying inflammatory disease. In patients with a history of IBD lasting ≥15 years who continue to experience exacerbations, the surgical removal of the colon can significantly reduce the risk for cancer and also eliminate the target organ for the underlying chronic gastrointestinal disorder. The value of such surveillance techniques as colonoscopy with mucosal biopsies and brushings for less symptomatic individuals with chronic IBD is uncertain. The lack of uniformity regarding the pathologic criteria that characterize dysplasia and the absence of data that such surveillance reduces the development of lethal cancers have made this costly practice an area of controversy.

■ OTHER HIGH RISK CONDITIONS

Streptococcus bovis Bacteremia For unknown reasons, individuals who develop endocarditis or septicemia from this fecal bacterium have a high incidence of occult colorectal tumors and, possibly, upper gastrointestinal cancers as well. Endoscopic or radiographic screening appears advisable.

Tobacco Use Cigarette smoking is linked to the development of colorectal adenomas, particularly after >35 years of tobacco use. No biologic explanation for this association has yet been proposed.

■ PRIMARY PREVENTION

Several orally administered compounds have been assessed as possible inhibitors of colon cancer. The most effective class of chemopreventive agents is aspirin and other NSAIDs, which are thought to suppress cell proliferation by inhibiting prostaglandin synthesis. Regular aspirin use reduces the risk of colon adenomas and carcinomas as well as death from large-bowel cancer; such use also appears to diminish the likelihood for developing additional premalignant adenomas following successful treatment for a prior colon carcinoma. This effect of aspirin on colon carcinogenesis increases with the duration and dosage of drug use. Emerging data linking adequate plasma levels of vitamin D with reduced risk of adenomatous polyps and colorectal cancer appear promising. The value of vitamin D as a form of chemoprevention is under study. Antioxidant vitamins such as ascorbic acid, tocopherols, and β-carotene are ineffective at reducing the incidence of subsequent adenomas in patients who have undergone the removal of a colon adenoma. Estrogen replacement therapy has been associated with a reduction in the risk of colorectal cancer in women, conceivably by an effect on bile acid synthesis and composition or by decreasing synthesis of IGF-I.

■ SCREENING

The rationale for colorectal cancer screening programs is that the removal of adenomatous polyps will prevent colorectal cancer, and that earlier detection of localized, superficial cancers in asymptomatic individuals will increase the surgical cure rate. Such screening programs are particularly important for individuals with a family history of the disease in first-degree relatives. The relative risk for developing colorectal cancer increases to 1.75 in such individuals and may be even higher if the relative was afflicted before age 60 years. The prior use of rigid proctosigmoidoscopy as a screening tool was based on the observation that 60% of early lesions are located in the rectosigmoid. For unexplained reasons, however, the proportion of large-bowel cancers arising in the rectum has been decreasing during the past several decades, with a corresponding increase in the proportion of cancers in the more proximal descending colon. As such, the potential for rigid proctosigmoidoscopy to detect a sufficient number of occult neoplasms to make the procedure cost-effective has been questioned.

Screening strategies for colorectal cancer that have been examined during the past several decades are listed in **Table 81-3**.

Many programs directed at the early detection of colorectal cancers have focused on digital rectal examinations and fecal occult blood (i.e., stool guaiac) testing. The digital examination should be part of any routine physical evaluation in adults aged >40 years, serving as a screening test for prostate cancer in men, a component of the pelvic examination in women, and an inexpensive maneuver for the detection of masses in the rectum. However, because of the proximal migration of colorectal tumors, its value as an overall screening modality for colorectal cancer has become limited. The development of the fecal occult blood test has greatly facilitated the detection of occult fecal blood. Unfortunately, even when performed optimally, the fecal occult blood test has major

limitations as a screening technique. About 50% of patients with documented colorectal cancers have a negative fecal occult blood test, consistent with the intermittent bleeding pattern of these tumors. When random cohorts of asymptomatic persons have been tested, 2–4% have fecal occult blood-positive stools. Colorectal cancers have been found in <10% of these “test-positive” cases, with benign polyps being detected in an additional 20–30%. Thus, a colorectal neoplasm will not be found in most asymptomatic individuals with occult blood in their stool. Nonetheless, persons found to have fecal occult blood-positive stool routinely undergo further medical evaluation, including sigmoidoscopy and/or colonoscopy—procedures that are not only uncomfortable and expensive but also associated with a small risk for significant complications. The added cost of these studies would appear justifiable if the small number of patients found to have occult neoplasms because of fecal occult blood screening could be shown to have an improved prognosis and prolonged survival. Prospectively controlled trials have shown a statistically significant reduction in mortality rate from colorectal cancer for individuals undergoing annual stool guaiac screening. However, this benefit only emerged after >13 years of follow-up and was extremely expensive to achieve because all positive tests (most of which were falsely positive) were followed by colonoscopy. Moreover, these colonoscopic examinations quite likely provided the opportunity for cancer prevention through the removal of potentially premalignant adenomatous polyps because the eventual development of cancer was reduced by 20% in the cohort undergoing annual screening.

With the appreciation that the carcinogenic process leading to the progression of the normal bowel mucosa to an adenomatous polyp and then to a cancer is the result of a series of molecular changes, investigators have examined fecal DNA for evidence of mutations associated with such molecular changes as evidence of the occult presence of precancerous lesions or actual malignancies. Such a strategy has been tested in >4000 asymptomatic individuals whose stool was assessed for occult blood and for 21 possible mutations in fecal DNA; these study subjects also underwent colonoscopy. Although the fecal DNA strategy proved to be more effective for suggesting the presence of more advanced adenomas and cancers than did the fecal occult blood testing approach, the overall sensitivity, using colonoscopic findings as the standard, was <50%, diminishing enthusiasm for further pursuit of the fecal DNA screening strategy.

The use of imaging studies to screen for colorectal cancers has also been explored. Air contrast barium enemas had been used to identify sources of occult blood in the stool prior to the advent of fiberoptic endoscopy; the cumbersome nature of the procedure and inconvenience to patients limited its widespread adoption. The introduction of CT scanning led to the development of virtual (i.e., CT) colonography as an alternative to the growing use of endoscopic screening techniques. Virtual colonography was proposed as being equivalent in sensitivity to colonoscopy and being available in a more widespread manner because it did not require the same degree of operator expertise as fiberoptic endoscopy. However, virtual colonography requires the same cathartic preparation that has limited widespread acceptance in association with endoscopic colonoscopy, is diagnostic but not therapeutic (i.e., patients with suspicious findings must undergo a subsequent endoscopic procedure for polypectomy or biopsy), and, in the setting of general radiology practices, appears to be less sensitive as a screening technique when compared with endoscopic procedures.

With the appreciation of the inadequacy of fecal occult blood testing alone, concerns about the practicality of imaging approaches, and the wider adoption of endoscopic examinations by the primary care community, screening strategies in asymptomatic persons have changed. At present, the American Cancer Society, the American College of Gastroenterology, and the National Comprehensive Cancer Network recommend either fecal occult blood testing annually coupled with flexible sigmoidoscopy every 5 years or colonoscopy every 10 years in asymptomatic individuals with no personal or family history of polyps or colorectal cancer. In view of the emerging increase in the incidence of colorectal cancer in individuals <50 years of age, guidelines issued from these organizations have recently lowered the age at which to begin such screening from age 50 to age 45 years. The recommendation

TABLE 81-3 Screening Strategies for Colorectal Cancer

Digital rectal examination
Stool testing
• Occult blood
• Fecal DNA
Imaging
• Contrast barium enema
• Virtual (i.e., computed tomography colonography)
Endoscopy
• Flexible sigmoidoscopy
• Colonoscopy

for the inclusion of flexible sigmoidoscopy is strongly supported by the recently published results of three randomized trials performed in the United States, the United Kingdom, and Italy, involving >350,000 individuals, which consistently showed that periodic (even single) sigmoidoscopic examinations, after more than a decade of median follow-up, lead to an ~21% reduction in the development of colorectal cancer and a >25% reduction in mortality from the malignant disease. Less than 20% of participants in these studies underwent a subsequent colonoscopy. In contrast to the cathartic preparation required before colonoscopic procedures, which is only performed by highly trained specialists, flexible sigmoidoscopy requires only an enema as preparation and can be accurately performed by nonspecialty physicians or physician-extenders. The randomized screening studies using flexible sigmoidoscopy led to the estimate that ~650 individuals needed to be screened to prevent one colorectal cancer death; this contrasts with the data for mammography where the number of women needing to be screened to prevent one breast cancer death is 2500, reinforcing the efficacy of endoscopic surveillance for colorectal cancer screening. Presumably the benefit from the sigmoidoscopic screening is the result of the identification and removal of adenomatous polyps; it is intriguing that this benefit has been achieved using a technique that leaves the proximal half of the large bowel unvisualized.

It remains to be seen whether surveillance colonoscopy, which has gained increasing popularity in the United States for colorectal cancer screening, will prove to be more effective than flexible sigmoidoscopy in reducing mortality from this disease. Ongoing randomized trials being conducted in Europe are addressing this issue. Although flexible sigmoidoscopy only visualizes the distal half of the large bowel, leading to the assumption that colonoscopy represents a more informative approach, colonoscopy has been reported as being less accurate for screening the proximal rather than the distal colon, perhaps due to technical considerations but also possibly because of a greater frequency of serrated (i.e., "flat") polyps in the right colon, which are more difficult to identify. Furthermore, the vast majority of colorectal cancers that have appeared in younger adults have arisen in the left colon (i.e., distal to the splenic flexure), within the visible range of a flexible sigmoidoscopy. At present, colonoscopy performed every 10 years has been offered as an alternative to annual fecal occult blood testing with periodic (every 5 years) flexible sigmoidoscopy. Colonoscopy has been shown to be superior to double-contrast barium enema and also to have a higher sensitivity for detecting villous or dysplastic adenomas or cancers than the strategy using occult fecal blood testing and flexible sigmoidoscopy. Whether colonoscopy performed every 10 years beginning at age 45 is medically superior and economically equivalent to flexible sigmoidoscopy remains to be determined.

■ CLINICAL FEATURES

Presenting Symptoms Symptoms vary with the anatomic location of the tumor. Because stool is relatively liquid as it passes through the ileocecal valve into the right colon, cancers arising in the cecum and ascending colon may become quite large without resulting in any obstructive symptoms or noticeable alterations in bowel habits. Lesions of the right colon commonly ulcerate, leading to chronic, insidious blood loss without a change in the appearance of the stool. Consequently, patients with tumors of the ascending colon often present with symptoms such as fatigue, palpitations, and even angina pectoris and are found to have a hypochromic, microcytic anemia, indicative of iron deficiency. Because the cancers may bleed intermittently, a random fecal occult blood test may be negative. As a result, the unexplained presence of iron-deficiency anemia in any adult (with the possible exception of a premenopausal, multiparous woman) mandates a thorough endoscopic and/or radiographic visualization of the entire large bowel (Fig. 81-1).

Because stool becomes more formed as it passes into the transverse and descending colon, tumors arising there tend to impede the passage of stool, resulting in the development of abdominal cramping, occasional obstruction, and even perforation. Radiographs of the abdomen often reveal characteristic annular, constricting lesions ("apple-core" or "napkin-ring") (Fig. 81-2).



FIGURE 81-1 Double-contrast air-barium enema revealing a sessile tumor of the cecum in a patient with iron-deficiency anemia and guaiac-positive stool. The lesion at surgery was a stage II adenocarcinoma.

Cancers arising in the rectosigmoid are often associated with hematicchezia, tenesmus, and narrowing of the caliber of stool; anemia is an infrequent finding. While these symptoms may lead patients and their physicians to suspect the presence of hemorrhoids, the development of rectal bleeding and/or altered bowel habits demands a prompt digital rectal examination and proctosigmoidoscopy.

Staging, Prognostic Factors, and Patterns of Spread The prognosis for individuals having colorectal cancer is related to the depth of tumor penetration into the bowel wall and the presence of both regional lymph node involvement and distant metastases. These variables are incorporated into a TNM classification method, in which T represents the depth of tumor penetration, N the presence of lymph node involvement, and M the presence or absence of distant metastases



FIGURE 81-2 Annular, constricting adenocarcinoma of the descending colon. This radiographic appearance is referred to as an "apple-core" lesion and is always highly suggestive of malignancy.

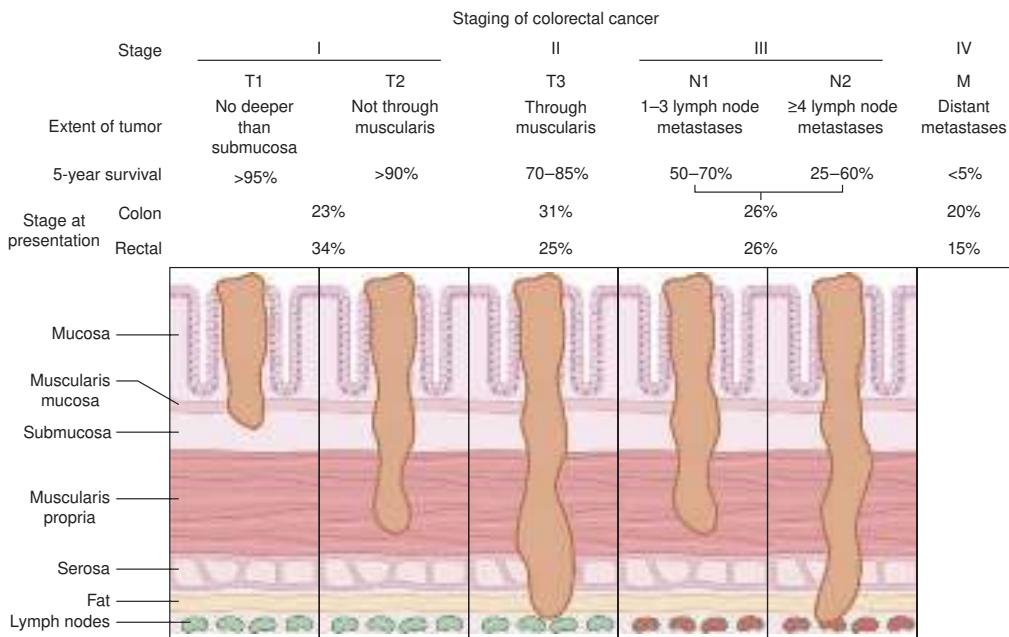


FIGURE 81-3 Staging and prognosis for patients with colorectal cancer.

(Fig. 81-3). Superficial lesions that do not involve regional lymph nodes and do not penetrate through the submucosa (T1) or the muscularis (T2) are designated as *stage I* (T1–2N0M0) disease; tumors that penetrate through the muscularis but have not spread to lymph nodes are *stage II* disease (T3–4N0M0); regional lymph node involvement defines *stage III* (TXN1–2M0) disease; and metastatic spread to sites such as liver, lung, or bone indicates *stage IV* (TXNM1) disease. Unless gross evidence of metastatic disease is present, disease stage cannot be determined accurately before surgical resection and pathologic analysis of the operative specimens.

Most recurrences after a surgical resection of a large-bowel cancer occur within the first 4 years, making 5-year survival a fairly reliable indicator of cure. The likelihood for 5-year survival in patients with colorectal cancer is stage-related (Fig. 81-3). That likelihood has improved during the past several decades when similar surgical stages have been compared. The most plausible explanation for this improvement is more thorough intraoperative and pathologic staging. In particular, more exacting attention to pathologic detail has revealed that the prognosis following the resection of a colorectal cancer is not related merely to the presence or absence of regional lymph node involvement; rather, prognosis may be more precisely gauged by the number of involved lymph nodes (one to three lymph nodes [“N1”] vs four or more lymph nodes [“N2”]) and the number of nodes examined. A minimum of 12 sampled lymph nodes is thought necessary to accurately define tumor stage, and the more nodes examined, the better. Other predictors of a poor prognosis after a total surgical resection include tumor penetration through the bowel wall into pericolic fat, poorly differentiated histology, perforation and/or tumor adherence to adjacent organs (increasing the risk for an anatomically adjacent recurrence), and venous invasion by tumor (Table 81-4). Regardless of the clinicopathologic stage, a preoperative elevation of the plasma carcinoembryonic antigen (CEA) level predicts eventual tumor recurrence. The presence of specific chromosomal aberrations, particularly a mutation in the *b-raf* gene in tumor cells, appears to predict for a higher risk for metastatic spread. Conversely, the detection of microsatellite instability in tumor tissue indicates a more favorable outcome. Tumors arising in the left colon are associated with a better prognosis than those appearing in the right colon, likely due to differences in molecular patterns. In contrast to most other cancers, the prognosis in colorectal cancer is not influenced by the size of the primary lesion when adjusted for nodal involvement and histologic differentiation.

Cancers of the large bowel generally spread to regional lymph nodes or to the liver via the portal venous circulation. The liver represents the most frequent visceral site of metastasis; it is the initial site of distant spread in one-third of recurring colorectal cancers and is involved in more than two-thirds of such patients at the time of death. In general, colorectal cancer rarely spreads to the lungs, supraclavicular lymph nodes, bone, or brain without prior spread to the liver. A major exception to this rule occurs in patients having primary tumors in the distal rectum, from which tumor cells may spread through the paravertebral venous plexus, escaping the portal venous system and thereby reaching the lungs or supraclavicular lymph nodes without hepatic involvement. The median survival after the detection of distant metastases has increased during the last 30 years from 6–9 months (hepatomegaly, abnormal liver chemistries) to 27–30 months (small liver nodule initially identified by elevated CEA level and subsequent CT scan) with increasingly effective systemic therapy improving this prognosis further.

Efforts to use gene expression profiles to identify patients at risk of recurrence or those particularly likely to benefit from adjuvant therapy have not yet yielded practice-changing results. Despite a burgeoning literature examining a host of prognostic factors, pathologic stage at diagnosis remains the best predictor of long-term prognosis. Patients with lymphovascular invasion and high preoperative CEA levels are likely to have a more aggressive clinical course.

TABLE 81-4 Predictors of Poorer Outcomes Following Total Surgical Resection of Colorectal Cancer

- Tumor spread to regional lymph nodes
- Number of regional lymph nodes involved
- Tumor penetration through the bowel wall
- Poorly differentiated histology
- Perforation
- Tumor adherence to adjacent organs
- Venous invasion
- Preoperative elevation of CEA titer (>5 ng/mL)
- Specific chromosomal deletion (e.g., mutation in the *b-raf* gene)
- Right-sided location of primary tumor

Abbreviation: CEA, carcinoembryonic antigen.

TREATMENT

Colorectal Cancer

Total resection of tumor is the optimal treatment when a malignant lesion is detected in the large bowel. An evaluation for the presence of metastatic disease, including a thorough physical examination, biochemical assessment of liver function, measurement of the plasma CEA level, and a CT scan of the chest, abdomen, and pelvis, should be performed before surgery. When possible, a colonoscopy of the entire large bowel should be performed to identify synchronous neoplasms and/or polyps. The detection of metastases should not preclude surgery in patients with tumor-related symptoms such as gastrointestinal bleeding or obstruction, but it often prompts the use of a less radical operative procedure. The necessity for a primary tumor resection in asymptomatic individuals with metastatic disease is an area of controversy. At the time of laparotomy, the entire peritoneal cavity should be examined, with thorough inspection of the liver, pelvis, and hemidiaphragm and careful palpation of the full length of the large bowel. Following recovery from a complete resection, patients should be observed carefully for 5 years by semiannual physical examinations and blood chemistry measurements. If a complete colonoscopy was not performed preoperatively, it should be carried out within the first several postoperative months. Some authorities favor measuring plasma CEA levels at 3-month intervals because of the sensitivity of this test as a marker for otherwise undetectable tumor recurrence. The value of periodically assessing plasma for the presence of circulating tumor DNA as a biomarker for residual or recurrent disease is under study. Subsequent endoscopic surveillance of the large bowel, probably at triennial intervals, is indicated, because patients who have been cured of one colorectal cancer have a 3–5% probability of developing an additional bowel cancer during their lifetime and a >15% risk for the development of adenomatous polyps. Anastomotic (“suture-line”) recurrences are infrequent in colorectal cancer patients, provided the surgical resection margins were adequate and free of tumor. The value of periodic CT scans of the abdomen, assessing for an early, asymptomatic indication of tumor recurrence, while uncertain, has been recommended annually for the first 3 postoperative years.

Radiation therapy to the pelvis is recommended for patients with rectal cancer because it reduces the 20–25% probability of regional recurrences following complete surgical resection of stage II or III tumors, especially if they have penetrated through the serosa. This alarmingly high rate of local disease recurrence is believed to be due to the fact that the contained anatomic space within the pelvis limits the extent of the resection and because the rich lymphatic network of the pelvic side wall immediately adjacent to the rectum facilitates the early spread of malignant cells into surgically inaccessible tissue. The use of sharp rather than blunt dissection of rectal cancers (*total mesorectal excision*) appears to reduce the likelihood of local disease recurrence to ~10%. Radiation therapy, either administered pre- or postoperatively, further reduces the likelihood of pelvic recurrences but does not appear to prolong survival. Combining radiation therapy with 5-fluorouracil (5-FU)-based chemotherapy, preferably prior to surgical resection, lowers local recurrence rates and improves overall survival. Radiation therapy alone is not effective as the primary treatment of colon cancer.

Systemic therapy for patients with colorectal cancer has become more effective. 5-FU remains the backbone of treatment for this disease. Partial responses are obtained in 15–20% of patients. The probability of tumor response appears to be somewhat greater for patients with liver metastases when chemotherapy is infused directly into the hepatic artery, but intraarterial treatment is costly and toxic and does not appear to appreciably prolong survival. The concomitant administration of folinic acid (leucovorin [LV]) improves the efficacy of 5-FU in patients with advanced colorectal cancer, presumably by enhancing the binding of 5-FU to its target enzyme, thymidylate synthase. 5-FU is generally administered

intravenously but may also be given orally in the form of capecitabine (Xeloda) with seemingly similar efficacy.

Irinotecan (CPT-11), a topoisomerase 1 inhibitor, has been added to 5-FU and LV (e.g., FOLFIRI) with resultant improvement in response rates and survival of patients with metastatic disease. The *FOLFIRI regimen* is as follows: irinotecan, 180 mg/m² as a 90-min infusion on day 1; LV, 400 mg/m² as a 2-h infusion during irinotecan administration; immediately followed by 5-FU bolus, 400 mg/m², and 46-h continuous infusion of 2.4–3 g/m² every 2 weeks. Diarrhea is the major side effect from irinotecan. Oxaliplatin, a platinum analogue, also improves the response rate when added to 5-FU and LV (FOLFOX) as initial treatment of patients with metastatic disease. The *FOLFOX regimen* is as follows: 2-h infusion of LV (400 mg/m² per day) followed by a 5-FU bolus (400 mg/m² per day) and 22-h infusion (1200 mg/m²) every 2 weeks, together with oxaliplatin, 85 mg/m² as a 2-h infusion on day 1. Oxaliplatin frequently causes a dose-dependent sensory neuropathy that often but not always resolves following the cessation of therapy. FOLFIRI and FOLFOX are equal in efficacy. In metastatic disease, these regimens may produce median survivals of 2 years.

Monoclonal antibodies are also effective in patients with advanced colorectal cancer. Cetuximab (Erbitux) and panitumumab (Vectibix) are directed against the epidermal growth factor receptor (EGFR), a transmembrane glycoprotein involved in signaling pathways affecting growth and proliferation of tumor cells. Both cetuximab and panitumumab, when given alone, have been shown to benefit a small proportion of previously treated patients, and cetuximab appears to have therapeutic synergy with such chemotherapeutic agents as irinotecan, even in patients previously resistant to this drug; this suggests that cetuximab can reverse cellular resistance to cytotoxic chemotherapy. The antibodies are not effective in the ~65% subset of colon tumors that contain mutations in *ras* or *b-raf* genes and appear to be less likely to prove beneficial in the treatment of tumors arising from the right rather than left colon. The use of both cetuximab and panitumumab can lead to an acne-like rash, with the development and severity of the rash being correlated with the likelihood of antitumor efficacy. Inhibitors of the EGFR tyrosine kinase such as erlotinib (Tarceva) or sunitinib (Sutent) do not appear to be effective in colorectal cancer.

Bevacizumab (Avastin) is a monoclonal antibody directed against the vascular endothelial growth factor (VEGF) and is thought to act as an antiangiogenesis agent. The addition of bevacizumab to irinotecan-containing combinations and to FOLFOX appears to significantly improve the outcome observed with chemotherapy alone. The use of bevacizumab can lead to hypertension, proteinuria, and an increased likelihood of thromboembolic events.

Emerging data suggest that the use of checkpoint inhibitors (i.e., PD-1 and PD-2) as immunotherapy is more effective than chemotherapy in the subset (15%) of patients with metastatic colorectal cancer whose tumors are mismatch repair protein deficient (i.e., microsatellite unstable). Patients with solitary hepatic metastases without clinical or radiographic evidence of additional tumor involvement should be considered for partial liver resection, because such procedures are associated with 5-year survival rates of 25–30% when performed on selected individuals by experienced surgeons.

The administration of 5-FU and LV for 6 months after resection of tumor in patients with stage III disease leads to a 40% decrease in recurrence rates and 30% improvement in survival. The likelihood of recurrence has been further reduced when oxaliplatin has been combined with 5-FU and LV (e.g., FOLFOX). Reducing the duration of such oxaliplatin-containing therapy from 6 months to 3 months in patients with less invasive tumors (T_{1–2}, N₁) has been shown to result in a similar therapeutic benefit with reduced side effects (i.e., neurotoxicity) whereas 6 months of such therapy continues to be recommended for optimally treating patients with more advanced stage III tumors (T_{3–4} and/or N₂). Unexpectedly, the addition of irinotecan to 5-FU and LV as well as the addition of either bevacizumab or cetuximab to FOLFOX did not significantly enhance outcome.

Patients with stage II tumors do not appear to benefit appreciably from adjuvant therapy, with the use of such treatment generally restricted to those patients having biologic characteristics (e.g., perforated tumors, T4 lesions, lymphovascular invasion) that place them at higher likelihood for recurrence.

In rectal cancer, the delivery of preoperative or postoperative combined-modality therapy (5-FU or capecitabine plus radiation therapy) reduces the risk of recurrence and increases the chance of cure for patients with stage II and III tumors, with the preoperative approach being better tolerated.

CANCERS OF THE ANUS

Cancers of the anus account for 1–2% of the malignant tumors of the large bowel. Most such lesions arise in the anal canal, the anatomic area extending from the anorectal ring to a zone approximately halfway between the pectinate (or dentate) line and the anal verge. Carcinomas arising proximal to the pectinate line (i.e., in the transitional zone between the glandular mucosa of the rectum and the squamous epithelium of the distal anus) are known as *basaloid*, *cuboidal*, or *cloacogenic* tumors; about one-third of anal cancers have this histologic pattern. Malignancies arising distal to the pectinate line have squamous histology, ulcerate more frequently, and constitute ~55% of anal cancers. The prognosis for patients with basaloid and squamous cell cancers of the anus is identical when corrected for tumor size and the presence or absence of nodal spread.

The development of anal cancer is associated with infection by human papillomavirus, the same organism etiologically linked to cervical and oro-pharyngeal cancers. The virus is sexually transmitted. The infection may lead to anal warts (*condyloma acuminata*), which may progress to anal intraepithelial neoplasia and on to squamous cell carcinoma. The risk for anal cancer is increased among homosexual males, presumably related to anal intercourse. Anal cancer risk is increased in both men and women with AIDS, possibly because their immunosuppressed state permits more severe papillomavirus infection. Vaccination against human papilloma viruses appears to reduce the eventual risk for anal cancer. Anal cancers occur most commonly in middle-aged persons and are more frequent in women than men. At diagnosis, patients may experience bleeding, pain, sensation of a perianal mass, and pruritis.

Radical surgery (abdominal-perineal resection with lymph node sampling and a permanent colostomy) was once the treatment of choice for this tumor type. The 5-year survival rate after such a procedure was 55–70% in the absence of spread to regional lymph nodes and <20% if nodal involvement was present. An alternative therapeutic approach combining external beam radiation therapy with concomitant chemotherapy (5-FU and mitomycin C) has resulted in biopsy-proven disappearance of all tumor in >80% of patients whose initial lesion was <3 cm in size. Tumor recurrences develop in <10% of these patients, meaning that ~70% of patients with anal cancers can be cured with nonoperative treatment and without the need for a colostomy. Surgery should be reserved for the minority of individuals who are found to have residual tumor after being managed initially with radiation therapy combined with chemotherapy. The use of checkpoint immunotherapy (i.e., PD-1 inhibition) has been beneficial in some patients with recurrent disease.

FURTHER READING

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82

Tumors of the Liver and Biliary Tree

Josep M. Llovet



Liver cancer is the sixth most common cancer worldwide, the fourth leading cause of cancer-related deaths, and the leading cause of death among cirrhotic patients. Liver cancer comprises a heterogeneous group of malignant tumors with different histologic features and unfavorable prognosis that range from hepatocellular carcinoma (HCC; 85–90% cases), intrahepatic cholangiocarcinoma (iCCA; 10%), and other malignancies accounting for <1% of tumors, such as fibrolamellar HCC, mixed HCC-iCCA, epithelioid hemangioendothelioma, and the pediatric cancer hepatoblastoma. The burden of liver cancer is increasing globally in almost all countries, and it is estimated to reach 1 million cases by 2025.

HEPATOCELLULAR CARCINOMA

■ EPIDEMIOLOGY AND RISK FACTORS

Overall, liver cancer accounts for 7% of all cancers (~850,000 new cases each year), and HCC represents 90% of primary liver cancers. The highest incidence rates of HCC occur in Asia and sub-Saharan Africa due to the high prevalence of hepatitis B virus (HBV) infection, with 20–35 cases per 100,000 inhabitants. Southern Europe and North America have intermediate incidence rates (10 cases per 100,000), whereas Northern and Western Europe have low incidence rates of <5 cases per 100,000 inhabitants. In the United States, liver cancer is ranked number one in terms of increased mortality during the past two decades, with an incidence of 35,000 cases per year (Fig. 82-1). HCC has a strong male preponderance with a male-to-female ratio estimated to be 2.5. The incidence increases with age, reaching a peak at 65–70 years old. In Chinese and in black African populations (where vertical transmission of HBV occurs), the mean age is 40–50 years. By contrast, in Japan, mean age in men is now around 75 years.

The risk factors for HCC are well established (Fig. 82-1). The main risk factor is cirrhosis—and associated chronic liver damage caused by inflammation and fibrosis—of any etiology, which underlies 80% of HCC cases worldwide and results from chronic infection by HBV or hepatitis C virus (HCV) infection, alcohol abuse, metabolic syndrome, and hemochromatosis (associated with *HFE1* gene germline mutations). Cirrhotic patients represent 1% of the human population, and one-third of them will develop HCC during their lifetime. Long-term follow-up studies have established an annual risk of HCC development of 3–8% in HBV- or HCV-infected cirrhotic patients. HCC is less common (1–3% per year) in cirrhosis associated with alcohol, nonalcoholic steatohepatitis (NASH), α_1 -antitrypsin deficiency, autoimmune hepatitis, Wilson's disease, and cholestatic liver disorders. Predictors of liver cancer development among cirrhotic patients have been associated with liver disease severity (platelet count of <100,000/ μ L, presence

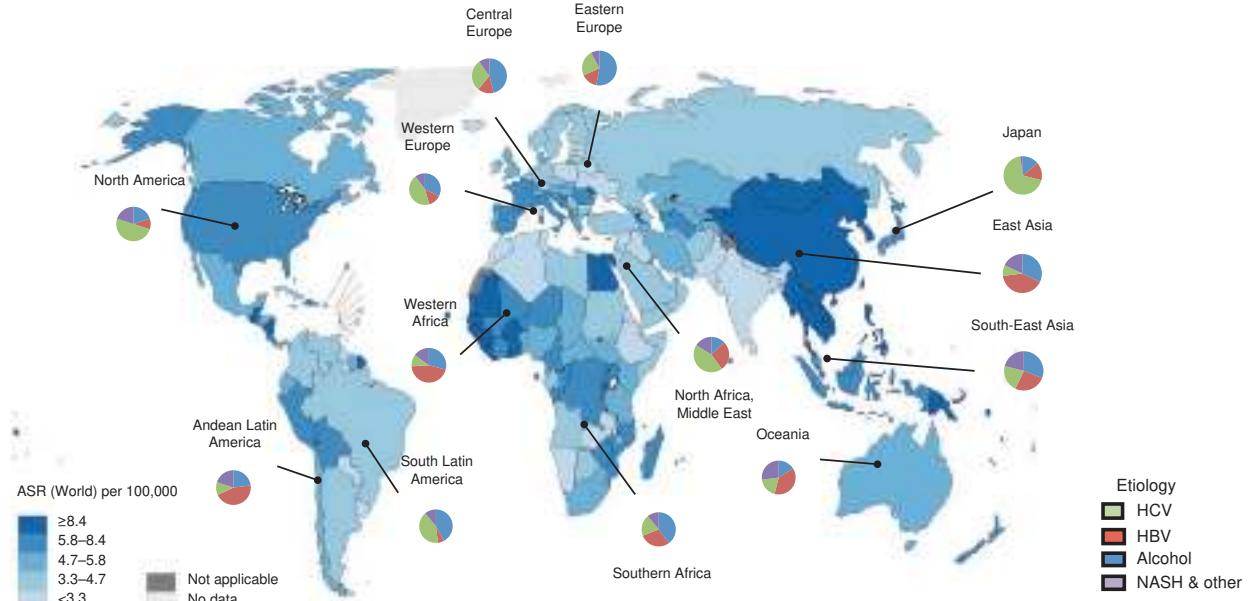


FIGURE 82-1 Distribution of hepatocellular carcinoma incidence according to geographical area and etiology. HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis. (Reproduced with permission from JM Llovet *et al*: Hepatocellular carcinoma. *Nat Rev Disease Primers* 6:7, 2021.)

of portal hypertension), the degree of liver stiffness as measured by transient elastography, and liver gene signatures capturing the *cancer field effect*.

In terms of attributable risk fraction, HBV infection—a DNA virus that can cause insertional mutagenesis and affects 400 million people globally—accounts for ~60% of HCC cases in Asia and Africa and 20% in the Western world. Among patients with HBV infection, a family history of HCC, HBeAg seropositivity, high viral load, and genotype C are independent predictors of HCC development. Chronic treatments with effective antiviral HBV therapies are able to significantly decrease the risk of cancer. HCV infection—an RNA virus that affects 170 million people—is responsible for ~30% of cases and is the main cause of HCC in Europe and North America. Among patients with HCV infection, HCC occurs almost exclusively when relevant liver damage is present (either advanced fibrosis—Metavir F3 [Metavir is a scoring system for hepatic histology that grades fibrosis from 0 to 4 with higher numbers indicating more fibrosis]—or cirrhosis), particularly if associated with HCV genotype 1b. In addition, a polymorphism that activates EGFR, the epidermal growth factor receptor, is associated with HCV-HCC in several studies. Antiviral therapies with interferon regimens are able to prevent cirrhosis development and HCC occurrence. Direct-acting antiviral agents (DAA) induce sustained virologic response, i.e., clearance of HCV infection, in most of cases, thus resulting in 50–80% reduction in HCC risk.

Alcohol consumption and metabolic syndrome due to diabetes and obesity are responsible for ~30% of cases. NASH is becoming the leading cause of cirrhosis in developed countries and currently represents ~15–20% of HCC cases in the West. The annual incidence of HCC in NASH-related cirrhosis (1–2%/year) justifies including patients at risk in surveillance programs. Nonetheless, it has to be taken into account that 25–30% of NASH-associated HCC occurs in the absence of cirrhosis. A PNPLA3 polymorphism is strongly associated with fatty and alcoholic chronic liver diseases and HCC occurrence. Other cofactors contributing to HCC development are tobacco and aflatoxin B1, a fungal carcinogen present in food supplies that induces TP53 mutations. Finally, infection with adeno-associated virus 2 is associated with HCC in individuals without cirrhosis. Aside from the associations described above, genome-wide association studies have not yet confirmed polymorphisms predisposing to HCC development.

■ MOLECULAR PATHOGENESIS

HCC development is a complex multistep process that starts with precancerous cirrhotic nodules, so-called low-grade dysplastic nodules (LGDNs) that evolve to high-grade dysplastic nodules (HGDNs) that can transform into early-stage HCC. Molecular studies support the pivotal role of adult hepatocytes as the cell of origin, either by directly transforming to HCC or by de-differentiating into hepatocyte precursor cells. Alternatively, progenitor cells also give rise to HCC with progenitor markers.

Genomic analysis has provided a clear picture of the main drivers responsible for HCC initiation and progression. This tumor results from the accumulation of around 40–60 somatic genomic alterations per tumor, of which 4–8 are considered driver cancer genes. HCC is a prototypical inflammation-associated cancer, where immune microenvironment and oxidative stress present in chronically damaged livers play pivotal roles in inducing mutations. In preneoplastic HGDN, mutations in telomere reverse transcriptase (*TERT*) gene (20% of cases) and gains in 8q have been described. Oncogenic transformation occurs upon additional genomic hits including Wnt/β-catenin pathway activation, reexpression of fetal genes, deregulation of protein folding machinery, and the response to oxidative stress. Genomic studies and next-generation sequencing conducted during the past decade have enabled a description of the landscape of mutations, signaling pathways, and molecular classification of the disease. Nonetheless, none of these data have yet translated into actual clinical benefits for any specific molecularly based subgroups.

Molecular Drivers The landscape of mutational drivers in HCC identified by deep-genome sequencing is detailed in Table 82-1. The most common mutations are in the *TERT* promoter (56%), *TP53* (27%), *CTNNB1* (26%), *ARID2* (7%), *ARID1A* (6%), and *AXIN1* (5%) genes. These mutated genes participate in cell-cycle control and senescence (*TERT* and *TP53*), cell differentiation (*CTNNB1* and *AXIN1*), and chromatin remodeling (*ARID2* and *ARID1A*). Genes commonly mutated in other solid tumors such as *EGFR*, *HER2*, *PIK3CA*, *BRAF*, or *KRAS* are rarely mutated in HCC (<5%). Overall, only ~20–25% of HCCs have at least one actionable mutation. Some risk factors have been associated with specific molecular aberrations. HBV integrates into the genome of driver genes, such as the *TERT* promoter, *MLL4*, and cyclin E1 (*CCNE1*). Alcohol abuse and HCV infection have been

TABLE 82-1 Molecular Aberrations Common in Hepatocellular Carcinoma (HCC)^a

PATHWAY	TARGET	PREVALENCE (%)
Mutations		
Telomere stability	<i>TERT</i> promoter	56
p53/cell-cycle control	<i>TP53</i>	27
	<i>ATM</i>	3
	<i>RB1</i>	3
Wnt/β-catenin signaling	<i>CTNNB1</i>	26
	<i>AXIN1</i>	5
Chromatin remodeling	<i>ARID1A</i>	6
	<i>ARID2</i>	7
	<i>KMT2A</i>	3
	<i>KMT2C</i>	3
Ras/PI3K/mTOR pathway	<i>RPS6KA3</i>	3
	<i>TSC1/TSC2</i>	3
Oxidative stress	<i>NFE2L2</i>	3
	<i>KEAP1</i>	3
High-level focal amplifications		
VEGFA signaling	<i>VEGFA</i>	3
FGF signaling	<i>FGF19</i>	6
Cell-cycle control	<i>CCND1</i> protein	7
Target with homozygous deletion		
TP53/cell-cycle control	<i>CDKN2A</i>	5
	<i>TP53</i>	4
	Retinoblastoma 1	4
Wnt/β-catenin signaling	<i>AXIN1</i>	3

^aRecurrent mutations, focal amplifications, or homozygous deletions in HCC based on next-generation sequencing analyses.

associated with *CTNNB1* mutations. *TP53* mutations are the most frequent alterations with a specific hotspot of mutation (R249S) in patients with aflatoxin B1 exposure.

Studies assessing copy number alterations in HCCs have consistently identified: (1) high-level amplifications at 5–10% prevalence containing oncogenes in 11q13 (*CCND1* and *FGF19*) and 6p21 (*VEGFA*), *TERT* focal amplification, and homozygous deletion of *CDKN2A*; and (2) common amplifications containing *MYC* (8q gain) and *MET* genes (focal gains of 7q31). Activation of the FGF19-FGFR4 pathway mediated by epigenetic mechanisms (~20%) or high-level amplifications of 11q13 (6%) or *VEGFA* gains (high-level gains of 6p21) are also potential therapeutic targets.

Signaling Pathways Several signaling pathways have been implicated in HCC progression and dissemination. Activation of these pathways can result from structural alterations (mutations and amplifications/losses) or epigenetic modifications. In brief, (1) *TERT* overexpression occurs in 90% of cases, particularly related to promoter *TERT* mutations or amplifications; (2) inactivation of p53 and alterations of cell cycle are major defects in HCC, particularly in cases related to HBV infection; (3) Wnt/β-catenin pathway activation occurs in 50% of cases, either as a result of β-catenin or *AXIN1* mutation or overexpression of Frizzled receptors or inactivation of E-cadherin; (4) PI3K/PTEN/Akt/mTOR pathway is activated in 40–50% of HCCs due to mutation and focal deletion of the tuberous sclerosis complex (*TSC1*/*TSC2* genes, *PTEN*, or ligand overexpression of EGF or insulin-like growth factor (IGF) upstream signals; (5) Ras MAPK signaling is activated in half of early and almost all advanced HCCs, and activation results from upstream signaling by EGF, IGF, and MET activation; (6) insulin-like growth factor receptor (IGFR) signaling is activated in 20% of cases through overexpression of the oncogenic ligand IGF2; (7) dysregulation of the c-MET receptor and its ligand HGF, critical for hepatocyte regeneration after liver injury, is a common event in advanced HCC (50%); (8) vascular endothelial growth factor (VEGF) signaling is the cornerstone of angiogenesis in HCC, along with activated angiogenic

pathways such as Ang2 and FGF signaling; and (9) chromatin remodeling complexes and epigenetic regulators are frequently altered in HCC due to *ARID1A* and *ARID2* mutations.

Molecular and Immune Classes Genomic studies have revealed two molecular subclasses of HCC, each representing ~50% of patients. The proliferative subclass is enriched by activation of Ras, mTOR, and IGF signaling and *FGF19* amplification and is associated with HBV-related etiologies, overexpression of α-fetoprotein, and poor outcomes. By contrast, the so-called nonproliferative subclass contains a subtype characterized by *CTNNB1* mutations and better outcome. Another classification based on immune status has been proposed. It defines an immune HCC class in ~25% of cases characterized by immune infiltrate with expression of PD-1/PD-L1, enrichment of T cell activation, and better outcome and an immune excluded class with activation of pathways related with immune escape (i.e., Wnt signaling) or absence of T cell infiltrate. This excluded class has been proposed to be associated with resistance to immune checkpoint inhibitors, although direct translation of molecular subclasses into clinical decision making has yet to be achieved.

■ PREVENTION AND EARLY DETECTION

Prevention Primary prevention of HCC can be achieved by vaccination against HBV and effective treatment of HBV and HCV infection. Studies assessing the impact of universal vaccination against HBV infection have reported a significant decrease of the incidence of HCC. HBV vaccination is recommended to all newborns and high-risk groups, following World Health Organization guidelines. Vaccination is also recommended in people with risk factors for acquiring HBV infection, such as health workers, travelers to areas where HBV infection is prevalent, injection drug users, and people with multiple sex partners.

Effective antiviral treatments for patients with chronic HBV infection—achieving undetectable viral titers (circulating HBV-DNA)—reduce the risk of HCC development. Evidence of this effect is supported by one randomized trial and several cohort studies. Treatment of HCV has dramatically advanced with the new DAAs, which yield >90% sustained virologic response (SVR) rates after 12 weeks of treatment. This effect has a direct implication in reducing HCC incidence in patients with cured chronic HCV infection. Once cirrhosis is established, the incidence of HCC is lower for patients with SVR than for those with active viral disease, although they continue to have persistent HCC risk (>1% per year). Additional putative chemopreventive agents have been proposed to reduce HCC incidence in at-risk populations. Aspirin is associated with HCC cumulative incidence reduction in large studies from 8% to 4%. Similarly, compelling cohort and case-control studies demonstrated a dose-dependent relationship between coffee consumption and reduced HCC incidence. As a result, coffee consumption is recommended as a chemoprevention strategy in patients with chronic liver disease.

Surveillance The aim of surveillance is to obtain a reduction in disease-related mortality. This is usually achieved through early detection that enhances the applicability and cost-effectiveness of curative therapies. U.S. and European guidelines recommend surveillance for patients at high risk for HCC on the basis of cost-effectiveness analyses. As a general rule, high-risk populations are considered those presenting an incidence cutoff >1.5% for patients with cirrhosis and 0.2% for patients with chronic hepatitis B. However, the strength of evidence supporting surveillance is modest and is based on two randomized studies conducted in China and a meta-analysis of observational studies. Overall, these studies conclude that surveillance identifies patients with smaller tumors who are more likely to undergo curative procedures. Because of lead time bias and length time bias, it cannot be concluded that surveillance ultimately reduces HCC-related mortality.

Surveillance is recommended for cirrhotic patients due to any cause, those with HCV-related advanced fibrosis (Metavir score of F3), and patients with chronic HBV infection if Asian and aged >40 years, if African and aged >20 years, if there is a family history of HCC, or if the

patient has sufficient risk by risk scores such as PAGE-B. In terms of liver dysfunction, the presence of advanced cirrhosis (Child-Pugh class C) prevents potentially curative therapies from being employed, and thus surveillance is not recommended. As an exception, patients on the waiting list for liver transplantation, regardless of liver functional status, should be screened for HCC in order to detect tumors exceeding conventional criteria and to define priority policies for transplantation. Complex scoring systems to identify at-risk populations are not yet recommended by guidelines.

Ultrasonography every 6 months, with or without serum α fetoprotein (AFP) levels, is the recommended method of surveillance. It has a sensitivity of 65–80% and a specificity of >90% for early detection. A 3-month interval does not enhance outcomes, and survival is lower with 12-month compared with 6-month intervals. A shorter follow-up interval (every 3–4 months) is recommended when a nodule of <1 cm has been detected. Computed tomography (CT) and magnetic resonance imaging (MRI) are not recommended as screening tools due to lack of data on accuracy, high cost, and possible harm (i.e., radiation with CT). Exceptionally, these techniques can be considered in patients with obesity and fatty liver, where visualization with ultrasound is difficult. Accurate tumor biomarkers for early detection need to be developed. Use of AFP levels as a stand-alone method identifies patients with HCC with 60% sensitivity but has high false-positive results. One main limitation of AFP is that only a small proportion of early tumors (~20%) present with abnormal AFP serum levels. Combining AFP with ultrasound might increase the HCC detection rate from 8–30% compared to ultrasound and depending on the performance by experienced personnel as a stand-alone method. The accuracy of other serum biomarkers proposed, such as des- γ -carboxyprothrombin (DCP) and the L3 fraction of AFP (AFP-L3), in early detection is not known.

Despite the fact that surveillance is cost-effective in HCC, the global implementation of such programs is estimated to engage ~50% of the target population in Europe and ~30% in the United States. Public health policies encouraging the implementation of such programs should lead to an increase in early tumor detection.

Diagnosis HCC is generally diagnosed at early or intermediate stages in Western countries but at advanced stages in most Asian (except Japan) and African countries. A surveillance program yields early diagnosis in 70–80% of cases. At these stages, the tumor is asymptomatic, and diagnosis can be made by noninvasive (radiologic) or invasive (biopsy) approaches. Without surveillance, HCC is discovered either as a radiologic finding or due to cancer-related symptoms. If symptoms are present, the disease is already at an advanced stage with a median life expectancy <1 year. Symptoms include malaise, weight loss, anorexia, abdominal discomfort, or signs related to advanced liver dysfunction.

NONINVASIVE (RADIOLOGIC) DIAGNOSIS Patients enrolled in a surveillance program are diagnosed by identification of a new liver nodule on abdominal ultrasound. Noninvasive diagnostic criteria can only be applied to cirrhotic patients and are based on imaging techniques obtained by four-phase multidetector CT scan (four phases are unenhanced, arterial, venous, and delayed) or dynamic contrast-enhanced MRI. A flowchart of diagnosis and recall policy recommended by U.S. and European guidelines is summarized in Fig. 82-2. Radiologic diagnosis is achieved with a high degree of confidence if the lesion is ≥ 1 cm in diameter and shows the *radiologic hallmarks of HCC* by one imaging technique. Using contrast-enhanced imaging techniques, the typical hallmark of HCC consists of vascular uptake of the nodule in the arterial phase with washout in the portal venous or delayed phases. This radiologic pattern captures the hypervascular nature characteristic of

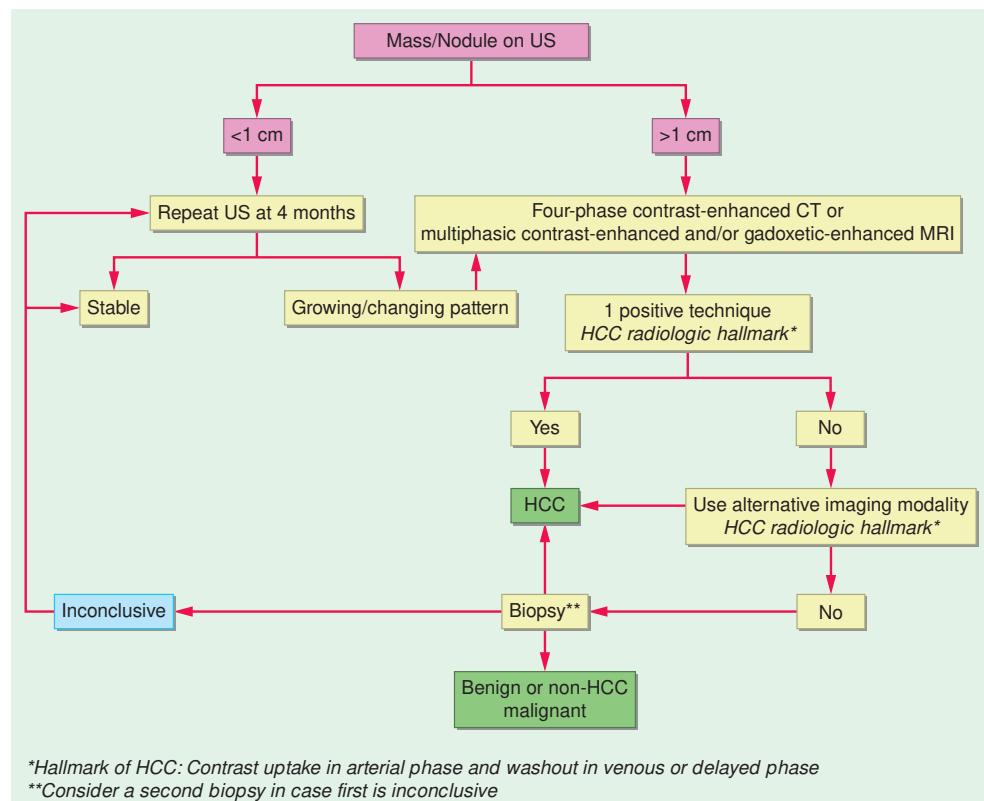


FIGURE 82-2 Recall diagnosis schedule for hepatocellular carcinoma (HCC) from the European Association for the Study of Liver Disease (EASL). **Pink color:** Size of the tumor at the time of detection by ultrasound (US). **Yellow color:** If a nodule of <1 cm is detected, repeated US at 4 months is recommended. If a nodule of >1 cm is detected, CT or MRI will be performed. Presence of *radiological hallmarks of HCC* by one imaging technique will suffice for diagnosis. This might require using one or two imaging techniques. If no diagnosis is established, then tissue biopsy would be recommended. **Green color:** Final diagnosis could be either HCC, benign tumor or non-HCC malignant. **Blue color:** If after 2 biopsies the is no conclusive diagnosis then consider follow-up with US at 4 months. (Reproduced with permission from European Association for the Study of the Liver: EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatology* 69:182-236, 2018.)

HCC. In these scenarios, the diagnostic specificity is ~95–100% and a biopsy is not necessary. Nodules <1 cm in size are unlikely to be HCC and would be very difficult to diagnose; thus, ultrasound follow-up at 3–4 months is recommended. MRI with liver-specific contrast agents is accepted as a diagnostic tool (Fig. 82-2). Contrast-enhanced ultrasound and angiography are less accurate for HCC diagnosis. Positron emission tomography (PET) scan performs poorly for early diagnosis. AFP levels ≥ 400 ng/dL are highly suspicious, but not diagnostic, of HCC according to guidelines.

The Liver Imaging Reporting and Data System (LI-RADS) has been proposed as a way of classifying radiologic findings. Essentially, nodules >10 mm visible on multiphase exams are assigned category codes reflecting their relative probability of being benign, HCC, or other hepatic malignant neoplasms. LI-RADS-1 lesions have a 0% probability of HCC, whereas lesions assigned to the LI-RADS-5 category have a 96% probability of HCC. LI-RADS-M category comprises lesions with malignant radiologic features but are not HCC malignancies in >50% of cases.

PATHOLOGIC DIAGNOSIS Pathologic diagnosis is required in two scenarios: (1) in patients without cirrhosis and (2) if imaging is not typical in at least one of two imaging techniques (CT and MRI). This occurs mainly with early-stage HCC lesions. Biopsy has not been used as the gold standard in clinical practice because of variation introduced by sampling and complications. Nonetheless, with the advent of molecular therapies and precision oncology, some guidelines advocate obtaining tissue samples in the setting of all research studies in HCC, even if radiologic criteria are met. Sensitivity of liver biopsies ranges between 70 and 90% for all tumor sizes but decreases to <50% in tumors 1–2 cm in size. The risk of complications such as tumor seeding and bleeding after liver biopsy is ~3%. Biopsies should be assessed by an expert

hepatopathologist. The use of special stains may help to resolve diagnostic uncertainties. Positive staining in two of four markers (glypican 3 [GPC3], glutamine synthetase, heat shock protein 70 [HSP70], and clathrin heavy chain) is highly specific for HCC. Gene expression blueprints (glypican 3, LYVE1, and survivin) are also able to differentiate HGDNs from early HCC. Additional staining can be considered to detect progenitor cell features (K19 and EpCAM) or assess neovascularization (CD34). A negative biopsy does not eliminate the diagnosis of HCC. A second biopsy is recommended in case of inconclusive findings or if growth or change in enhancement pattern is identified during follow-up (Fig. 82-2).

TREATMENT

Overview The landscape of management of HCC has substantially changed during the past decade. Several treatments have been adopted as standard of care according to clinical practice guidelines. For early stages, resection, liver transplantation, and local ablation have substantially improved life expectancy, with median overall survival (OS) times beyond 5 years (Fig. 82-3). For intermediate stages, transarterial chemoembolization (TACE) has improved OS from 16 months (natural history) to 20–30 months. Finally, systemic drugs for advanced tumors (atezolizumab plus bevacizumab, sorafenib, lenvatinib, regorafenib, cabozantinib, and ramucirumab) have improved median survival times from 8 months to 19 months in first-line settings and 10 months in second-line settings. Currently, several unmet needs, such as adjuvant therapies after resection or local ablation and improving outcomes at intermediate/advanced stages with combination therapies including immunotherapies, are being addressed in the setting of phase 3 investigations (Fig. 82-3).

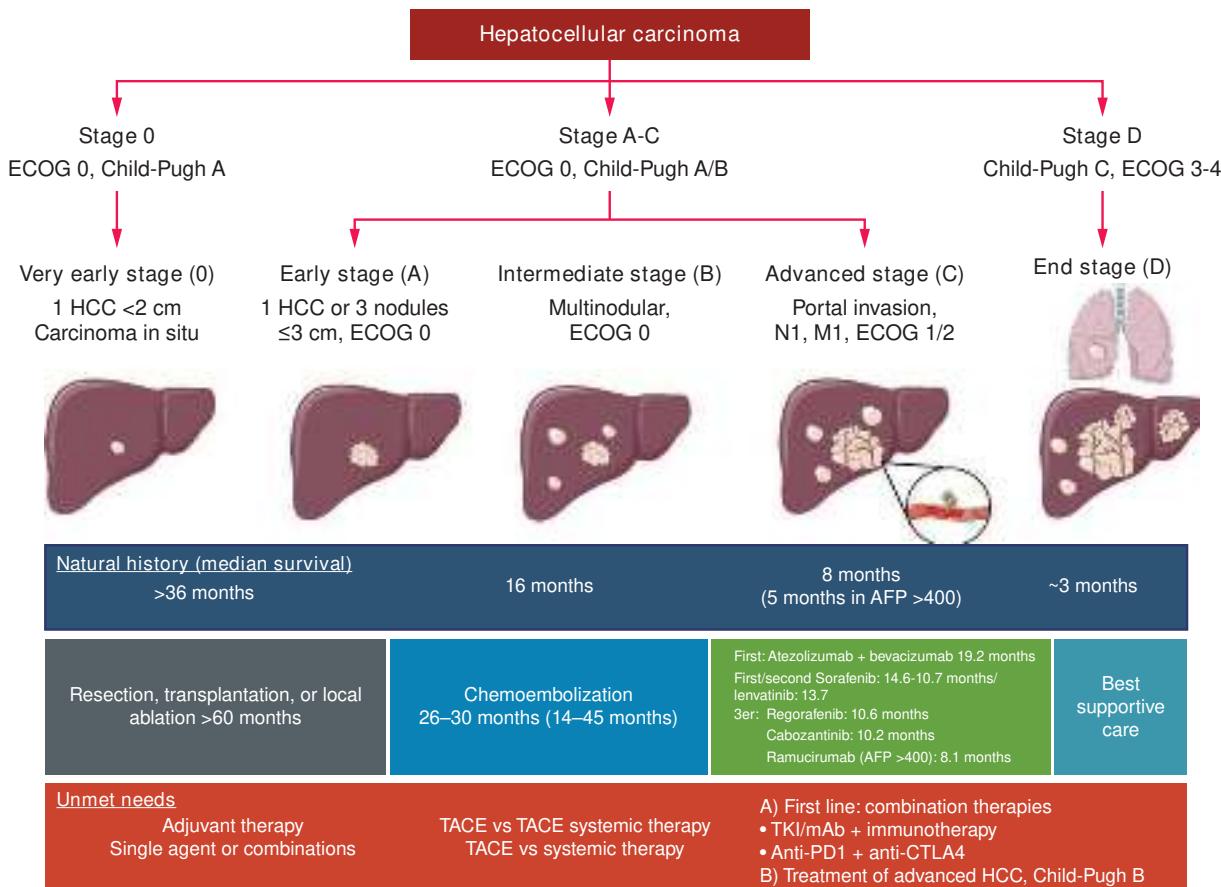


FIGURE 82-3 Natural history, impact of therapies, and unmet needs in hepatocellular carcinoma (HCC). AFP, α fetoprotein; ECOG, Eastern Cooperative Oncology Group performance status; mAb, monoclonal antibody; TACE, transarterial chemoembolization; TKI, tyrosine kinase inhibitor.

Hepatocellular carcinoma

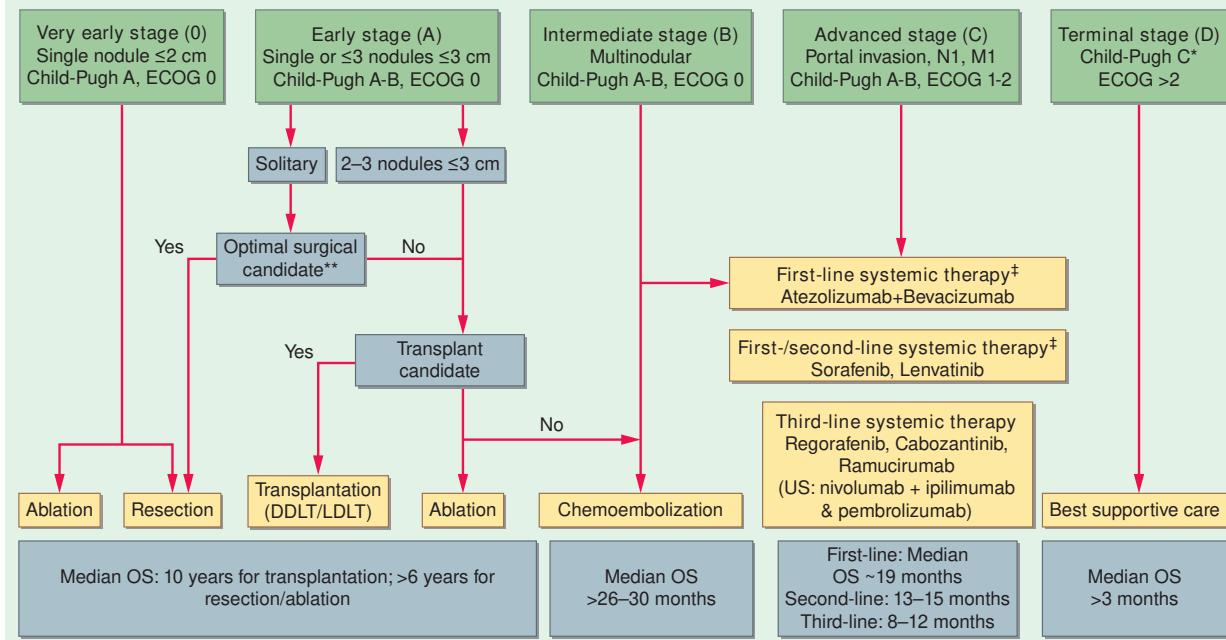


FIGURE 82-4 Staging system and therapeutic strategy. Barcelona Clinic Liver Cancer (BCLC) classification comprises five stages that select the best candidates for therapies according to evidence-based data. Patients with asymptomatic early tumors (stages 0–A) are candidates for radical therapies (resection, transplantation, or local ablation). Asymptomatic patients with multinodular hepatocellular carcinoma (HCC) (stage B) are suitable for transcatheter arterial chemoembolization (TACE), whereas patients with advanced symptomatic tumors and/or an invasive tumoral pattern (stage C) are candidates to receive systemic therapies. End-stage disease (stage D) includes patients with poor prognosis who should be treated by best supportive care. Patients with end-stage liver disease if Child-Pugh class C should first be considered for liver transplantation. [‡]Atezolizumab plus bevacizumab has been approved as new first-line treatment for advanced HCC. Nonetheless, sorafenib and lenvatinib are still considered first line options when there is a contraindication for the combination treatment. DDLT, deceased donor liver transplantation; ECOG, Eastern Cooperative Oncology Group performance status; LDLT, living donor liver transplantation; OS, overall survival. (Reproduced with permission from JM Llovet et al: Trial Design and Endpoints in Hepatocellular Carcinoma: AASLD Consensus Conference 73:158, 2021.)

Staging Systems and Treatment Allocation Staging systems are aimed at stratifying patients according to prognostic factors and outcome and allocating the best available therapies according to evidence. The most accepted staging system is the Barcelona Clinic Liver Cancer (BCLC) Classification, which is endorsed by U.S. and European clinical practice guidelines (Fig. 82-4). This staging system defines five prognostic subclasses and allocates specific treatments for each stage. The BCLC staging system has been externally validated by numerous studies. It is an evolving system that allows incorporation of new therapies and treatment-dependent variables as new evidence emerges. Ten treatments have been shown to improve survival in HCC and thus have been incorporated in the therapeutic algorithm: surgical resection, liver transplantation, radiofrequency (RF) ablation, chemoembolization, and systemic therapies (atezolizumab-bevacizumab, sorafenib, lenvatinib, regorafenib, cabozantinib, and ramucirumab). The BCLC assigns each patient to a given treatment allocation. Treatment stage migration is also applied by this scheme, meaning that if patients are not candidates for the selected therapy, the next effective therapy at more advanced stages can be given.

In HCC, three parameters are relevant for defining treatment strategy: tumor status, cancer-related symptoms, and liver dysfunction. The BCLC staging captures all three variables and allocates patients to treatments according to evidence. Since >80% of patients have two diseases, HCC and cirrhosis, a clear measurement of liver dysfunction should be in place. The prognosis of chronic liver disease is commonly assessed using the Child-Pugh score, which uses five clinical measures—total bilirubin, serum albumin, prothrombin time, ascites severity, and hepatic encephalopathy grade—to classify patients into one of three groups (A–C) of predicted survival rates. In brief, Child-Pugh class A reflects well-preserved liver function, Child-Pugh class B indicates moderate liver dysfunction with a median life expectancy of ~3 years, and Child-Pugh class C indicates severe liver dysfunction with life

expectancy of ~1 year. At early BCLC stages, more granular criteria to define patients with very-well-preserved liver function (Child-Pugh hyper-A class; those patients with normal bilirubin and without portal hypertension) need to be in place to select candidates for resection. Modifications of Child-Pugh scoring or the Model for End-Stage Liver Disease (MELD) score have not been adopted for treatment allocation, except for prioritization on the waiting list for liver transplantation (MELD score). The ALBI score, which is based only on serum albumin and bilirubin levels, has been shown to accurately stratify patients with HCC, particularly those with less severe liver dysfunction. Performance status is assessed using the Eastern Cooperative Oncology Group (ECOG) performance scale (a 5-point system where higher numbers indicate greater disability), and the presence of cancer-related symptoms (ECOG 1–2) is considered a sign of advanced stage. Patients with severe liver dysfunction (Child-Pugh class C) or performance status impairment (ECOG 3–4) are offered supportive care management.

Considering all of these prognostic and predictive variables and evidence-based treatment efficacy, five BCLC stages have been defined (Fig. 82-4). Patients with liver-only neoplastic disease, no symptoms (ECOG 0), and mild to moderate liver dysfunction (Child-Pugh A–B) can be classified as very early (stage 0) or early (stage A) or intermediate (stage B) stages depending on tumor size and number. Very early HCC (BCLC 0) is defined by single tumors ≤2 cm (if pathology is available, they should be well differentiated with absence of microvascular invasion or satellites). Early HCC (BCLC A) includes either single tumors or a maximum of three nodules of ≤3 cm in diameter. Intermediate stage (BCLC B) is defined by all other liver-only tumors. Conversely, HCC is considered at advanced stages (BCLC C) when patients present with cancer-related symptoms (ECOG 1–2) or tumors with macrovascular invasion (of any type, including branch, hepatic, or portal vein), lymph node involvement, or extrahepatic spread. Finally,

end-stage disease (BCLC D) is considered in cases of several impairment of quality of life/cancer-related symptoms (ECOG 3–4) or severe liver dysfunction (Child-Pugh C).

Around 40% of patients are diagnosed at stages 0 and A and, hence, are eligible for potentially curative therapies, resection, transplantation, or local ablation. These treatments provide median survival rates of 60 months and beyond, which are in sharp contrast with outcomes of 36 months reported in historical controls (Fig. 82-3, Table 82-2). No adjuvant therapy is recommended. Patients at intermediate stage (stage B) with preserved liver function have a documented natural history of around 16 months. These patients benefit from TACE as reported in two randomized studies and one meta-analysis and achieve an estimated survival of 25–30 months. None of the combination therapies with TACE have shown outcome advantages. Patients progressing on TACE or at advanced stage (stage C) benefit from systemic treatments. Sorafenib extends survival by ~3 months compared to placebo (from 7.9 to 10.7 months), whereas lenvatinib showed noninferiority compared to sorafenib (13.6 months vs 12.3 months, respectively). Atezolizumab (an anti-PD-L1 antibody) plus bevacizumab showed superiority compared to sorafenib (median survival 19.2 months vs 13.4 months). Three additional targeted therapies have shown improved survival compared to placebo in patients with HCC progressing on sorafenib: regorafenib, cabozantinib, and ramucirumab (only in patients with AFP >400 ng/mL). Therefore, these treatments have been adopted by guidelines and incorporated into the treatment algorithm. Patients with end-stage disease (BCLC D) should be considered for nutritional and psychological support and proper management of pain.

Although the BCLC establishes validated stages and treatment assignment according to evidence, clinical practice is not always aligned with this classification. In large cohort studies and surveys, only half of patients, or even less in Asia, are treated accordingly. Alternative

staging or scoring systems have been proposed, but none of them has acquired global consensus. In contrast to BCLC, some proposed systems capture the standard of practice in Asia, such as the Hong Kong classification or the Japan Integrated Staging score. These systems capture extended indications for resection and TACE applied in clinical practice in Asia. Finally, the tumor-node-metastasis (TNM) staging system is not used in HCC since it does not incorporate the main prognostic variables related to liver function and performance status.

Due to the complexities of HCC diagnosis and management, it is recommended that patients be sent to a referral center where all the armamentarium of therapies can be offered. In principle, patient management and outcome benefit from liver cancer multidisciplinary programs that include a hepatologist, oncologist, hepatobiliary and transplant surgeons, interventional and body imaging radiologist, hepatopathologist, and specialized nurses.

SURGICAL THERAPIES

Resection Surgical resection is the first-line option for noncirrhotic patients with early-stage HCC (BCLC 0 or A) with solitary tumors (Fig. 82-4). In cirrhotic patients, ablation competes with resection for BCLC 0 tumors (<2 cm in diameter). Which treatment is better is not defined. Cost-effectiveness approaches report a benefit for local ablation with RF. For single tumors >2 cm (BCLC A), resection remains the mainstay of treatment in patients with Child-Pugh hyper-A class, i.e., those patients with normal bilirubin and absence of portal hypertension (portal hypertension is defined by hepatic venous pressure gradient ≥10 mmHg). Surrogate measures of portal hypertension are presence of esophageal varices or platelet count <100,000/ μ L associated with splenomegaly. Anatomic resections following the functional segments of the liver are recommended to spare uninvolved

TABLE 82-2 Summary of Key Results of Randomized and Cohort Studies in the Management of Hepatocellular Carcinoma

TREATMENT OF EARLY- AND INTERMEDIATE-STAGE HCC				
TREATMENTS	HCC STAGE	TREATMENT ARMS	OUTCOMES (OS)	
Treatment for early HCC				
Resection	Early	Optimal (single nodule; no portal hypertension)	5 years: 50–70%	
		Suboptimal (multinodular or portal hypertension)	5 years: 35–55%	
Liver transplantation	Early	Milan (1 nodule <5 cm, 2–3 nodules ≤3 cm, no MVI, no EHS)	5 years: 70–80%	
	Early/intermediate	Downstaged (1 nodule ≤6.5 cm, ≤3 nodules ≤4.5 cm and total diameter ≤8 cm, no MVI, no EHS)	5 years: 60–70%	
Ablation	Early	RFA	Median: 50–60 months	
Treatments for intermediate HCC				
Transarterial therapies	Intermediate	TACE	Median: 20–32 months	
TREATMENT OF ADVANCED-STAGE HCC				
STUDY	TREATMENT	MEDIAN OS, MONTHS (HR, 95% CI)	MEDIAN PFS, MONTHS (HR, 95% CI)	ORR: M RECIST/RECIST
First-line therapies				
IMbrave150	Atezolizumab-bevacizumab	19.2 (HR 0.66, 0.52–0.85)	6.9 (HR 0.65, 0.53–0.81)	35.4%/29.8%
SHARP/REFLECT/ CheckMate-459	Sorafenib	10.7–14.6 (HR 0.69, 0.55–0.87)	3.7–3.8	2–9.2%/12.4%
REFLECT	Lenvatinib	13.6 (HR 0.92, 0.79–1.06)	7.4 (HR 0.66, 0.57–0.77)	24.1%/18.8%
Second-line therapies				
RESORCE	Regorafenib	10.6 (HR 0.63, 0.5–0.79)	3.1 (HR 0.46, 0.37–0.56)	11%/7%
CELESTIAL	Cabozantinib	10.2 (HR 0.76, 0.63–0.92)	5.2 (HR 0.44, 0.36–0.52)	NA/4%
REACH-2	Ramucirumab	8.5 (HR 0.71, 0.53–0.95)	2.8 (HR 0.45, 0.34–0.6)	NA/5%

Abbreviations: CI, confidence interval; EHS, extrahepatic spread; HCC, hepatocellular carcinoma; HR, hazard ratio; M RECIST, Modified Response Evaluation Criteria in Solid Tumors; MVI, microvascular invasion; NA, not available; NE, not evaluable; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

liver parenchyma and to remove satellite tumors. Predictors of recurrence are tumor size and number and presence of microsatellites or microvascular invasion at the specimen analysis. Outcomes in suboptimal candidates lead to 5-year survival rates of ~35–55%, as opposed to 60–70% for ideal candidates (Table 82-2). Macrovascular invasion, extrahepatic involvement, and liver dysfunction (Child-Pugh B-C) are major contraindications for resection.

ADJUVANT TREATMENTS Tumor recurrence represents the major complication of resection (and local ablation) and occurs in 70% of cases at 5 years. Most recurrences are intrahepatic metastases, but at least one-third are considered de novo tumors, new clones developing in the cirrhotic carcinogenic field. The type of recurrence can only be defined by molecular studies. So far, no adjuvant therapies have been proven to improve outcome or prevent recurrence after resection/ablation. Randomized trials testing adjuvant sorafenib, retinoids, chemotherapies, or chemoembolization have been negative, and thus, no adjuvant therapy recommendation has been established for patients after resection or local ablation.

Liver Transplantation Liver transplantation is the first treatment choice for cirrhotic patients with single tumors \leq 5 cm and portal hypertension (including Child-Pugh B and C) or with small multinodular tumors (\leq 3 nodules, each \leq 3 cm) (Fig. 82-4). These so-called Milan criteria have been validated over the years, and a meta-analysis reported 5- and 10-year survival rates of ~70 and ~50%, respectively, similar to outcomes achieved in non-HCC transplantation indications. Perioperative mortality rates have been reduced to <3%. Transplantation simultaneously cures the tumor and the underlying cirrhosis, and it is associated with a low risk of recurrence, around 10–15% at 5 years. No immunosuppressive regimens or antitumor therapies after transplantation have demonstrated any preventive effect on recurrence. Milan criteria are integrated in the treatment strategy (BCLC 0 and A) and have also been adopted by the United Network for Organ Sharing (UNOS) pretransplant staging for organ allocation in the United States (stage T2). Aside from size and number, conventional contraindications for organ transplantation procedures (e.g., ABO incompatibility, comorbidities) are applied in this setting.

Liver transplantation has a couple of factors, such as cost and donor availability, that limit this procedure to <5% of HCC cases. The scarcity of donors represents a major drawback of liver transplantation. Donor scarcity varies geographically, and deceased liver donation is almost zero in some Asian countries. Due to the shortage of donors, median waiting times in Western programs is ~6–12 months, leading to 20% of candidates dropping off the list due to tumor progression before receiving the procedure. Predictors of dropout are neoadjuvant treatment failure, baseline AFP >400 ng/mL, and steady increase of AFP level >15 ng/mL per month. Several strategies have been proposed to overcome this limitation. First, apply neoadjuvant therapies in patients on the waiting list. Neoadjuvant treatments such as TACE or RF ablation have been assessed in the setting of cohort and cost-effectiveness studies. In principle, the use of these therapies is recommended when the waiting time exceeds 6 months, even though impact on long-term outcome is uncertain. Second, a priority policy has been established for patients enlisted. UNOS has implemented a scoring system based on the dropout risk.

The Milan criteria are universally used as the basis for transplant eligibility, and adherence to these criteria yields good posttransplant survival. Modest expansion of Milan criteria applying the “up-to-seven” criterion (i.e., those HCCs having the number 7 as the sum of the size of the largest tumor and the number of tumors) in patients without microvascular invasion achieves competitive outcomes. These pathologically defined criteria are being used in clinical practice to predict the expected outcome after transplantation. Similarly, *downstaging to Milan criteria* is currently defined as the reduction of HCC burden by locoregional treatments to achieve Milan staging before transplantation. This strategy leads to long-term 10-year survival rates of ~50%. Since policies for enhancing organ donation have reached a ceiling during the past several years, alternatives to donation have emerged. Living donor liver transplantation represents a plausible alternative that accounts of ~5% of total transplants performed globally.

Outcomes reported are similar to those with deceased liver donors, and it is recommended as an alternative option in patients on a waiting list exceeding 6 months. The risks and benefits of this procedure should take into account both donor (death is estimated in 0.3%) and recipient, a concept known as *double equipoise*. Due to the complexity of this treatment, it must be restricted to centers of excellence in hepatobiliary surgery and transplantation.

■ LOCOREGIONAL THERAPIES

LOCAL ABLATION RF ablation is recommended as the primary ablative technique (Fig. 82-4). The energy generated by RF ablation (heating of tissue at 80–100°C) induces coagulative necrosis of the tumor, producing a *safety ring* in the peritumoral tissue, which might eliminate small undetected satellites. Treatment consists of one or two sessions performed using a percutaneous approach, although in some instances, ablation with laparoscopy is needed. RF ablation is more effective in response rate and time to recurrence compared with the once-conventional percutaneous ethanol injection. HCC patients treated by RF ablation have 5-year survival rates of ~60% (Table 82-2). In tumors <2 cm, RF ablation achieves complete responses in >90% of cases with good long-term outcome and is competitive with resection in cost-effectiveness as first-line option. For BCLC A cases, RF ablation is the first-line treatment for single tumors 2–5 cm or up to three nodules, each \leq 3 cm in diameter, unsuitable for surgery.

The failure rate of RF ablation increases in tumors >3 cm because of the heat loss due to perfusion-mediated tissue cooling within the area ablated. In tumors 3–5 cm in diameter, complete pathologic tumor necrosis of <50% has been reported. In particular, ~10–15% of tumors with difficult-to-treat locations, such as a subcapsular location or adjacent to the gallbladder, have a higher risk of incomplete ablation or major complications and can be approached by ethanol injection. Several approaches have been proposed to enhance the antitumor activity of RF ablation. Microwave ablation is the most widely used local image-guided technique alternative to RF. Theoretically, it provides major efficacy but higher complication rates in tumors >3 cm. Randomized trials comparing both techniques are needed. Other treatments, such as high-intensity focused ultrasound or stereotactic body radiotherapy for small tumors, have been studied in early clinical trials and are under investigation.

Chemoembolization TACE is the most widely used primary treatment for unresectable HCC worldwide and the first-line indication for patients with intermediate BCLC B stage (Fig. 82-4). Conventional chemoembolization (c-TACE) consists of the local hepatic artery administration of chemotherapy (either doxorubicin 50 mg/m² or cisplatin) mixed with an emulsion of lipiodol followed by obstruction of the feeding artery with sponge particles. c-TACE mainly benefits patients with liver-only disease, Child-Pugh A class or B class without ascites, good performance status (ECOG 0), and absence of branch or trunk vascular invasion. Median survival is ~20 months (compared to 16 months for pooled control arms). The best randomized phase 3 investigations have provided median survivals for TACE of 20–30 months in properly selected populations. Median objective response rates are 50–70%. In randomized studies, the treatment is either performed at a regular schedule of 0, 2, and 6 months (median number of sessions: 3) or on demand according to tumor response. TACE procedures should be stopped upon tumor progression or any other contraindication. Exceptionally, occurrence of a new small untreated nodule as the only progression feature can be considered for treatment. Around 50% of patients present with a limited postembolization syndrome of fever and abdominal pain related to ischemic injury and release of cytokines. Less than 5% of patients present with major complications (liver abscess, ischemic cholecystitis, or liver failure), and in <2% of cases, treatment-related death occurs.

Applicability of c-TACE in patients at intermediate stage is limited to half of cases, mostly as a result of the presence of liver failure (Child B or ascites or encephalopathy), technical contraindications to the procedure (i.e., impaired portal vein blood flow), or infiltrative/massive tumor burden (i.e., generally main tumor size >10 cm). Super-selective

TACE minimize the ischemic insult to nontumor tissue. According to guidelines, treatment-stage migration allows performing TACE on patients at early stages not suitable for surgical or ablative therapies. In selective studies, median survival times of 5 years have been reported in patients with single HCC treated by super-selective TACE. On the other hand, TACE performed beyond guidelines as a conventional practice in patients with advanced HCC yields poor outcomes.

Drug-eluting bead chemoembolization (DEB-TACE) differs from c-TACE in the use of more standardized embolic spheres of regular size embedded with chemotherapy. This strategy ensures drug release over a 1-week period, resulting in an enhancement of drug concentration within the tumor. DEB-TACE achieves similar antitumor activity (objective responses of ~60%) as c-TACE and is associated with significantly less systemic cytotoxic effects and better tolerance, but with no clear differences in clinical outcomes. Phase 2 and 3 studies have compared DEB-TACE with the combination of DEB-TACE plus sorafenib, orantinib, or brivanib, which are VEGF receptor inhibitors. Median survival in both arms of these international trials was 25–30 months.

Radioembolization and Other Intraarterial Therapies
Radioembolization using beads coated with yttrium-90 (Y-90)—an isotope that emits short-range β radiation—is the most promising alternative to TACE. Several phase II studies reported objective responses and overall outcome with a safe profile. Due to the lack of phase III trials, this treatment is currently not recommended in guidelines. Radioembolization requires prevention of severe lung shunting and intestinal radiation before the procedure. Around 20% of patients present with liver-related toxicity and 3% experience treatment-related death. Due to the minimally embolic effect of Y-90 microspheres, treatment can be

safely used in patients with portal vein thrombosis, a setting where survival results in phase II were encouraging. However, three randomised controlled trials, including two head-to-head trials against sorafenib and one trial combining radioembolization plus sorafenib versus sorafenib alone, did not show OS endpoint superiority. Thus, these treatments are not indicated in the advanced-stage scenario.

TACE should be distinguished from other intraarterial therapies, such as chemo-lipiodolization, which involves the delivery of an emulsion of chemotherapy mixed with lipiodol; bland transcatheter embolization, where no chemotherapeutic agent is delivered; and intraarterial chemotherapy, where no embolization is performed. None of these approaches is recommended due to the lack of survival benefit.

■ SYSTEMIC THERAPIES

Conventional systemic chemotherapy and radiotherapy have not produced survival advantages. Randomized studies also failed to show benefit with antiestrogen therapies and vitamin D derivatives. External-beam liver-directed radiotherapy (stereotactic body radiotherapy) efficacy is currently being tested with and without sorafenib in phase III trials. In 2007, a phase III trial demonstrated survival benefits for patients with advanced-stage disease treated with sorafenib, and lenvatinib showed similar effects to sorafenib in first-line treatment. Recently, the combination of atezolizumab with bevacizumab demonstrated survival benefits in the advanced setting when compared to sorafenib and has now become the standard first-line treatment. Three additional therapies, regorafenib, cabozantinib, and ramucirumab (only in patients with AFP >400 ng/mL), have been shown to benefit patients progressing on sorafenib (Fig. 82-5).

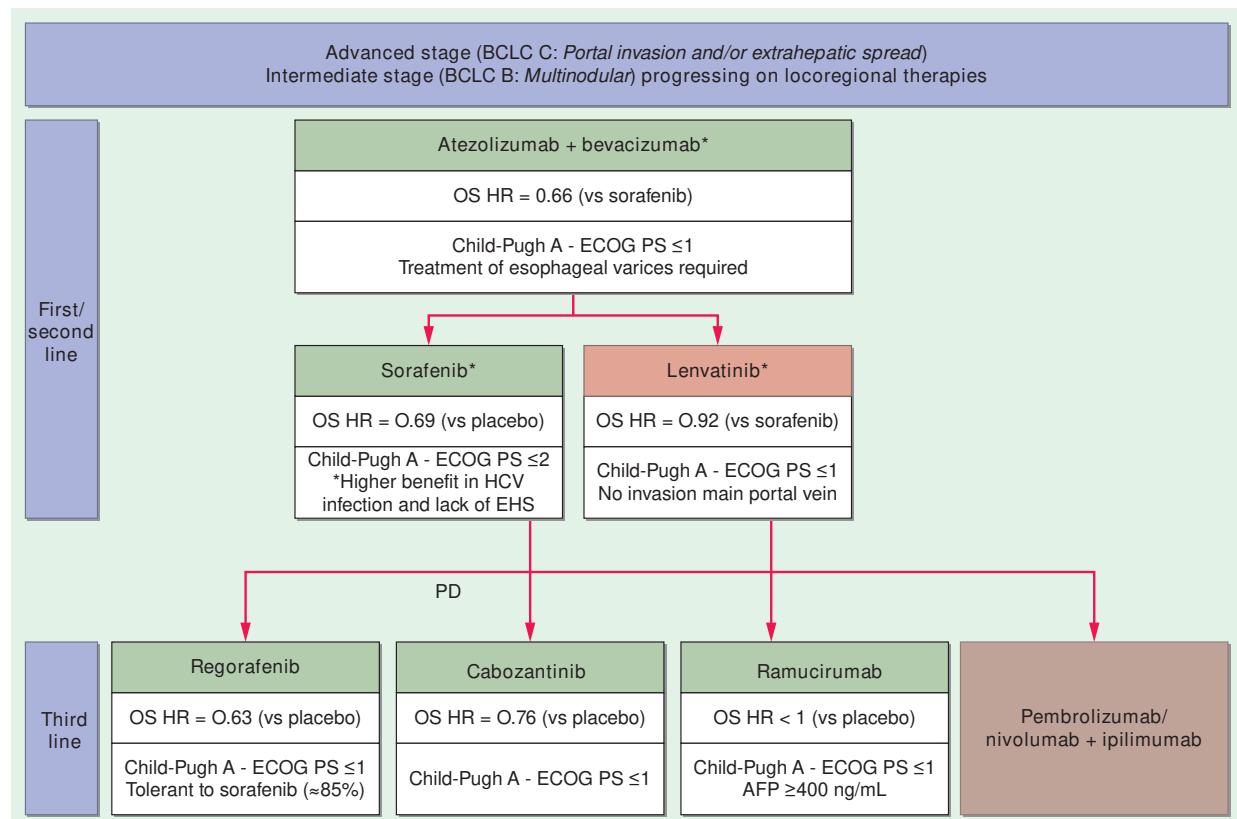


FIGURE 82-5 Treatment strategy for advanced hepatocellular carcinoma with systemic therapies. Drugs in green have positive results from phase 3 trials with a superiority design (atezolizumab plus bevacizumab, sorafenib, regorafenib, cabozantinib, and ramucirumab). Drugs in orange have positive results from phase 3 trials with a noninferiority design (lenvatinib vs sorafenib). Drugs in red have received accelerated approval from the U.S. Food and Drug Administration (FDA) based on promising efficacy results in phase 2 trials in the second-line setting (nivolumab, pembrolizumab, and nivolumab ipilimumab). Key details of the patient populations are provided. *Around 20% of patients can receive sorafenib or lenvatinib in first line due to contraindications to atezolizumab + bevacizumab. AFP, α fetoprotein; BCLC, Barcelona Clinic Liver Cancer (classification); ECOG PS, Eastern Cooperative Oncology Group performance status; EHS, extrahepatic spread; HCV, hepatitis C virus; HR, hazard ratio; mRECIST, modified Response Evaluation Criteria in Solid Tumors; OS, overall survival. (Reproduced with permission from JM Llovet: Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol* 15:599, 2018.)

652 Molecular Targeted Therapies Atezolizumab (anti-PD-L1 checkpoint inhibitor) plus bevacizumab (antibody against VEGFA) has become the standard of care in first-line treatment for advanced HCC as a result of a positive phase 3 trial indicating superiority versus sorafenib in terms of survival (Fig. 82-5). Median survival with the combination was 19.2 months compared with 13.4 months for sorafenib. Combination treatment also improved progression-free survival and patient-reported quality of life outcomes. Objective response to the combination was 35.4% versus 13.9% for sorafenib. Adverse events also favored the combination (grade 3–4 adverse events, 36% vs 50% for sorafenib). The most common side effects associated with the combination were hypertension, proteinuria, and low-grade diarrhea, whereas autoimmune events were infrequent. Treatment-related adverse events leading to discontinuation of these two drugs was 15%. Upper gastrointestinal endoscopies are required before initiating the combination therapy for detection and treatment of varices to mitigate the risk of bleeding associated with bevacizumab. Thus, screening for varices is becoming standard before first-line therapy in HCC management.

Alternatively, sorafenib and lenvatinib are indicated for HCC in patients with well-preserved liver function (Child-Pugh class A) and with advanced tumors either as first-line treatment in patients with contraindications or with progression to the combination therapy

(Fig. 82-5). A phase III study comparing sorafenib versus placebo showed increased survival from 7.9 months to 10.7 months (hazard ratio [HR] 0.69; 31% reduction in risk of death). Patients with HCV-related HCC achieve significantly better outcomes with sorafenib, with a median survival of 14 months. No predictive biomarkers of responsiveness to sorafenib have been identified. The recommended daily dose of sorafenib is 800 mg. Median treatment duration is about 6 months. Treatment is associated with adverse events, such as diarrhea, hand-foot skin reactions, fatigue, and hypertension. These toxicities lead to treatment discontinuation in 15% of patients and dose reduction in up to half. This therapy cannot be administered to around one-third of the targeted patients due to primary intolerance, advanced age, or liver failure (ascites or encephalopathy). Active vascular disease, either coronary or peripheral, is considered a formal contraindication.

The efficacy of sorafenib probably results from a balance between targeting cancer cells and the microenvironment by blocking up to 40 kinases, including antiangiogenic (VEGF receptor [VEGFR], platelet-derived growth factor receptor [PDGFR]) and antiproliferative drivers (serine/threonine-protein kinase B-raf [BRAF] and mast/stem cell growth factor receptor [c-Kit]) (Fig. 82-6). Median time to progression on sorafenib is 4–5 months in phase III trials.

Another alternative to sorafenib is the multikinase inhibitor lenvatinib; it was noninferior in a phase 3 investigation (13.6 months vs

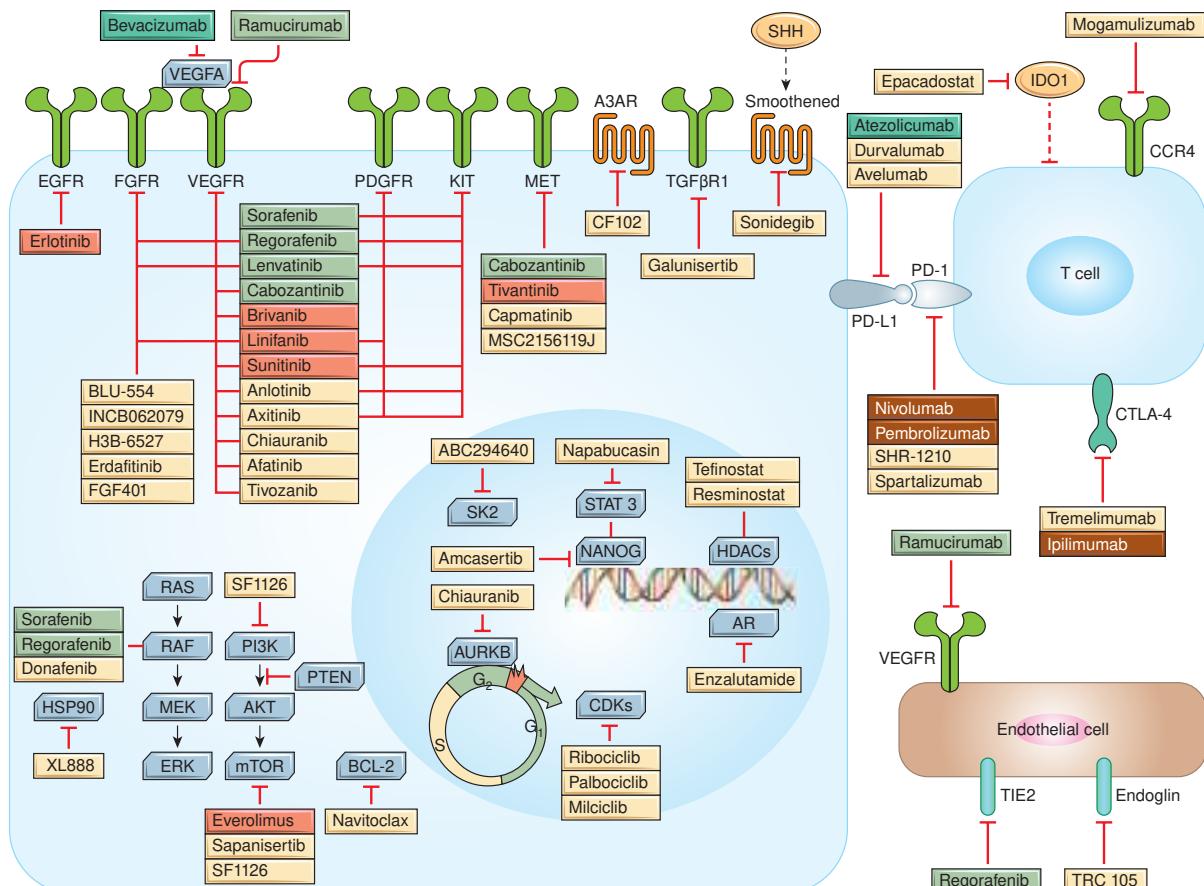


FIGURE 82-6 Molecularly targeted therapies for hepatocellular carcinoma and their target signaling pathways. Green boxes indicate drugs with positive results from phase 3 trials (atezolizumab plus bevacizumab, sorafenib, lenvatinib, regorafenib, lenvatinib, cabozantinib, and ramucirumab). Red boxes indicate drugs with negative results from phase 3 trials (everolimus, sunitinib, linifanib, erlotinib, brivanib, and tivantinib). Drugs in yellow boxes are currently in development for hepatocellular carcinoma in phase 1, 2, or 3 clinical trials. Brown boxes indicate drugs approved based on phase 2 trial data (pembrolizumab, nivolumab + ipilimumab). Dashed arrows and lines indicate indirect activities. A3AR, adenosine receptor A3; AR, androgen receptor; AURKB, aurora kinase B; BCL-2, apoptosis regulator BCL-2; COP4, CC-chemokine receptor 4; CDKs, cyclin-dependent kinases; CTLA-4, cytotoxic T lymphocyte protein 4; HDAC, histone deacetylase; HSP90, heat shock protein 90; IDO1, indoleamine 2,3-dioxygenase 1; NANOG, homeobox protein NANOG; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; SHH, Sonic hedgehog; STAT3, signal transducer and activator of transcription 3; TIE-2, angiopoietin 1 receptor. (Reproduced with permission from JM Llovet: Molecular therapies and precision medicine for hepatocellular carcinoma. Nat Rev Clin Oncol 15:599, 2018.)

12.3 months; HR 0.92) (Fig. 82-5). Lenvatinib induces objective responses in 24% of cases. The main side effects are hypertension, proteinuria, asthenia, diarrhea, and weight loss. This treatment induced grade 3–4 drug-related adverse events in 55% of patients, resulting in a withdrawal rate of ~15%.

Three drugs (regorafenib, cabozantinib, and ramucirumab) have shown survival benefits versus placebo in patients progressing on sorafenib, and two additional immune-based treatments have been approved by the U.S. Food and Drug Administration (FDA) based on promising phase 2 data (pembrolizumab and nivolumab plus ipilimumab) (Fig. 82-5). The median survival of patients progressing on first-line treatment is 8 months (obtained from patients allocated to the placebo arm).

A phase III study comparing regorafenib (a more potent multikinase inhibitor than sorafenib targeting similar kinases) versus placebo in patients progressing on sorafenib reported a benefit in survival from 7.8 to 10.6 months (HR 0.62; 38% reduction in risk of death) (Fig. 82-5). Response rate was 10%. Median time on treatment was 3.5 months. Prevalence of toxicity (hand-foot reaction, fatigue, and hypertension) was higher compared with reported toxicity from sorafenib, but adverse events only led to treatment discontinuation in 10% of cases. Cabozantinib, a multikinase VEGFR inhibitor with activity against both AXL and c-MET (Fig. 82-6), improves survival compared to placebo after progression on sorafenib (10.2 months for cabozantinib vs 8.0 months in the placebo arm; HR 0.76). The most common grade 3–4 adverse events were palmar-plantar erythrodysesthesia, hypertension, increased aspartate aminotransferase level, fatigue, and diarrhea. Ramucirumab, an anti-VEGFR-2 monoclonal antibody, is the only biomarker-guided therapy in HCC based on AFP levels. The randomized, placebo-controlled, phase 3 REACH-2 study selected patients with advanced HCC in second line with baseline AFP ≥ 400 ng/dL. Median survival for patients treated with ramucirumab was 8.1 months, compared to 5 months for patients receiving placebo. The most common grade 3–4 adverse events were hypertension, hyponatremia, and increased aspartate aminotransferase. Patients progressing after second-line therapy and patients with BCLC D stage should receive best supportive palliative care, including management of pain, nutrition, and psychological support.

Immunotherapy and Combinations The combination of the anti-PD-L1 antibody atezolizumab with the VEGFA inhibitor bevacizumab is the first regimen to improve survival in the first-line setting compared to sorafenib. In addition, two additional treatment regimens involving immunotherapies have been approved by the FDA as second-line therapies based on phase 2 data. Single-agent checkpoint inhibitor treatments, such as nivolumab and pembrolizumab, are associated with objective responses of 15–20%, which are durable in time, generally beyond 12 months. Less than 30% of patients experience grade 3–4 treatment-related adverse events. Neither regimen hit the primary endpoint of improved survival in phase 3 investigations compared with sorafenib (nivolumab) or placebo (pembrolizumab). The median survival for nivolumab of 16.4 months in first-line treatment was not superior to the survival time of 14.7 months for sorafenib. Similarly, in the second-line setting, the median survival for pembrolizumab of 13.9 months was not superior to the median survival of 10.6 months for placebo. Emerging regimens have shown signals of efficacy, such as lenvatinib plus pembrolizumab in first-line patients with advanced HCC and the combination of an anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) in second-line patients.

CHOLANGIOPAPILLARY CARCINOMA

Cholangiocarcinoma (CCA) is classified according to its anatomic location as intrahepatic (iCCA; ~20–30%), perihilar (pCCA; ~50–60%), and distal (dCCA; ~20–30%). The latter two are also known as extrahepatic cholangiocarcinomas (eCCAs), with the second-order bile ducts acting as the separation point (Fig. 82-7). This classification is endorsed by the eighth edition of the *American Joint Committee on Cancer (AJCC) Staging Manual*. In addition, iCCA has been recognized as a distinct entity with specific ad hoc clinical practice guidelines. Treatment options beyond surgery are limited, and few molecular targeted therapies have been approved for its treatment. The three subtypes of CCA differ in their anatomic location, epidemiology and risk factors, cell of origin, pathogenesis, and treatment. iCCA originates from adult cholangiocytes, trans-differentiation of adult hepatocytes, and hepatic progenitor cells (cholangiocyte precursors) (Fig. 82-8), as opposed to HCC, which originates only from hepatic progenitor cells or adult hepatocytes. Mixed HCC-iCCA originates from hepatic progenitor cells, whereas eCCA arises from the biliary epithelium and peribiliary glands. Moreover, their mutational profiles also differ. *FGFR2* fusions and *IDH1/2* mutations mostly occur in iCCA, whereas *ERBB2/3* amplifications and *SMAD4* aberrations are characteristic of eCCA. Thus, clinical management and trials testing molecular therapies should be tailored according to each biological/anatomical subtype of CCA, as opposed to a common approach for all biliary tract cancers.

■ EPIDEMIOLOGY, RISK FACTORS, AND MOLECULAR TRAITS

CCA is the second most common liver cancer after HCC, with a 5-year survival of 10%. iCCA has globally increasing incidence and mortality rates. The incidence of iCCA varies according to exposure to risk factors, ranging from 1–2 cases per 100,000 inhabitants in Europe and North America to the highest incidence in some areas of Southeast Asia, particularly in Thailand (>80 cases per 100,000 inhabitants). The male-to-female ratio is 1.2. Overall, most cases occur with unknown risk factors. The classical risk factors for CCA development include primary sclerosing cholangitis (PSC), biliary duct cysts, hepatolithiasis, and Caroli's disease (congenital cystic dilation of the intrahepatic biliary tree). Parasitic biliary infestation with flukes (i.e., most common is *Opisthorchis viverrini* and *Clonorchis sinensis*) is a prevalent etiology in Asia that can be prevented with the antihelminth therapy praziquantel. PSC is a clear risk factor for iCCA and pCCA development, with a

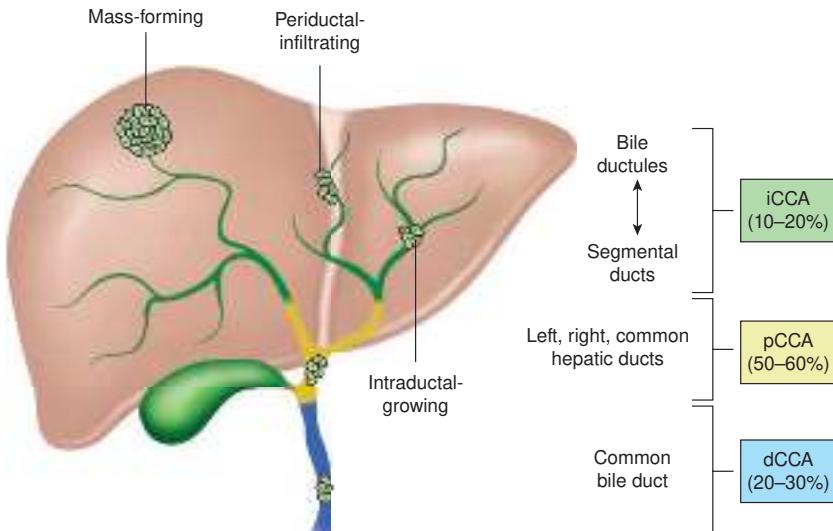


FIGURE 82-7 Anatomical classification of cholangiocarcinoma. Cholangiocarcinoma (CCA) is classified as intrahepatic (iCCA) and extrahepatic (eCCA). eCCA can be subclassified as perihilar (pCCA) and distal (dCCA). (Reprinted with permission from JM Banales et al: Cholangiocarcinoma 2020: The next horizon in mechanisms and management. *Nat Rev Gastroenterol Hepatol* 17:557, 2020.)

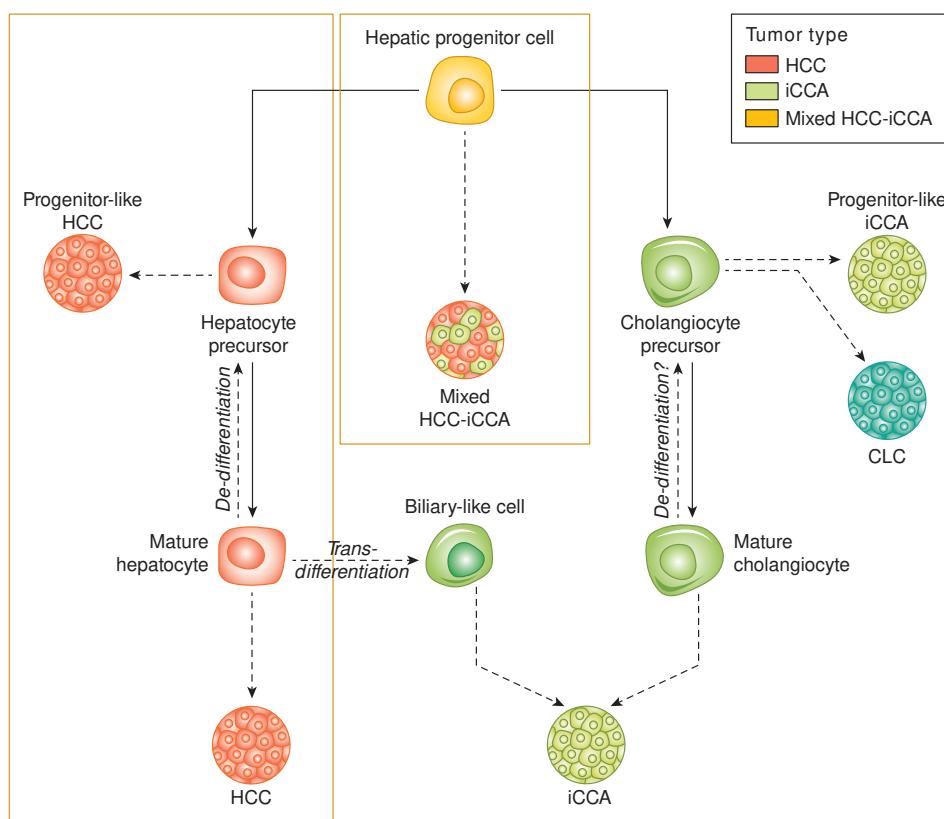


FIGURE 82-8 Cell of origin of liver cancer. Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) can develop from the neoplastic transformation of mature hepatocytes and cholangiocytes, respectively. There is evidence showing that hepatic progenitor cells (HPCs), their intermediate states, or de-differentiated hepatocytes can originate liver cancers with progenitor-like features, including mixed HCC-iCCA (e.g., cholangiocellular carcinoma [CLC]). Mature hepatocytes can be also reprogrammed into cells that closely resemble biliary epithelial cells and induce the onset of iCCA. (Printed with permission from ©Mount Sinai Health System.)

lifetime incidence ranging from 5 to 10%. Surveillance in PSC patients is recommended with annual imaging techniques and CA 19-9 serum determination. Common risk factors for HCC, such as HBV and HCV infection and cirrhosis, have been associated with iCCA development. Sweetened beverages were reported to constitute an additional risk factor in the development of eCCA and gallbladder carcinoma in a population cohort study.

Molecular Classification and Drivers No molecular classification of CCA has been established. Genomic studies have provided insight on two subclasses of iCCA, a proliferation subclass—characterized by activation of oncogenic signaling pathways (including RAS and MET)—and an inflammation subclass, characterized by activation of inflammatory pathways, overexpression of cytokines, and STAT3 activation. Similarly, a molecular classification of eCCA has been proposed, dividing tumors into four categories (metabolic, proliferation, mesenchymal, and immune) based on molecular traits. The hypothesis that the proliferation class with enrichment of *ERBB2/3* mutations might respond to monoclonal antibodies against this receptor and the immune class might respond to checkpoint inhibitors has not been tested or confirmed. The iCCA mutation portrait is characterized by ~50–60% of tumors having at least one targetable driver including *FGFR2* fusion events (~25%); mutations in *IDH1/2* (15%), *KRAS* (15%), *BRAF* (5%), and *EGFR* (3%); and amplifications in *FGF19/CCDN1* (4%). Although mutations in *TP53* (~30%) and *KRAS* (~25%) are more common in eCCA than in iCCA, some molecular drivers are specific for subtypes, such as fusion of *PRKACA* or *PRKACB* for eCCA or *ERBB2* amplifications (~20%) for gallbladder cancer. Liver fluke-associated CCAs have a higher incidence of *TP53* and *SMAD4* mutations. Host genetic polymorphisms predisposing to CCA have not been established.

■ INTRAHEPATIC CHOLANGIOPRINCIPAL CANCER

Diagnosis Diagnosis of iCCA requires pathologic confirmation. Guidelines currently do not recommend surveillance for early diagnosis because at-risk populations are ill-defined. Cirrhotic patients at risk of HCC development are enrolled in surveillance programs and can benefit from early detection of iCCA. Otherwise, incidental diagnosis occurs due to cross-sectional imaging performed for other reasons. In most cases, iCCA is diagnosed at advanced stages where symptoms such as weight loss, malaise, abdominal discomfort, or jaundice are present. Pathologic diagnosis of iCCA is based on the World Health Organization (WHO) criteria. Differential diagnosis should be established with metastatic adenocarcinoma and mixed iCCA-HCC tumors, which may require evaluation of markers such as Hep-Par-1, GPC3, HSP70, and glutamine synthetase markers. Imaging studies with CT/MRI are not accurate enough to establish iCCA noninvasive diagnosis. Dynamic CT scanning characterizes 80% of iCCAs as liver mass-forming tumors with progressive contrast uptake from the arterial to the venous/delayed phase. MRI dynamic images also show peripheral enhancement in the arterial phase followed by progressive filling in of the tumor. Atypical radiologic behavior with arterial enhancement recapitulating HCC occurs in 10% of cases. MRI with cholangiopancreatography is useful to visualize the ductal system and vascular structures. Guidelines do not recommend PET scan for diagnosis. Tumor biomarker CA 19-9 at a cutoff level of 100 U/mL has prognostic significance but lacks accuracy (sensitivity and specificity of ~60%) for early diagnosis.

Radiologic criteria are inadequate for iCCA diagnosis in cirrhotic patients. However, in noncirrhotic patients, guidelines endorse a presumed diagnosis of iCCA (i.e., venous phase contrast enhancement on dynamic CT/MRI) if resection is considered. Assessment of disease

extent (venous or arterial invasion and extrahepatic disease) and resectability is best accomplished with CT and/or MRI studies. Doppler ultrasound is accurate in defining vascular invasion. Before surgery, PET scanning may be considered to rule out an occult primary or metastatic site.

Staging System The staging system for iCCA resected cases is based on the TNM staging as per the eighth edition of the AJCC/International Union Against Cancer (UICC) staging. T1 tumors are solitary without vascular invasion and can be divided into T1a or T1b if tumor size is ≤ 5 cm or >5 cm, respectively; T2 disease includes multiple tumors (e.g., multifocal disease, satellitosis, intrahepatic metastasis) or presence of vascular invasion (microvascular or major vascular invasion); T3 tumors perforate the visceral peritoneum; and T4 disease includes tumors involving local extrahepatic structures by direct invasion. Regional lymph node metastasis in the hilar, periduodenal, and peripancreatic nodes is considered N1 disease, while distant spread is considered M1 disease. TNM stages IA, IB, II, and IIIA overlap with T status, whereas stage IIIB includes T4N0 or N1M0 disease and stage IV includes M1 disease.

TREATMENT

After adopting the TNM staging system, the International Liver Cancer Association (ILCA) guidelines for management of iCCA proposed a treatment algorithm (Fig. 82-9), adapted and updated with the new treatment modalities accepted. Overall, most of the treatments endorsed have a modest level of evidence. Surgical resection represents the sole curative treatment option in 30–40% of patients, with

a median survival of 51 months in properly selected candidates. In noncirrhotic individuals, the best candidates for resection are patients at TNM stage I–II, whereas in patients with cirrhosis, liver function should be assessed as previously described for HCC. Preoperative disease assessment should discard vascular invasion, N1, and M1. Lymphadenectomy of regional nodes is recommended given its prognostic value. The main predictors of recurrence (~50–60% at 3 years) and survival are identified at the pathologic examination, including presence of vascular invasion, lymph node metastases, and poor differentiation. A phase 3 trial (BILCAP trial) including all types of CCA in a prespecified per-protocol analysis reported improved survival with adjuvant therapy (53 months vs 36 months; adjusted HR 0.75). Based on this trial, American Society of Clinical Oncology guidelines recommend adjuvant capecitabine for a period of 6 months. Other adjuvant regimens, such as gemcitabine monotherapy or a combination of gemcitabine and oxaliplatin, did not improve OS. Liver transplantation remains controversial, and few studies have reported good outcomes for single tumors ≤ 5 cm.

Nonsurgical candidates have a dismal life expectancy. Overall, patients at stage III might be considered for locoregional therapies, such as chemoembolization or radioembolization, but the level of evidence is low and is mostly based on cohort studies. A meta-analysis of 14 trials testing locoregional therapies reported median survival times of 15 months. External-beam radiation therapy is not recommended as standard therapy. At more advanced stages (stage IV) in patients with an ECOG of 0–1, systemic chemotherapy with the combination of gemcitabine and cisplatin is considered the standard of practice, yielding median survival times of 11.7 months compared to 8 months

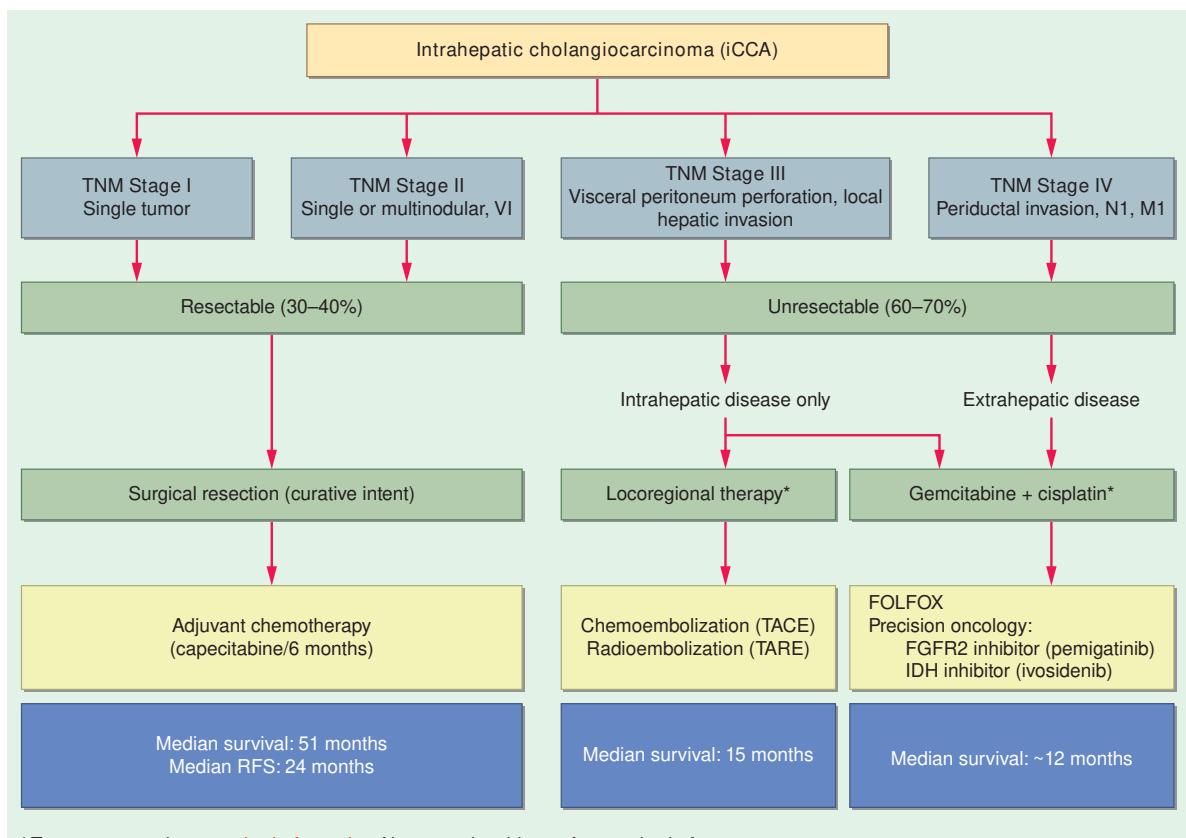


FIGURE 82-9 Staging and treatment schedule for intrahepatic cholangiocarcinoma (iCCA) proposed by the International Liver Cancer Association. FOLFOX, leucovorin, fluorouracil, and oxaliplatin; RFS, recurrence-free survival; TACE, transcatheter arterial chemoembolization; TARE, transarterial radioembolization; TNM, tumor-node-metastasis. (Reproduced with permission from J Bridgewater et al: Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma. *J Hepatol* 60:1268, 2014.)

for gemcitabine alone. This recommendation for first-line treatment of advanced tumors is based on a subgroup analysis of 80 iCCA patients included in a large randomized phase III trial (n = 410, ABC-02 Trial) of patients with advanced biliary tract tumors. In the second-line setting, a phase 3 study randomized patients who had progressed on cisplatin and gemcitabine to mFOLFOX (leucovorin, fluorouracil, and oxaliplatin) versus best supportive care. The chemotherapy regimen showed an improvement in median OS to 6.2 months (adjusted HR 0.69).

Two molecular targeted therapies have been approved in the second-line setting in iCCA patients with *IDH1/2* mutations or *FGFR2* aberrations. A phase 3 trial compared ivosidenib, an *IDH-1* inhibitor, versus placebo; ivosidenib improved progression-free survival (2.7 vs 1.4 months; HR 0.37) and OS. A single-arm phase 2 study assessing pemigatinib (*FGFR2* inhibitor) in iCCA patients with *FGFR2* fusions showed a median survival of 21 months and an objective response rate of 35%.

Mixed HCC-iCCA is a rare neoplasm accounting for <0.5% of all primary liver cancers. Diagnosis is based on pathology. The 2010 WHO classification defined two subtypes: the classical and the stem cell feature type. Molecular data have defined a third unique entity, cholangiocellular carcinoma, with distinct molecular traits and better outcome. Due to its low incidence, the demographic features and clinical behavior of these tumors remain ill-defined. Survival and management are similar to iCCA.

■ EXTRAHEPATIC CHOLANGIOCARCINOMA

Perihilar and Distal Cholangiocarcinoma The eighth edition AJCC/UICC TNM staging classification has established pCCAs as tumors that arise between the second-order bile ducts up to the insertion of the cystic duct, whereas dCCAs arise from this point to the ampulla of Vater (Fig. 82-7). Thus, dCCA can be difficult to distinguish from early pancreatic cancer. Both entities have a similar diagnostic approach. Acute onset of painless jaundice occurs in 90% of patients with pCCA, and 10% present with cholangitis. Primary biliary cholangitis with a cutoff for CA 19-9 >129 U/mL is suspicious for CCA. Imaging assessment starts with CT and MRI; they have a good sensitivity and specificity (>85%) for detecting the degree of bile duct involvement and hepatic and portal vein invasion. MRI cholangiography is optimal for defining the extent of the bile duct lesion. Ruling out IgG4 cholangiopathy by assessing serum IgG4 is mandatory. As a second step, endoscopic retrograde cholangiography with brushing to explore cytology and fluorescence in situ hybridization (FISH) for exploring polysomy are recommended. FISH enhances the sensitivity of cytology from 20 to ~40%.

Diagnosis is based on pathology. The treatment algorithm for pCCA indicates that in cases of a dominant stricture with positive cytology/biopsy or polysomy, a lymph node biopsy via endoscopic ultrasound should be obtained. pCCA with negative lymph node involvement is best treated by surgery, resection, or transplantation, the sole curative options. Staging laparoscopy is recommended to exclude metastatic disease before surgery; metastases occur in 15% of cases. Resection entails hepatic and bile duct removal and Roux-en-Y-hepaticojunostomy with regional lymphadenectomy. Bilobular involvement is considered a surgical contraindication. Perioperative mortality is as high as 10%, mostly as a result of liver failure. In a few referral centers, unresectable single pCCA <3 cm without dissemination can be considered for liver transplantation with neoadjuvant chemoradiation. This procedure is associated with 5-year survival rates of ~70%. If lymph node involvement is present, systemic chemotherapy can be considered along with biliary tract stenting. Surgical resection (Whipple procedure) is the primary option for management of dCCA, a procedure that achieves a median survival of 2 years and 5-year survival rates of ~25%. Main contraindications for resection are presence of distant lymph node involvement, metastases, or major vascular invasion. At the pathologic examination, perineural invasion, lymph node metastasis, R0 resection (absence of residual tumor at pathologic examination), and tumor differentiation are predictors of survival. Adjuvant therapy with capecitabine for 6 months is accepted based on the BILCAP study.

Consensus statements endorse first- and second-line chemotherapy strategies for unresectable eCCA similar as for iCCA. No molecular targeted therapies are available for these entities.

■ GALLBLADDER CANCER

Gallbladder cancer is the most common cancer of the biliary tract worldwide. The estimated cases of gallbladder cancer in the United States in 2020 were 11,980, more than CCA. The female-to-male ratio is 3:1. Cholelithiasis is the major risk factor, but <1% of patients with cholelithiasis develop this cancer. Gallbladder polyps at risk of transformation are those ≥10 mm in diameter. Early cases are discovered incidentally at routine cholecystectomy. Clinical symptoms, such as jaundice, pain, and weight loss, are associated with advanced stages. Staging of gallbladder cancer follows the TNM classification. The most accurate technique to define staging and vascular and biliary tract invasion is magnetic resonance cholangiopancreatography. CT and PET scans can be also useful for preoperative staging.

The mainstay of treatment is surgical, either simple or radical cholecystectomy (partial hepatectomy and regional lymph node dissection) for stage I or II disease, respectively. Only ~20% of patients are candidates for surgery with curative intent. Survival rates are near 80–90% at 5 years for stage I disease and range from 60 to 90% at 5 years for stage II disease. Regional nodal status and the depth of tumor invasion (T status) are the two most important prognostic factors. Adjuvant therapy with capecitabine is recommended in R0 cases. Gallbladder cancers at stage III and IV are considered unresectable. For patients with ECOG of 0–1, chemotherapy with gemcitabine and cisplatin is the standard of practice based on data from the subgroup analysis including 181 patients with gallbladder cancer in the setting of two clinical trials. Overall, median survival is 10–12 months in advanced cases. Percutaneous transhepatic drainage is indicated in case of biliary obstruction. Radiotherapy is not effective.

OTHER MALIGNANT LIVER TUMORS

■ FIBROLAMELLAR HEPATOCELLULAR CARCINOMA

Fibrolamellar hepatocellular carcinoma (FLC) is a rare form of primary liver cancer that typically affects children and young adults (10–30 years of age) without background liver disease. FLC accounts for 0.85% of all primary hepatic malignancies in the United States, and its incidence rate is 0.02 cases per 100,000 inhabitants. FLC is considered a unique entity with a specific fusion oncogene *PRKACA-DNAJB1* present in 80–100% of cases. A few mutations have been described, all at a level of <10%. FLC has a better prognosis than HCC, probably due to the absence of cirrhosis and the earlier age of presentation. Surgical resection is the mainstay of treatment, and indications are less restrictive than for HCC. A retrospective series of 575 FLC cases reported a median survival of 70 months after resection. At advanced stages, the expected outcome is <20 months. Chemotherapy is not effective, and there is no standard of care.

■ HEPATOBLASTOMA

Hepatoblastoma (HB) is the most frequent primary liver tumor in children. The incidence of the disease is 1.5 cases per 1,000,000. Background liver disease is rare in these patients. WNT signaling plays a major role, with *CTNNB1* mutations (70%) as the most frequently reported molecular event. Overexpression of IGF2 and genes in the 14q32 *DLK1/DIO3* locus are also prevalent. Resection followed by chemotherapy with doxorubicin is the mainstay treatment strategy. A study including 1605 patients randomized in eight clinical trials reported better outcome for patients with stage I-II of the PRETEXT (Pretreatment Extent of Tumor) classification (out of four stages), age <3 years, AFP >1000 ng/mL, and absence of metastases. As opposed to HCC, low AFP indicates poor prognosis. The best candidates (stage I or II with small tumors, age <3 years, and AFP >100 ng/mL) achieve 5-year disease-free survival after resection of 90%, compared with 5-year disease-free survival of 20–30% in the worst candidates (metastatic disease and AFP <100 ng/mL).

BENIGN LIVER TUMORS

The most common benign liver tumors are hemangiomas, focal nodular hyperplasia (FNH), and hepatocellular adenomas (HCA). Most benign tumors are identified incidentally by abdominal ultrasound or other imaging techniques. *Hemangiomas* are present in ~5% of the general population and are diagnosed by ultrasound, except in cirrhotic patients or oncology patients in whom contrast-enhanced imaging (contrast-enhanced ultrasound, CT, or MRI) is required. Conservative management is appropriate and follow-up is not recommended. Exceptionally, growing lesions causing symptoms by compression can be considered for resection. FNH is a benign tumor present in <2% of the population and occurs mostly in females aged 40–50 years. FNH is a polyclonal hepatocellular proliferation due to an arterial malformation. MRI has the highest diagnostic accuracy with a specificity of 100% when typical imaging features are present (homogeneous enhancement in the arterial phase with a central scar). Atypical FNH requires biopsy for diagnosis. Treatment is not recommended since these tumors do not degenerate or cause complications. In exceptional cases of expanding symptomatic lesions, surgery is the treatment of choice.

Hepatic adenomas are clonal benign proliferations resulting from single-gene driver mutations. HCAs have a low prevalence of 0.001% of the population and are frequently diagnosed in women aged 35–40 years. The female-to-male ratio is 10:1, and the main risk factors are oral contraceptives in females and use of anabolic androgenic steroids in male body builders. HCAs have the potential for hemorrhage and HCC development, particularly when >5 cm. Molecular classification of HCA is defined as follows: (1) HCA with *CTNNB1* mutations (10–20%) are at risk of HCC development and are present in men treated with androgens; (2) inflammatory adenomas (50–60%) are associated with single mutations (*Gp130*: 65%) and are more prevalent in females with obesity or diabetes; and (3) adenomas with inactivated *HNF1A*. Diagnosis is based on MRI, which correlates with molecular subtypes in 80% of cases (inflammatory and HNF1A type). For defining HCA with *CTNNB1* mutations, biopsy is required. Upon diagnosis, discontinuation of oral contraceptives and weight loss are recommended. Resection is indicated in all cases of >5 cm, in men, or in those with *CTNNB1* mutation. For HCA <5 cm, 1-year follow-up is recommended. In case of active HCA bleeding, embolization followed by resection is the treatment of choice. The presence of multiple HCAs is common, and guidelines endorse treating them based on the size of the main nodule.

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83

Pancreatic Cancer

Daniel D. Von Hoff



Pancreatic cancer is the third leading cause of death from cancer in the United States, with >57,000 Americans diagnosed and >47,000 dying from the disease each year. Unfortunately, pancreatic cancer is projected to be the second leading cause of death from cancer in the United States by 2030. Worldwide, pancreatic cancer is the eleventh most common cancer with 458,000 new patients diagnosed and >432,000 deaths (seventh cause of cancer deaths). Pancreatic cancer currently has the worst survival rate of any cancer with an overall 5-year survival (regardless of stage) of ~8.2%. However, that situation is changing. In particular, (1) knowledge about specific molecular subsets of the disease has become crucial to provide the best possible care for patients, and (2) the application of treatment that improves survival for patients with advanced disease used either after surgery or even earlier in the disease has improved survival.

EPIDEMIOLOGY

Pancreatic cancer accounts for 3.2% of all new cancer cases in the United States and for 7.8% of all deaths from cancer in the United States. The lifetime risk of developing pancreatic cancer is ~1.7%. The incidence of pancreatic cancer has been increasing about 1.03% per year. Pancreatic cancer is more common with increasing age and more common in men than in women. The 5-year survival rate for all stages has only increased from 3% in 1975 to 9% in 2015. The latest information from the U.S. Surveillance, Epidemiology, and End Results (SEER) database predicts that the 5-year survival for patients with localized pancreatic cancer is about 37%, 12% for those with regional disease, and 3.1% for patients with advanced metastatic disease. Pancreatic cancer is more common in developed countries (although generally it tracks with the prevalence of smoking). The incidence is highest in Western Europe and North America followed by other areas in Europe, Australia, New Zealand, and South-Central Asia. The population at greatest risk are women living in Scandinavian countries, while the lowest risk is seen for women living in middle Africa.

RISK FACTORS

Age is one of the greatest risk factors for pancreatic cancer with median age at diagnosis of 70 years (the disease is most frequently diagnosed in the 65–79 age group; for men, 65–69; for women, 75–79). The number of new cases per 100,000 persons and the number of deaths per 100,000 persons are higher for males and for blacks of both sexes. Both the number of cases and the number of deaths per 100,000 people are lower for American Indian/Alaskan natives and Asian Pacific Islanders. Both the number of cases and deaths are intermediate for the Hispanic population. People who have a non-O blood type are at higher risk of developing pancreatic cancer.

Environment The greatest risk factor for pancreatic cancer is cigarette smoking. The risk correlates with the increased number of cigarettes smoked and persists for at least 10 years after smoking cessation. About 30% of pancreatic cancer is caused by smoking. Exposure to cadmium as part of cigarette smoking or via exposure to welding,

soldering, or dietary exposure has been weakly associated with an increased risk of pancreatic cancer.

Although dietary factors are often difficult to interpret, high intakes of fat or meat (particularly well-done barbequed meat) are risk factors. High intakes of citrus fruits and vegetables are associated with a decreased risk. Coffee and low-to-moderate alcohol consumption are not associated with an increased risk for pancreatic cancers, while consumption of sugary carbonated drinks has been associated with an increased risk.

Microbiome To date, no solid evidence links *Helicobacter pylori* infection and pancreatic cancer. Some data link the oral microbiome associated with poor dentition to pancreatic cancer, but the evidence is very thin.

Hereditary/ Genetics Hereditary factors may account for 10–16% of all pancreatic cancers. Family members of patients with pancreatic cancer should seek participation in an early detection program with genetic counseling, definition of risk, and if appropriate, periodic MRI screening of the abdomen, although this recommendation is not yet based on research data. In addition, the identification of any pancreatic cancer-associated germline mutations could lead to specific and effective new therapeutics for patients with these abnormalities in their tumors. Table 83-1 identifies the various germline mutations along with their familial cancer syndromes where an increased risk for pancreatic cancer is known.

Knowing the patient has a *BRCA2* or *PALB2* germline mutation or any of the above mutations should lead one to not only refer the patient's relatives to an early detection or high-risk individual clinic but also realize that for patients with a *BRCA2/PALB2* germline mutation consideration for treatment with a poly (ADP-ribose) polymerase (PARP) inhibitor should be considered (see below). Other germline mutations are under study to determine their increased risk of pancreatic cancer, including *CFTR*, *PRSS2*, *CDK4*, *FANCC*, *PALLD*, *APC*, *ATM*, *BMPRIA*, *BRCA1*, *EPCAM*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *NFI*, *PMS2*, *SMAD4*, *TP53*, *TSC1*, *TSC2*, and *VHL*. Some of these mutations are associated with pancreatic neuroendocrine tumors (Chap. 84).

In addition to the recognized genetic syndromes, other possible familial pancreatic cancer genes have not yet been discovered. For example, a family history of pancreatic cancer is associated with a 13-fold increase in the disease. If you have one first-degree relative, the risk is increased 4.6-fold, having two first-degree relatives increases the risk 6.4-fold, and three or more first-degree relatives confers a 32-fold increased risk. The risk is also increased if a relative developed pancreatic cancer at <55 years old.

TABLE 83-1 Germline Mutations, Their Familial Cancer Syndrome, and Fold Risk of Pancreatic Cancer

GERMLINE MUTATION	FAMILIAL CANCER SYNDROME	ESTIMATED INCREASED RISK (FOLD) OF PANCREATIC CANCER
<i>BRCA2</i> ^a	Familial breast/ovarian cancer	2–6
<i>PALB2</i> (partner and localizer of <i>BRCA2</i>)	Familial breast cancer and others	~sixfold
<i>p16/CDKN2A</i>	Familial atypical multiple mole melanoma (FAMMM)	15–18
<i>STK11 (LKB1)</i>	Peutz-Jeghers syndrome	76–140
<i>PRSS1</i> or <i>SPIN1</i> ^b	Hereditary (familial) pancreatitis	53
<i>ATM</i>	Ataxia-telangiectasia	Not yet established
<i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i> , <i>PMS2</i>	Heredity nonpolyposis colorectal syndrome or Lynch syndrome ^c	9–30

^aParticularly common in individuals with Ashkenazi Jewish heritage. ^bForty percent chance of pancreatic cancer by the age of 70. ^cVery important because this is associated with microsatellite instability, which is a marker for response to an anti-PD-1/PD-L1 agent.

Other Considerations Most patients with pancreatic cancer relate that they have had developing symptoms over the past few years. Thus, early detection of the disease is possible when the index of suspicion is high.

Medical Conditions Chronic pancreatitis that is nonfamilial is also associated with an increased risk of pancreatic cancer (2.3–16.5-fold increase). Risk is also increased in people with chronic pancreatitis associated with cystic fibrosis or tropical pancreatitis.

A clear association exists between diabetes mellitus (both type 1 and type 2) and pancreatic cancer. Whether this is a causal association or whether the diabetes is the result of the cancer is not exactly clear. What is clear is that when a person presents with new-onset type 2 diabetes, they should be considered at risk for having pancreatic cancer. The excessive insulin or insulin-like growth factors associated with adult-onset diabetes and metabolic syndrome may promote pancreatic carcinogenesis.

Obesity is considered a possible risk factor for pancreatic cancer. A high body mass index (BMI) ≥30 is associated with a doubling of the risk of pancreatic cancer. Since obesity is a risk factor for diabetes, the contribution of obesity alone is unclear. Interestingly, patients with severe obesity who undergo a gastric bypass experience a reduction in the incidence of gastrointestinal (GI) cancer, including pancreatic cancer, by >30% in the first 3 years (along with a dramatic decrease in their hemoglobin A_{1c} and blood glucose). Physical inactivity also has been associated with an increased risk in pancreatic cancer.

■ PATHOLOGY AND MOLECULAR CONSIDERATION

Location The posterior location of the pancreas in the abdomen is likely one of the issues that leads to a late diagnosis (Fig. 83-1A).

Pathology Cancers of the pancreas can be divided into neoplasms of the endocrine pancreas (Chap. 84) and tumors of the exocrine pancreas. The most common neoplasm of the exocrine pancreas and most deadly is pancreatic infiltrating ductal adenocarcinoma. These tumors arise in the head, body, or tail of the pancreas and are characterized by infiltrating desmoplastic stromal reactions (Fig. 83-1B).

Other subtypes of nonneuroendocrine pancreatic cancers include acinar cell carcinoma (tumors of the exocrine enzyme producing cell), medullary carcinoma, adenosquamous carcinoma, and other rare subtypes. Each of these is different in behavior and in their molecular characteristics and often requires other specific types of treatment.

Molecular Characteristics The molecular characteristics of pancreatic ductal adenocarcinoma reveal four genes that are commonly mutated or inactivated (sometimes referred to as the “four horsemen”). The most common is *KRAS* (usually in codon 12). It is critical to determine the specific mutation in *KRAS* because specific mutations may indicate specific therapies that should be considered. *KRAS* mutations are seen in virtually 100% of pancreatic adenocarcinomas. In fact, with the deep sequencing now available, if a *KRAS* mutation is not detected in the patient's tumor, one should consider that the tumor is likely of a different origin (e.g., small bowel, gallbladder, or cholangiocarcinoma—all of which could require different treatments). *p16/CDKN2A* is also noted in >90% of invasive pancreatic adenocarcinomas. *TP53* and *DPC4/MADH4* are mutated in about half of these tumors. As a reference point, the *BRCA2* gene noted in Table 83-1 is mutated in 7–10% of pancreatic adenocarcinomas.

Precursor Lesions Many pancreatic adenocarcinomas seem to arise from noninvasive epithelial precursor lesions. Detection of these could allow for early diagnosis of pancreatic cancer. These pancreatic intraepithelial neoplasias (PanINs) have varying degrees of dysplasia designated as PanINs 1–3 (and constitute a progression model for pancreatic cancer). Genetic alterations become more frequent as the PanIN grade increases (e.g., grade 3). Not all PanIN lesions progress to invasive malignancy. PanINs that are ≥1 cm are called *intraductal papillary neoplasms* and are usually noninvasive. If the intraductal tumor is in a branch duct, it is usually noninvasive; however, if the intraductal tumor is in a main duct and is large and nodular, it is more likely to have malignant behavior.

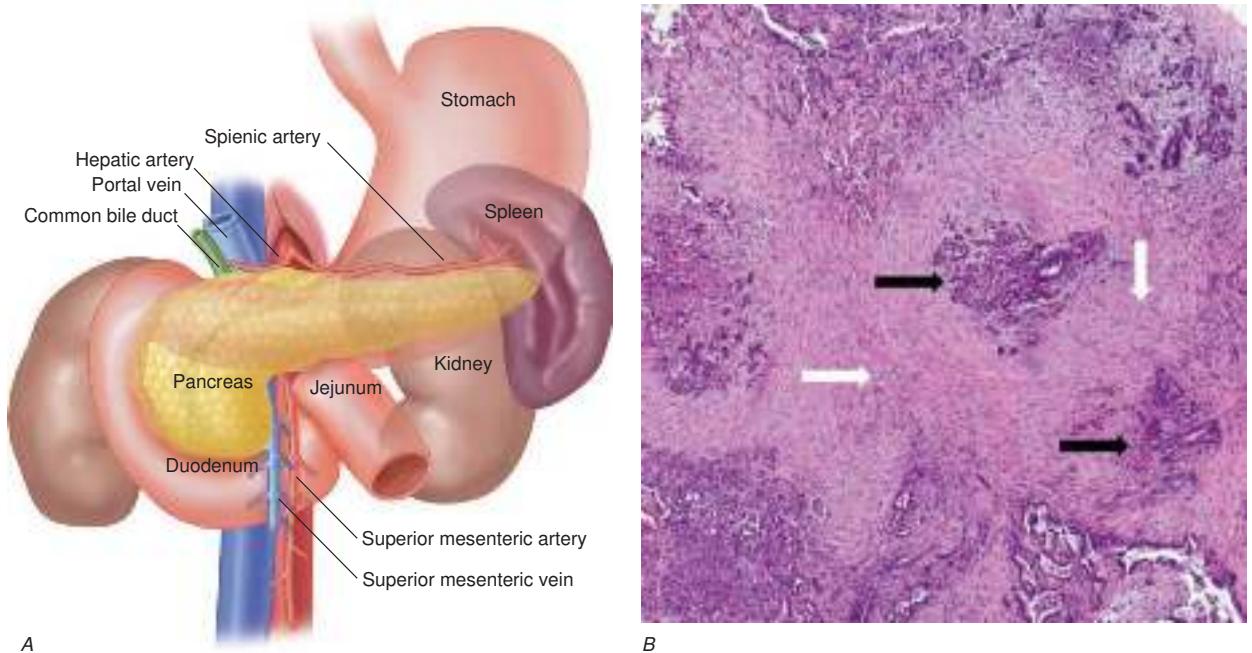


FIGURE 83-1 *A.* Note the relationship of the pancreas to the major vessels of the retroperitoneum. *B.* Ductal adenocarcinoma of the pancreas (black arrows), with intense stromal component (white arrows). (*Part A* is courtesy of Mary Kay Washington, MD, PhD, Vanderbilt University. *Part B* is courtesy of Haiyong Han, PhD, Translational Genomics Research Institute [TGen].)

One other pancreatic tumor is the mucinous cystic neoplasm; they may be seen as incidental findings on scans. These lesions are less likely invasive (20%) unless they are large and have nodules in them.

■ CLINICAL FEATURES

History and Physical The classic presentation for a patient with pancreatic cancer has been abdominal pain and weight loss with or without jaundice. The pain is midepigastric (sometimes described as a “boring-like” pain). Often the pain is in the back (due to retroperitoneal invasion of the splanchnic nerve plexus). The pain may be exacerbated by eating or lying flat. Other items of note in a history are light stool color from the absence of bile (steatorrhea also causes malodorous stools) and the onset of diabetes in the prior year. Jaundice, first detectable with a bilirubin of 2.5–3.0 mg/dL, is usually associated with tumor in the head of the pancreas. In some instances, depression is noted (with a higher subsequent number of suicides). Pruritis may be seen when the bilirubin reaches 6–8 mg/dL.

Physical signs include jaundice, signs of weight loss, a palpable gallbladder (Courvoisier’s sign), hepatomegaly, an abdominal mass, and even an enlarged spleen (usually indicating a portal vein thrombosis). Migratory superficial thrombophlebitis can also be seen (Trouseau’s syndrome). Signs of late disease include a lymph node palpable in the supraclavicular fossa (usually on the left where the thoracic duct enters the subclavian vein). This is clinically referred to as Virchow’s node. Occasionally, one can palpate subcutaneous metastases in the perumbilical area referred to as a Sister Mary Joseph’s node—named after one of the scrub nurses on the Mayo Clinic Operative Team who noted that when she prepped that area and felt those nodules, the patient often had peritoneal metastases.

The history and symptoms noted above may lead a person to see a physician; often CT and MRI scanning detects the disease before advanced disease symptoms appear.

■ DIAGNOSTIC WORKUP

Imaging Diagnostic imaging plays a major role in diagnosing pancreatic cancer and other intraabdominal diseases. The best technique

is the use of a dual-phase contrast-enhanced spiral CT using the pancreatic cancer protocol, which allows arterial phase enhancement and portal venous phase enhancement. This special protocol can provide helpful prospective staging and assessment of resectability. **Figure 83-2** demonstrates such a CT scan (with vascular involvement). **Figure 83-3** demonstrates the use of an 18F glucose positron emission tomography (PET) scan.

Histologic Diagnosis A histologic (tissue) diagnosis is essential and should be obtained with a cutting biopsy needle (not a skinny needle with cytology). Misdiagnosis is more common based on only fine-needle aspirates. Obtaining a tissue diagnosis allows not only for accuracy but also for molecular testing for KRAS mutations, microsatellite instability, and other important molecular abnormalities. Those molecular abnormalities and others will be increasingly important as more targeted therapies are developed for patients with pancreatic cancer.

The core needle (16–18 gauge) biopsy can be obtained via endoscopic ultrasound-guided techniques for a tumor localized to the pancreas or, if there are liver lesions or Virchow’s node, via percutaneous biopsy by interventional radiologists.

Serum Markers Before treatment, a serum sample should be obtained for levels of CA19-9, carcinoembryonic antigen (CEA), or if both are negative, for CA125 (can be positive when the CA19-9 is negative due to the patient not being a Lewis antigen secretor). These markers are not useful for staging but can be useful in following the course of pancreatic cancer.

■ IMPORTANT IMMEDIATE CONSIDERATIONS IN PATIENT CARE

While the patient is being evaluated and staged, one must be alert for biliary tract obstruction (and the attendant risk for sepsis from the biliary tree). A stent can be placed (plastic if temporary or metal if needed longer) to relieve the jaundice and pruritus. If surgery is being contemplated, an early surgical consultation is in order as some surgeons may want to proceed to surgery without placement of a stent. This immediate surgical approach is becoming less common as many multidisciplinary teams want consideration of use of chemotherapy

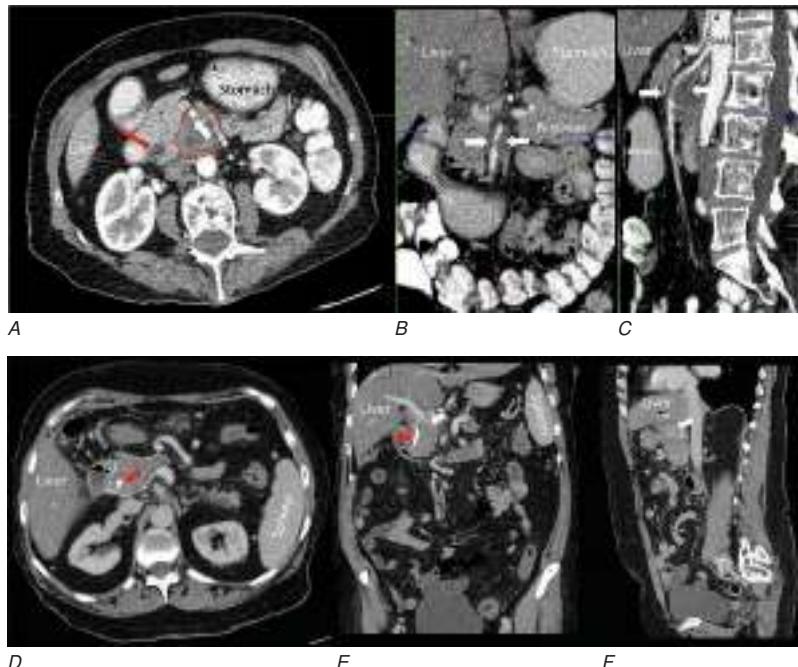


FIGURE 83-2 Selected images from contrast-enhanced CT in patients with locally advanced adenocarcinoma of the pancreas. A high-quality contrast-enhanced CT scan (arterial phase in panels A–C and portal venous phase in panels D–F) is required for optimal staging of pancreas cancer. Panel A demonstrates the typical features of adenocarcinoma of the pancreas on arterial phase axial CT scans (*dotted outline*) with tumor encasement of the superior mesenteric artery (*white arrow*). Note the dilatation of the common bile duct (*red arrow*). Panels B (magnified coronal) and C (sagittal) show reconstruction of CT images into additional orthogonal planes with exquisite details to confirm the unresectable nature of the tumor due to vascular encasement. Panel D demonstrates the typical features of adenocarcinoma of the pancreas on portal venous phase axial CT scans in a different subject. The dotted line outlines a pancreas cancer lesion in the pancreatic head, which is encasing the portal splenic confluence (*dotted outline*). Panels E (*white arrow*) and F show the pinched appearance of the portal splenic confluence by tumor abutment and invasion of the superior mesenteric vein (*white arrow*) on coronal and sagittal views. Note the presence of a stent in the common bile duct (*red arrow*) to help relieve biliary obstruction caused by the tumor. CA, celiac axis; SMA, superior mesenteric artery.

with or without radiation therapy (called neoadjuvant therapy) before a patient is taken to surgery.

Patients with pancreatic cancer are often hypercoagulable and frequently have migratory thrombophlebitis (Trousseau's sign) as well as deep vein thrombosis with pulmonary emboli (a frequent cause of

death). Appropriate examinations plus being alert to thromboses on the routine workup are mandatory so appropriate management can be put in place.

Control of pain or of any symptoms should be pursued to help patients be as comfortable as possible for their decision-making. Sometimes simple approaches like the use of a replacement pancreatic enzyme (at good therapeutic doses) can relieve the bloating, cramping, and diarrhea. Early involvement of a palliative care team can improve a patient's quality of life and sometimes even its length.

■ CLINICAL STAGING

The clinical staging of pancreatic cancer according to the American Joint Commission on cancer staging is presented in [Table 83-2](#).

[Table 83-3](#) presents another clinical way to express extent of disease as well as therapeutic approaches (to be discussed later).

For proper staging, some physicians believe that a laparoscopy either before or at the time of surgery is important. If metastatic disease is found at laparoscopy, one can avoid surgery that would not be helpful because disease is already advanced.

TREATMENT

Resectable Disease

Even for patients with resectable disease, the patient should be presented to a combined-modality conference. Some clinicians feel the best approach for patients with resectable disease (as defined in [Table 83-3](#)) is surgery. Only a small percentage of patients are in this category (10–20%). Some clinicians feel neoadjuvant therapy (chemotherapy before surgery) should be given before surgery (for controlling potential micrometastatic disease and shrinking the primary tumor). The surgery for patients with tumors in the head or

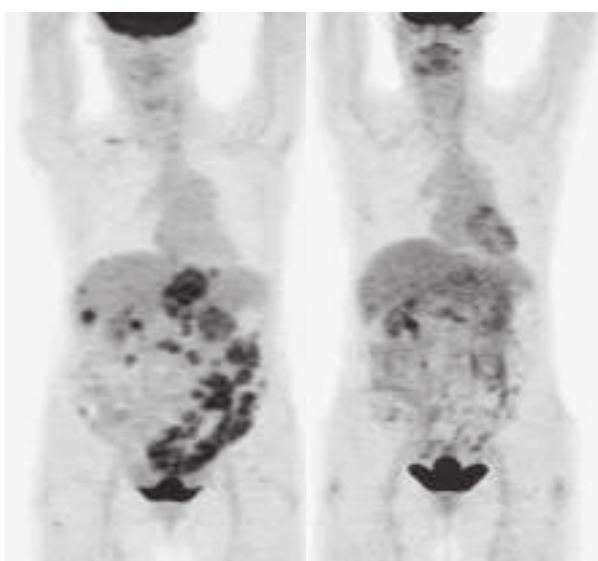


FIGURE 83-3 PET scan demonstrating metastatic disease—baseline and after 6 weeks of chemotherapy with some resolution of liver metastases.

TABLE 83-2 Definition of Primary Tumor (T)

T CATEGORY	T CRITERIA		
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
Tis	Carcinoma in situ This includes high-grade pancreatic intraepithelial neoplasia (PanIN-3), intraductal papillary mucinous neoplasm with high-grade dysplasia, intraductal tubulopapillary neoplasm with high-grade dysplasia, and mucinous cystic neoplasm with high-grade dysplasia		
T1	Tumor ≤2 cm in greatest dimension		
T1a	Tumor ≤0.5 cm in greatest dimension		
T1b	Tumor >0.5 cm and <1 cm in greatest dimension		
T1c	Tumor 1–2 cm in greatest dimension		
T2	Tumor >2 cm and ≤4 cm in greatest dimension		
T3	Tumor >4 cm in greatest dimension		
T4	Tumor involves celiac axis, superior mesenteric artery, and/or common hepatic artery, regardless of size		
M CATEGORY	M CRITERIA		
M0	No distant metastasis		
M1	Distant metastasis		
N CATEGORY	N CRITERIA		
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastases		
N1	Metastasis in one to three regional lymph nodes		
N2	Metastasis in four or more regional lymph nodes		
AJCC Prognostic Stage Groups			
WHEN T IS...	AND N IS...	AND M IS...	THEN THE STAGE GROUP IS....
Tis	N0	M0	0
T1	N0	M0	IA
T1	N1	M0	IIB
T1	N2	M0	III
T2	N0	M0	IB
T2	N1	M0	IIB
T2	N2	M0	III
T3	N0	M0	IIA
T3	N1	M0	IIB
T3	N2	M0	III
T4	Any N	M0	III
Any T	Any N	M1	IV

Source: Used with the permission of American College of Surgeons. MB Amin et al (eds): *AJCC Cancer Staging Manual*, 8th ed. Springer, 2017.

uncinate body of the pancreas is usually a pylorus-sparing pancreaticoduodenectomy (a modified Whipple procedure). For tumors in the body or tail, a distal pancreatectomy is usually performed. Clinical and pathologic findings of the resection are defined as either an R0 resection (no macroscopic or microscopic disease left

after surgery) or an R1 resection, which refers to residual disease likely left behind. Patients with smaller tumors and lymph node-negative disease have a better survival (median of about 18–23 months with 5-year survival of about 20%).

Two approaches are being explored to try to improve on this.

- Postoperative adjuvant therapy. The standard of care is to use 24 weeks of adjuvant treatment with a modified folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin (FOLFRINOX) regimen. In the definitive clinical trial, the median survival was 54 months for the combination of modified FOLFRINOX versus 35 months for gemcitabine alone (hazard ratio [HR] 0.64; 95% confidence interval [CI] 0.48–0.86; $p = .003$). Toxicities were manageable.
- A newer approach is the use of neoadjuvant chemotherapy (chemotherapy given before surgery) to try to shrink the tumor and normalize the patient's serum CA19-9 level. Data suggest that patients who have borderline resectable/locally advanced disease can benefit from neoadjuvant therapy. Studies of neoadjuvant chemotherapy with or without radiation therapy are ongoing.

LOCALLY ADVANCED DISEASE (30% OF PATIENTS)

For patients with locally advanced disease, the median survival is also quite poor (6–10 months) because many of the patients die with local problems (e.g., portal vein thrombosis with bleeding

TABLE 83-3 Extent of Disease and Therapeutic Approach

DESIGNATION (MEDIAN SURVIVAL)	THERAPEUTIC APPROACHES
1. Resectable (localized): (18–23 mo) <ul style="list-style-type: none"> No encasement of celiac axis or superior mesenteric artery (SMA) Patent superior mesenteric—portal veins No extrapancreatic disease 	Surgical option (or preoperative-neoadjuvant therapy first) Surgery is followed by postsurgery adjuvant therapy <ul style="list-style-type: none"> Currently mFOLFRINOX
2. Locally advanced: (6–10 mo) <ul style="list-style-type: none"> Encasement of arteries Venous occlusion (superior mesenteric vein [SMV] or portal) No extrapancreatic disease 	Either chemotherapy or chemotherapy + radiation therapy
3. Metastatic: (8.3–12.8 mo)	Systemic chemotherapy

Abbreviation: mFOLFRINOX, modified FOLFRINOX (folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin (T Conroy et al: *N Engl J Med* 379:2395, 2018).

varices, obstruction, sepsis). The approach has been to try to reduce the bulk of the disease with use of radiation therapy plus chemotherapy or chemotherapy alone, with the goal that the disease could become resectable. No standard therapy has been agreed upon, but experimental approaches are applying some of the treatments that show promise in advanced metastatic disease.

ADVANCED METASTATIC DISEASE (60% OF PATIENTS)

Only a few of the many phase 3 randomized trials in patients with advanced pancreatic cancer have led to meaningful increases in survival. We have learned that a regimen needs to have at least a 50% improvement in overall survival or 90% improvement in 1-year survival in a pilot phase 2 trial to predict for any degree of success in large randomized phase 3 trials.

Patients with the best chance of receiving a benefit from treatment have a good performance status (functioning up and around at least 70% of the day), have a reasonable albumin level (≥ 3.0 g/dL), and a neutrophil/lymphocyte ratio of ≤ 5.0 .

Single-agent gemcitabine achieves a median survival of 6 months and a 1-year survival rate of 18%. Table 83-4 details three combination regimens that have further improved survival modestly. Median overall survival still ranges from 6 to 11 months. However, 1-year survival is now approaching 35% for these combination regimens with some long-term 4+ year survivors.

Also of note in Table 83-4, liposomal irinotecan has U.S. Food and Drug Administration (FDA) approval in combination with 5-fluorouracil and leucovorin for patients whose tumors have progressed on gemcitabine (e.g., second-line therapy for stage IV disease) based on improved overall survival.

FOR PATIENTS WITH A SPECIFIC MOLECULAR PROFILE IN THEIR TUMOR/GERM LINE

PARP inhibitors have clinical activity against pancreatic cancers having mutations in *BRCA2*, *BRCA1*, or *PALB2* (i.e., defective DNA repair proteins). In addition, their tumors might be more sensitive to specific combinations of chemotherapy like gemcitabine plus cisplatin. In addition, tumors with microsatellite instability often have more mutations, and such tumors appear to have a higher response

TABLE 83-4 Combination Chemotherapy Regimens That Have an Impact on Survival

STUDY DESIGN (AUTHOR/REF)	NO. OF PATIENTS	MEDIAN SURVIVAL (MONTHS)
Gemcitabine + erlotinib vs gemcitabine (Moore et al: J Clin Oncol 26:1960, 2007)	569	6.24 vs 5.91 (HR 0.82; 95% CI 0.69–0.99; $p = .038$)
FOLFIRINOX (folinic acid + 5-fluorouracil + irinotecan + oxaliplatin) vs gemcitabine (Conroy et al: N Engl J Med 364:1817, 2011)	342	11.1 vs 6.8 (HR 0.57; 95% CI 0.45–0.70; $p < .001$)
Nap-paclitaxel + gemcitabine vs gemcitabine, (Von Hoff et al: N Engl J Med 369:1691, 2013)	861	8.5 vs 6.7 (HR 0.72; 95% CI 0.62–0.83; $p < .001^a$)
Nanoliposomal irinotecan + fluorouracil + folinic acid vs nanoliposomal irinotecan monotherapy vs fluorouracil + folinic acid (Wang-Gillam et al: Lancet 387:545, 2015)	417	6.1 vs 4.2 (HR 0.67; 95% CI 0.49–0.92; $p = .012^a$)

^aThe 2-year survival rate with this regimen is 9% and the 3+ year rate is 4%. Other studies have not reported on these parameters.^bHR is for nanoliposomal irinotecan + 5-fluorouracil + folinic acid vs 5-fluorouracil + folinic acid.

Abbreviations: CI, confidence interval; HR, hazard ratio.

rate to immunotherapy with checkpoint inhibitors and anti-PD-1 (pembrolizumab, nivolumab) and anti-PD-L1 antibodies.

MAINTENANCE THERAPY FOR PATIENTS RESPONDING TO TREATMENT

For patients with germline *BRCA1* or *BRCA2* mutations whose disease has not progressed during a first-line platinum-based regimen, the PARP inhibitor olaparib has been shown to improve progression-free survival (7.4 vs 3.8 months; HR 0.53; 95% CI 0.35–0.82; $p = .004$) with no change in quality of life.

OTHER POTENTIAL FACTORS INFLUENCING SURVIVAL

Preclinical studies have suggested that vitamin D can inhibit the development and growth of cancer. In models of pancreatic cancer, synthetic analogues of vitamin D had an effect on both tumor cells and on the tumor microenvironment. Clinical studies are conflicting as to whether circulating levels of plasma 25-hydroxyvitamin D (25[OH]D) affect the incidence of pancreatic cancer. However, patients with prediagnostic levels of 25(OH)D that are in the normal range have a longer survival than those who have reduced levels (35% lower hazard for death).

FUTURE DIRECTIONS

Death from pancreatic cancer is often due to progressive inanition. The metabolic consequences of this cancer are being examined. The tumor can be fatal at a modest level of tumor burden based on the profound metabolic effects. Other promising areas of investigation include addressing the florid stromal reaction around the tumor cells (believed to act as a physical barrier to drug delivery and as an immune sanctuary for the tumor cells). Improvements in outcomes for pancreatic cancer would accompany earlier detection. A small decrease in the percentage of patients being diagnosed with stage IV pancreatic cancer has been noted. The reason for this encouraging sign is unknown. The 5-year survival for earlier stage patients has increased from 44.7% in 2004 to 83.7% in 2012. There is emerging evidence that the surveillance to detect *CDKN2A* mutation carriers can detect pancreatic ductal cancer at a resectable stage.

A

Thank you to Nicole Harkey, for assistance in the preparation of this chapter, and Drs. Elizabeth Washington, Ron Korn, and Haiyong Han and the American Joint Committee on Cancer for providing the figures and tables.

FURTHER READING

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Gastrointestinal (GI) neuroendocrine tumors (NETs) can be broadly grouped according to their site of origin, as either extrapancreatic NETs, historically called carcinoid tumors, or pancreatic NETs. While NETs can pursue a broad range of clinical behaviors, they classically follow a course that is more indolent than many other malignancies. NETs also have the ability to synthesize peptides, growth factors, and bioactive amines that may be ectopically secreted, giving rise to a range of unique clinical syndromes.

INCIDENCE AND PREVALENCE

The diagnosed incidence of NETs has been steadily increasing over the past several decades (Fig. 84-1). An analysis of data from the Surveillance, Epidemiology, and End Results (SEER) program, comprising population-based data in the United States from 1973 to 2012, showed that the incidence had increased 6.4-fold over this time period and that the estimated prevalence of patients who had been diagnosed with a NET was >170,000. This analysis also found that overall survival durations for patients with NETs had improved significantly. The increasing incidence and improved survival durations for patients with NETs likely reflect, at least in part, advances in both diagnosis and treatment. While environmental or other factors leading to an increased incidence of NETs cannot be excluded; common cancer risk factors such as tobacco or alcohol use and dietary patterns have not been clearly linked to NET development.

A minority of NETs develop in the context of autosomal inherited genetic syndromes associated with mutations in specific tumor-suppressor genes. The most common of these is multiple endocrine neoplasia type 1 (MEN 1), due to mutation and loss of function of the *menin* gene, located on chromosome 11q13 (Chap. 388). Patients with MEN 1 are at risk for developing pancreatic NETs as well as hyperparathyroidism and pituitary adenomas; less commonly, they may develop bronchial and thymic NETs. Other inherited syndromes

associated with NETs include von Hippel-Lindau disease (VHL), von Recklinghausen's disease (neurofibromatosis type 1), and tuberous sclerosis (Bourneville's disease). Inherited mutations in the VHL gene, located on chromosome 3p25, are associated with the development of cerebellar hemangioblastomas, renal cancer, and pheochromocytomas and, less commonly, pancreatic NETs. Mutations in neurofibromin (*NFI*) are associated with neurofibromatosis (von Recklinghausen's disease); patients with neurofibromatosis are at risk of developing both pancreatic and extrapancreatic NETs. Tuberous sclerosis is caused by mutations that alter either hamartin (*TSC1*) or tuberin (*TSC2*). Both hamartin and tuberin function as inhibitors of the phosphatidylinositol 3-kinase and the mechanistic target of rapamycin (mTOR) signaling cascades, and pancreatic NETs have been reported in these patients. Rare cases of familial small intestine NETs have also been reported; in these cases, multiple synchronous tumors generally arise within the small intestine. A characteristic inherited mutation, however, has not been identified to date in the majority of these cases.

HISTOLOGIC CLASSIFICATION AND MOLECULAR FEATURES

The histologic features of NETs vary widely and are one of the most important determinants of both clinical behavior and treatment. NETs are classified based on the degree tumor differentiation (well or poorly differentiated), as assessed by a pathologist, and tumor grade (grades 1–3) (Table 84-1). Tumor grade closely correlates with mitotic count and Ki-67 proliferative index. Classic, well-differentiated NETs are composed of monotonous sheets of small round cells with uniform nuclei and only rare mitoses. Immunocytochemical staining for chromogranins and synaptophysin is typical. Ultrastructurally, these tumors contain electron-dense neurosecretory granules containing peptides and bioactive amines that may be ectopically secreted, giving rise to a range of clinical syndromes. These classic well-differentiated NETs have low-grade features and generally have a mitotic index of <2 mitoses per 10 high-power field (HPF) and a Ki-67 proliferative index of <3%. Less commonly, well-differentiated NETs have an intermediate histologic grade and pursue a somewhat more aggressive clinical course. These intermediate-grade tumors typically have a mitotic count of 2–20 per 10 HPF and a mitotic index of 3–20%. Well-differentiated high-grade tumors are rare and have mitotic counts that exceed 20 per 10 HPF and a Ki-67 proliferative index of >20%. Poorly

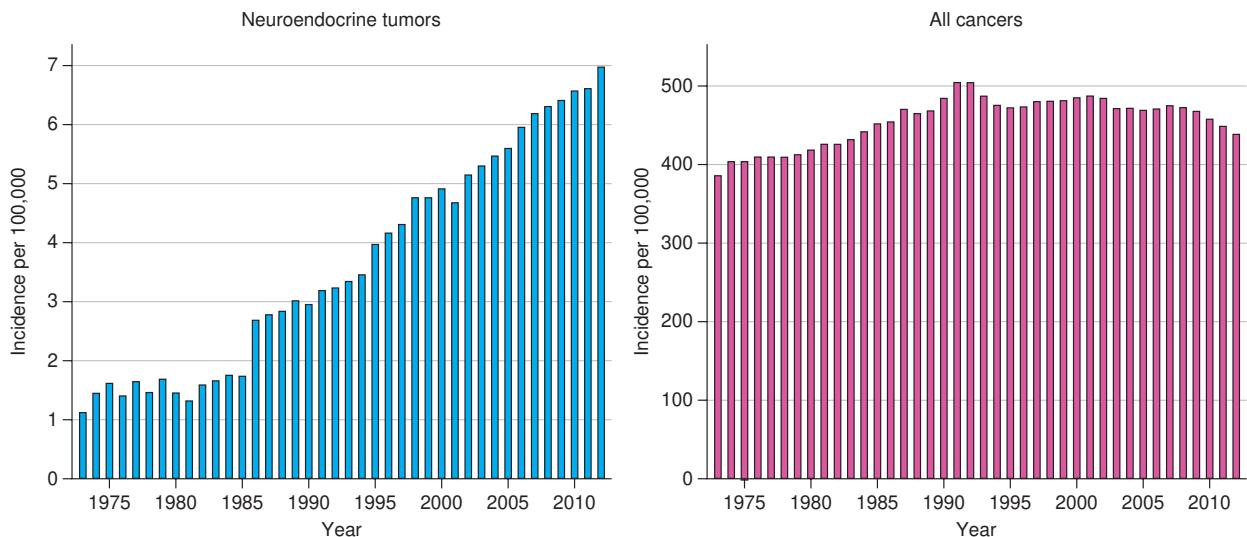


FIGURE 84-1 Incidence of neuroendocrine tumors (NETs). The incidence of neuroendocrine tumors has been increasing over the past several decades, an observation that has been attributed in part to improved diagnosis and classification. (Adapted from A Dasari et al: Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. JAMA Oncol 3:1335, 2017.)

TABLE 84-1 Histologic Classification of Neuroendocrine Tumors

CLASSIFICATION	DIFFERENTIATION	GRADE	MITOTIC COUNT	KI-67
Neuroendocrine tumor	Well differentiated	Low grade (grade 1)	<2 per 10 HPF	<3%
Neuroendocrine tumor	Well differentiated	Intermediate grade (grade 2)	2–20 per 10 HPF	3–20%
Neuroendocrine tumor	Well differentiated	High grade (grade 3)	>20 per 10 HPF	>20%
Neuroendocrine carcinoma	Poorly differentiated	High grade (grade 3)	>20 per 10 HPF	>20%

Abbreviation: HPF, high-power field.

differentiated high-grade tumors form the most clinically aggressive category; prognosis and treatment for these tumors differ markedly from their well-differentiated counterparts.

Whole exome sequencing of sporadic pancreatic NETs found that the most frequently altered gene was *MEN1*, occurring in 44% of tumors. In addition, 43% of tumors had mutations in genes encoding two subunits of a transcription/chromatin remodeling complex consisting of DAXX (death-domain-associated protein) and ATRX (α -thalassemia/mental retardation syndrome X-linked). Mutations in genes associated with the mTOR pathway were identified in 15% of tumors. In contrast, recurrent mutations in extrapancreatic NETs appear to be rare. In one study that evaluated 180 small intestinal NETs using a combination of whole exome and more targeted genome-sequencing analysis, recurrent mutations were only observed in the *CDKN1B* gene (cyclin-dependent kinase inhibitor 1B [p27^{Kip1}]) in 8% of cases. Loss of chromosome 18 is a common finding in small-bowel NETs. Small-intestinal GI carcinoids commonly have epigenetic changes; however, the clinical significance of these alterations remains uncertain. Initial studies have suggested that well-differentiated pancreatic and extrapancreatic NETs express only low levels of the immune checkpoint markers PD-1 and PD-L1.

CLINICAL PRESENTATION AND MANAGEMENT OF LOCALIZED PANCREATIC NEUROENDOCRINE TUMORS

Pancreatic NETs have been subcategorized as either “functional,” meaning associated with symptoms of hormone secretion, or non-functional, in which case they may be clinically silent until they cause anatomic symptoms. The clinical presentation of functional pancreatic NETs depends on the type of hormone secreted and can sometimes lead to dramatic clinical presentations (Table 84-2). The most common functional pancreatic NETs are insulinomas, followed in incidence by glucagonomas and gastrinomas. Pancreatic NETs secreting other hormones, including somatostatin, vasoactive intestinal peptide (VIP), adrenocorticotrophic hormone (ACTH), and parathyroid hormone (PTH) have also been described but are uncommon. Only ~20% of pancreatic NETs are associated with symptoms of hormone hypersecretion; the majority of pancreatic NETs are “nonfunctional” and are diagnosed either incidentally or after patients present with abdominal pain, weight loss, or other anatomic symptoms related to tumor bulk.

GASTRINOMA

Patients with gastrinoma typically present with Zollinger-Ellison syndrome (ZES) (Chap. 324). The most common symptoms associated with this syndrome are abdominal pain, diarrhea, gastroesophageal reflux disease (GERD), and peptic ulcer disease. Peptic ulcer disease manifesting as multiple ulcers with associated diarrhea is a classic presentation. Up to 25% of patients with ZES have MEN 1, and a diagnosis of gastrinoma should prompt a family history as well as an assessment for concurrent hyperparathyroidism. Fasting hypergastrinemia is a nearly universal finding in patients with gastrinoma. Importantly, however, proton pump inhibitors (PPIs) can suppress acid secretion sufficiently to cause hypergastrinemia and can confound the diagnosis. Achlorhydria, usually in the context of chronic atrophic gastritis, will also elevate serum gastrin levels but can usually be easily distinguished from gastrinoma given the absence of other evidence of acid hypersecretion.

While often classified as pancreatic NETs, the majority of gastrinomas in fact arise in the “gastrinoma triangle,” an anatomic region

bounded by the duodenum, pancreas, and confluence of the cystic and common bile ducts. Most gastrinomas (50–90%) in sporadic ZES arise in the duodenum. They are frequently small and may be difficult to localize. Imaging studies generally include either CT or MRI; endoscopic ultrasound or somatostatin scintigraphy may also be helpful.

PPIs are generally highly effective in the treatment of symptoms related to gastrinoma and are considered the initial treatment of choice. Rapid resolution of both abdominal pain and diarrhea related to acid hypersecretion is common. Somatostatin analogues may also be helpful in controlling symptoms in refractory cases. Once symptoms are controlled, surgical resection is generally recommended for patients with sporadic gastrinomas, both to eliminate the cause of gastrin secretion and to decrease the risk of developing metastatic disease. The technique used for resection depends in large part on the precise location of the tumor. In some cases where preoperative imaging is not successful but a diagnosis is strongly suspected, exploratory laparotomy with intraoperative ultrasound may be undertaken. In gastrinoma patients who have underlying MEN 1, tumors are generally small and multiple; the role of routine surgery in this setting remains more controversial but generally is still recommended in patients with larger tumors measuring ≥ 1.5 –2 cm in diameter.

INSULINOMA

Patients with insulinoma generally present with symptoms of hypoglycemia, which may include confusion, headache, disorientation, visual difficulties, irrational behavior, and even coma. In some cases, the diagnosis of insulinoma may not be immediately evident, and patients with

TABLE 84-2 Clinical Presentation and Management of Secretory Syndromes Associated with Neuroendocrine Tumors

	CLINICAL SYMPTOMS AND MANIFESTATIONS	TREATMENT OPTIONS TO CONTROL SECRETORY SYMPTOMS
Pancreatic Neuroendocrine Tumors		
Gastrinoma (generally located in “gastrinoma triangle”)	Zollinger-Ellison syndrome: gastroesophageal reflux, peptic ulcer disease, diarrhea	Proton pump inhibitors, somatostatin analogues
Insulinoma	Hypoglycemia leading to confusion, lethargy, coma; weight gain	Diazoxide, everolimus
Glucagonoma	Skin rash (necrolytic migratory erythema), glucose intolerance, weight loss	Somatostatin analogues
VIPoma	Verner-Morrison syndrome: watery diarrhea, hypokalemia, achlorhydria	Somatostatin analogues
ACTHoma	Cushing’s syndrome: hyperglycemia, weight gain, hypokalemia	Ketoconazole, consider adrenalectomy
Extrapancreatic gastrointestinal neuroendocrine tumors		
Typically in setting of advanced disease from small intestine or appendiceal primary tumors	Carcinoid syndrome: flushing, diarrhea, right-sided valvular heart disease, mesenteric fibrosis	Somatostatin analogues, telotristat ethyl

insulinoma may initially be diagnosed with psychiatric illnesses that in retrospect were hypoglycemic symptoms. The diagnosis of insulinoma is generally confirmed with elevated fasting insulin levels in conjunction with elevated proinsulin and C-peptide. Fasting hypoglycemia can also be caused by severe liver disease, alcoholism, and poor nutrition. Postprandial hypoglycemia may also occur after gastric bypass surgery. Surreptitious use of insulin or hypoglycemic agents may be difficult to distinguish from an insulinoma. Evaluation of proinsulin and C-peptide levels, both of which should be normal in patients using exogenous insulin, and measurement of sulfonylurea levels in serum or plasma are helpful in such cases.

The hypoglycemia associated with insulinomas can be severe and challenging to manage. Diazoxide has historically been used in the initial management of patients with insulinoma and results in inhibition of insulin release, though it can also be associated with side effects including sodium retention and nausea. Everolimus, in addition to its antitumor effect (see below), is highly effective in improving glycemic control in patients with insulinoma. The benefits of everolimus in this setting may be related both to induction of insulin resistance and a direct antitumor effect. While somatostatin analogues are usually effective in treating symptoms of hormone hypersecretion associated with other types of NETs, they should be used with caution in patients with insulinoma. Somatostatin analogues may suppress counterregulatory hormones, such as growth hormone (GH), glucagon, and catecholamines, and precipitously worsen hypoglycemia.

Insulinomas may be difficult to localize, as they are less consistently avid on somatostatin scintigraphy than other pancreatic NETs. Insulinomas are also generally small, with the majority measuring <2 cm in diameter. Because of their generally small size, insulinomas are best localized with endoscopic ultrasound (EUS). In the absence of metastatic disease, surgical resection is usually recommended. The primary treatment for exophytic or peripheral insulinomas is enucleation. If enucleation is not possible because of invasion or the location of the tumor within the pancreas, then pancreateoduodenectomy for tumors in the head of the pancreas or distal pancreatectomy with preservation of the spleen for smaller tumors not involving splenic vessels may be considered.

■ GLUCAGONOMA

Patients with glucagonoma most commonly present with a characteristic dermatitis, called necrolytic migratory erythema (Fig. 84-2). The rash usually involves intertriginous sites, especially in the groin or buttock, and can wax and wane. Other common presenting symptoms of glucagonoma include glucose intolerance and weight loss. The diagnosis of glucagonoma can be confirmed by demonstrating an increased plasma glucagon level, generally in excess of 1000 pg/mL. Somatostatin analogues are usually highly effective as an initial treatment to alleviate the symptoms and rash associated with glucagon hypersecretion. The majority of glucagonomas are large in size at presentation and arise in the tail of the pancreas. For patients with localized disease, distal pancreatectomy and splenectomy are recommended. A hypercoagulable state has been reported in up to 33% of patients with glucagonoma, and perioperative anticoagulation should generally be employed.

■ SOMATOSTATINOMA

Patients with somatostatinoma typically present with diabetes mellitus, gallbladder disease, diarrhea, and steatorrhea. Somatostatinomas occur primarily in the pancreas or duodenum, are usually large, and are commonly metastatic at presentation. They are only rarely associated with MEN 1. The diagnosis of somatostatinoma is based on the demonstration of elevated plasma somatostatin levels, and as such, the potential benefits of using somatostatin analogs as a treatment for patients with somatostatinoma is questionable. Surgery is recommended for patients with localized disease.

■ VIPOMA

VIPomas are associated with a distinct syndrome that has been variously called Verner-Morrison syndrome, pancreatic cholera, and WDHA syndrome (watery diarrhea, hypokalemia, and achlorhydria).



FIGURE 84-2 Glucagonoma syndrome. Patients with glucagonoma may present with a classic skin rash, necrolytic migratory erythema (shown). Other presenting symptoms include glucose intolerance and weight loss.

VIP is a 28-amino-acid peptide that mimics the effects of the cholera toxin by stimulating chloride secretion in the small intestine and increasing smooth-muscle contractility, resulting in profound diarrhea. Treatment of dehydration, hypokalemia, and electrolyte losses with fluid and electrolyte replacement is the most critical initial treatment for patients with VIPoma. VIPomas are usually solitary and arise in the pancreatic tail. Elevated plasma levels of VIP are typical but should not be the only basis of the diagnosis of VIPomas because they can occur with some diarrheal states including inflammatory bowel disease, in the setting of small bowel resection, and radiation enteritis. Chronic surreptitious use of laxatives/diuretics can be particularly difficult to detect clinically. Somatostatin analogues are usually highly effective in controlling the diarrhea; surgical resection is recommended for patients with localized disease.

■ OTHER SECRETORY PANCREATIC NETS

Pancreatic NETs secreting GH-releasing factor (GRF), calcitonin, ACTH, and PTH-related protein have also been described; it is also possible for pancreatic NETs to secrete more than one hormone or for the secretory profiles to evolve over time. Gastrinomas, in particular, may evolve and may be associated with secretion of ACTH, resulting in ectopic Cushing's syndrome. Tumors secreting these hormones may not be as responsive to treatment with somatostatin analogues as the more common pancreatic NETs and the associated hormonal symptoms may cause significant morbidity. As with other pancreatic NETs, patients with localized disease are generally treated with surgical resection. In patients with ACTH-secreting tumors, the associated symptoms of Cushing's syndrome can be alleviated with adrenalectomy if resection of the primary tumor is not possible or in the setting of metastatic disease.

■ PANCREATIC NETS ARISING IN THE SETTING OF MEN 1

Pancreatic NETs occurring in patients with MEN 1 are typically multiple and often pursue a relatively indolent course. Because of the high probability of multiple tumors, surgical resection of confirmed pancreatic NETs in patients with MEN 1 is usually undertaken with caution given the likelihood of tumors arising in the remaining pancreas if

partial pancreatectomy is undertaken as well as the significant morbidities associated with total pancreatectomy. However, for symptomatic tumors or for growing tumors >2 cm in size, surgical resection may still be considered.

■ NONFUNCTIONING PANCREATIC NETS

As noted above, the majority of pancreatic NETs are not associated with symptoms of hormone hypersecretion and are considered “non-functional.” As a result, they often remain clinically silent and either are diagnosed incidentally or are not diagnosed until widespread, metastatic disease is present resulting in anatomic symptoms. If they are localized at diagnosis, the general treatment recommendation is surgical resection; however, the management of small, asymptomatic pancreatic NETs is debated. Assuming tumors are low grade, patients with incidentally discovered, low-grade tumors measuring <1 cm in size can be safely followed; other retrospective studies have suggested nonoperative management for nonfunctioning pancreatic NETs measuring up to 3 cm. In contrast, however, an analysis of the SEER database suggested that at least some tumors measuring <2 cm in size can pursue a more aggressive course. Management of small, incidentally discovered, asymptomatic, low-grade pancreatic NETs is therefore based on clinical judgement, taking into account surgical risk and patient comorbidities.

CLINICAL PRESENTATION AND MANAGEMENT OF LOCALIZED EXTRAPANCREATIC GASTROINTESTINAL NEUROENDOCRINE TUMORS

Extrapancreatic GI NETs, historically called carcinoid tumors, may arise virtually anywhere in the GI tract and differ significantly in their clinical characteristics depending on their location. The most common locations for extrapancreatic NETs are the stomach, distal small intestine, appendix, and rectum.

■ GASTRIC NETS

Gastric NETs can be categorized into three groups: type 1 (associated with chronic atrophic gastritis); type 2 (associated with gastrinomas and ZES), and type 3 (sporadic, gastric NETs). Type 1 gastric NETs are the most common of the three types. In type 1 gastric NETs, chronic atrophic gastritis results in loss of acid secretion with consequent loss of the negative feedback loop on gastrin-producing cells in the antrum of the stomach. Pernicious anemia is also commonly associated with this condition; classic laboratory findings are a markedly elevated gastrin level and low levels of vitamin B₁₂. Unchecked gastrin secretion in these patients results in hyperplasia of the endocrine cells in the gastric fundus. A typical finding on endoscopy is diffuse endocrine cell hyperplasia with multiple gastric carcinoid tumors (Fig. 84-3). These tumors generally pursue a benign course and can be monitored with serial endoscopy. In cases where tumors continue to grow or become symptomatic, antrectomy to remove the source of gastrin production



FIGURE 84-3 Multifocal gastric neuroendocrine tumor. (Courtesy of Christopher Huang MD, Boston Medical Center.)

can result in tumor regression. Type 2 tumors are rare and usually occur in the setting of gastrinoma; as with type 1 gastric NETs, elevated gastrin levels result in diffuse gastric neuroendocrine hyperplasia and multifocal gastric NETs. Resection of the gastrinoma, removing the source of gastrin production, is the treatment of choice.

In contrast to type 1 and type 2 gastric NETs, type 3 gastric NETs are generally solitary, arise in the setting of normal gastrin levels, and may pursue a far more aggressive course. For early-stage, smaller tumors, endoscopic or wedge resection may be performed. For larger tumors, partial gastrectomy with lymphadenectomy is recommended.

■ NETS OF THE SMALL INTESTINE

Small-bowel NETs occur most commonly in the terminal ileum and are notoriously difficult to diagnose at an early stage. One reason for this is that they arise within the muscularis, and their submucosal location makes them difficult to see during routine colonoscopy (Fig. 84-4A). Small-bowel NETs are also often multifocal; multifocal tumors appear to arise independently throughout the small intestine, although the mechanisms underlying this phenomenon remain uncertain.

Small-bowel NETs are often associated with desmoplasia and mesenteric fibrosis, likely as a result of fibroblast proliferation stimulated by tumor serotonin secretion. Mesenteric fibrosis frequently

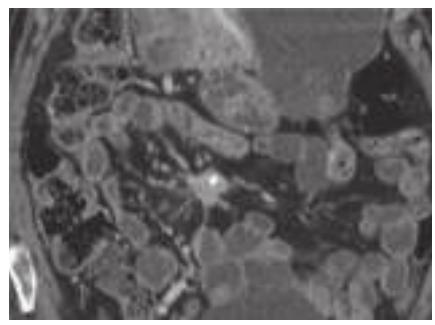


FIGURE 84-4 Small intestine neuroendocrine tumor. *A*, Small intestine neuroendocrine tumors arising in submucosal location. The submucosal location of small intestine neuroendocrine tumors, together with their location beyond the ileocecal valve in the terminal ileum, can make endoscopic detection challenging. *B*, Classic “spoke and wheel” appearance of calcified mesenteric mass associated with small intestine primary neuroendocrine tumor. Mesenteric fibrosis commonly leads to intermittent obstructive symptoms and can also lead to ischemia when the mesenteric vasculature is involved. (Fig. B: Courtesy of Christina LeBedis MD, Boston Medical Center.)

results in intermittent small-bowel obstruction and, in some cases, bowel ischemia due to involvement of the mesenteric vessels. Patients may experience symptoms of intermittent abdominal pain and associated diarrhea, sometimes for months or years before diagnosis, that because of the difficulty in diagnosis are often attributed to irritable bowel syndrome. One classic finding that can aid in diagnosis is that the lymph node metastases associated with small intestine NETs are usually larger than the primary tumor and may be calcified, which, together with the tethering of the small intestine caused by the associated fibrosis, results in a classic “spoke and wheel” appearance on computed tomography (Fig. 84-4B).

Surgical resection of the primary tumor and associated metastases is recommended when feasible and is performed with curative intent when distant metastatic disease is not already present. Resection should also be considered in patients with metastatic disease experiencing intermittent obstruction or abdominal discomfort thought to be related to the primary tumor or associated mesenteric disease. Some have also advocated the routine resection of asymptomatic small-bowel primary tumors in patients with distant metastatic disease, with the rationale that this may be a way to prevent the future development of fibrosis and obstruction and preempt the development of unresectable disease due to tumor involvement of the mesenteric vessels. However, the available data on the benefits of resecting an asymptomatic primary tumor in this context are conflicting. Some studies have suggested that this practice results in an overall survival benefit, but the retrospective nature of these studies makes the data difficult to interpret given the high potential for selection bias in patients taken to surgery compared with those who were not. Other studies have suggested that prophylactic primary tumor resection confers no survival benefit and that surgery can be safely delayed until it is indicated based on the development of symptoms.

■ NETS OF THE APPENDIX

NETs are one of the most common tumors arising in the appendix. They are typically discovered incidentally in younger individuals undergoing appendectomy for acute appendicitis and not uncommonly are identified only at the time of pathology review. While the unexpected diagnosis of an appendiceal NET in such situations can cause considerable anxiety, in the majority of cases, the prognosis is excellent. Indeed, the clinical behavior of appendiceal NETs has been inferred from multiple large retrospective surgical series that suggest that the risk of lymph node or distant metastases from appendiceal NETs with well-differentiated histology and a tumor diameter measuring <2 cm is extremely low. In such cases, appendectomy alone is felt to be a sufficient surgical procedure.

In contrast, the risk of metastases for tumors measuring 2–3 cm is ~20–30% and is even greater for tumors measuring >3 cm. For patients with larger tumors, more formal staging studies with either cross-sectional imaging or somatostatin scintigraphy are generally recommended to assess for distant metastases, and a subsequent right colectomy to remove regional lymph nodes is performed if no distant metastases are observed. Whether right colectomy should be performed for tumors measuring <2 cm with features such as mesoappendiceal invasion or tumor origin at the appendiceal base, which in some series have suggested a poorer prognosis, remains uncertain. Additionally, tumors may arise in which neuroendocrine cells are admixed with mucin-producing cells or cells exhibiting features of frank adenocarcinoma. In such mixed neuroendocrine-adenocarcinoma tumors, sometimes termed “adenocarcinoids,” treatment recommendations are generally dictated by the more aggressive component of the tumor and align with typical recommendations for colorectal adenocarcinoma.

■ RECTAL NETS

With the increased use of screening colonoscopy, the diagnosis of rectal NET has also become more common. For unclear reasons, the incidence of rectal carcinoid tumors shows geographic variation. In European studies, they compose up to 14% of all NETs, while in some Asian series (Japan, China, Korea), they compose up to 90% of all NETs. The majority of rectal NETs are small, measuring <1 cm in diameter, and have well-differentiated histology. These tumors rarely

metastasize and can usually be safely removed endoscopically with subsequent endoscopic monitoring. In contrast, up to one-third of rectal NETs between 1 and 2 cm are associated with metastases, and those >2 cm, though uncommon, metastasize in >70% of patients. When identified early, these tumors generally require a surgical resection. In contrast to NETs of the appendix and small intestine, hormone secretion from rectal NETs, even when metastatic, is exceedingly rare.

CLINICAL PRESENTATION, DIAGNOSIS, AND EVALUATION OF PATIENTS WITH METASTATIC NEUROENDOCRINE TUMORS

While patients who undergo resection of localized NETs may be at risk of developing tumor recurrence or metastatic disease, postoperative treatment has not yet been shown to alter the risk of recurrence, and systemic adjuvant therapy is not recommended following resection of well-differentiated NETs, as it is for some other cancers. Whether adjuvant systemic therapy may be of benefit following resection of high-grade NETs is uncertain, and an approach utilizing platinum-based chemotherapy with or without external-beam radiation, analogous to that used in small-cell carcinoma, is sometimes considered.

The evaluation of patients with known or suspected metastatic disease generally includes both standard cross-sectional imaging such as CT or MRI and somatostatin scintigraphy. Somatostatin scintigraphy in this setting is based on the fact that >90% of NETs express somatostatin receptors. Gallium-68 (⁶⁸GA) dotate, a radioligand bound to a somatostatin analogue, can be used as a nuclear medicine tracer to perform PET scanning and is highly sensitive in detecting both primary NETs and metastases (Fig. 84-5). Because of the sensitivity of this approach, false-positive results can occur due to somatostatin receptor expression in other tissues. Physiologic uptake in the pancreatic uncinate process is common; uptake can also occur in the setting of sarcoidosis, in meningiomas, and in thyroid goiter or thyroiditis. Standard fluorodeoxyglucose (FDG) positron emission tomography (PET) scans are often negative in well-differentiated NET due to their low metabolic activity but can show uptake in higher-grade tumors; conversely, rates of somatostatin expression tend to be lower in higher-grade tumors, and ⁶⁸GA dotate scans may be negative in this setting.

The utility of blood-based tumor markers in NETs is controversial. The circulating tumor marker chromogranin A is commonly used as a screen for the presence of NETs and also to monitor for both

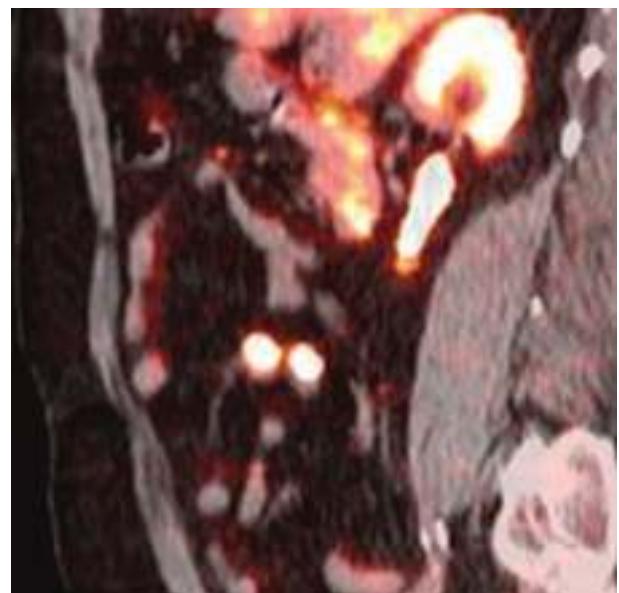


FIGURE 84-5 Gallium-68 Dotate PET scan demonstrating a small bowel neuroendocrine tumor and associated mesenteric mass. (Courtesy of Sara Meibom, MD, Boston Medical Center.)

recurrence and progression of disease in patients with known metastases. While chromogranin A is elevated in patients with metastatic NETs, it is neither particularly sensitive nor specific. A broad range of different assays for chromogranin A have also posed challenges in interpreting results in a standardized fashion. Chromogranin A is often elevated in a number of nonmalignant conditions, including in patients with impaired renal function and in patients who are taking PPIs. Elevated values of chromogranin A should be interpreted with caution in patients in whom a NET is being considered but in whom a diagnosis has not been established.

The overall survival durations for patients with metastatic NETs vary significantly, depending on both the primary location of the tumor and the histologic grade. Median survival durations for patients with well-differentiated NETs have markedly increased in recent years, likely reflecting both earlier diagnoses and improved treatments. For example, in early analyses of the SEER database, the median survival for patients with advanced pancreatic NETs was ~2 years; this had increased to 4 years in a more recent analysis. Similar increases were observed in patients with advanced small intestine NETs, where the median survival for patients with well-differentiated small intestine NETs exceeds 5 years. The sometimes prolonged survival of patients with NETs can sometimes make it challenging to determine at what point to initiate treatment. In patients with symptoms of hormone secretion, decisions to initiate therapy are straightforward. In asymptomatic patients, on the other hand, observation off treatment can sometimes be appropriate. Nevertheless, the natural course of NETs is ultimately to progress, and if treatment is not initiated, close monitoring is essential to ensure patients maximize access to available treatment options over the course of their disease.

MANAGEMENT OF SYMPTOMS OF HORMONE HYPERSECRETION AND THE CARCINOID SYNDROME

Patients with advanced NETs may in some cases experience more symptoms from hormone hypersecretion than from tumor bulk. The management of hormonal symptoms associated with pancreatic NETs depends on the hormone being secreted (see above). Patients with GI NETs, particularly those with small intestine or appendiceal primaries, may develop the carcinoid syndrome. Flushing and diarrhea are the two most common symptoms associated with carcinoid syndrome. The characteristic flush is of sudden onset; it is a deep red or violaceous erythema of the upper body, especially the neck and face, often associated with a feeling of warmth. Flushes may be precipitated by stress, alcohol, exercise, and certain foods such as cheese. Flushing episodes initially are brief, lasting 2–5 min, though later in the disease course, they may last hours. The diarrhea associated with carcinoid syndrome may or may not be associated with flushing and is described as watery in nature. Diarrhea can be profound, sometimes occurring in excess of 10 times daily and is one of the symptoms that most significantly interferes with activities of daily living. Less common manifestations of the carcinoid syndrome include wheezing or asthma-like symptoms. Impaired cognitive function has also been described in particularly advanced cases.

The main secretory product implicated in the carcinoid syndrome is serotonin (5-HT). Serotonin is synthesized from tryptophan by the enzyme tryptophan hydroxylase (Fig. 84-6). Up to 50% of dietary tryptophan can be used in this synthetic pathway by tumor cells, resulting in inadequate supplies for conversion to niacin; hence, some patients develop symptoms of niacin deficiency and pellagra-like lesions. Serotonin has numerous biologic effects, including the stimulation of intestinal secretion, increasing intestinal motility, and the stimulation of fibroblast growth. Other secreted products contributing to carcinoid syndrome symptoms are thought to include histamines and tachykinins, including substance P.

DIAGNOSIS AND TREATMENT OF THE CARCINOID SYNDROME

While the carcinoid syndrome can develop in patients with NETs from almost any site, it is most commonly associated with appendiceal or

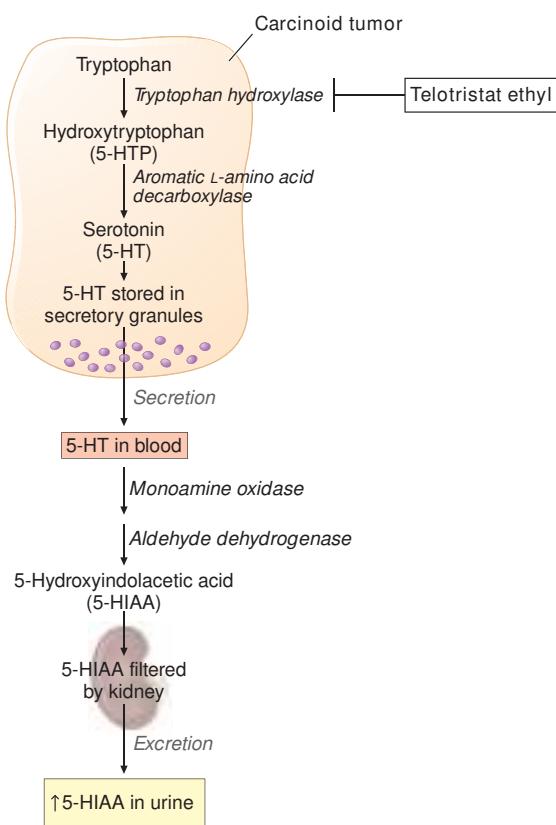


FIGURE 84-6 Serotonin synthesis and secretion in neuroendocrine tumors. Tryptophan is converted to hydroxytryptophan by tryptophan hydroxylase within the tumor cell and, subsequently, to serotonin (5-HT). Serotonin can be subsequently converted to 5-hydroxyindole acetic acid (5-HIAA), which can be measured in a 24-h urine collection and can facilitate the diagnosis of carcinoid syndrome. Telotristat ethyl inhibits tryptophan hydroxylase and can be used as a treatment for carcinoid syndrome.

small intestine NETs. In these patients, the syndrome usually develops only after the development of hepatic metastases or retroperitoneal lesions, allowing entry of serotonin and other vasoactive substances into the systemic circulation. While serotonin levels can be measured in plasma, such measurements are frequently highly variable. Evidence of excess serotonin secretion can be more reliably confirmed by measuring levels of the serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA), commonly using a 24-h urine collection. Urine collections can be challenging, and false-positive elevations may occur if the patient is eating serotonin-rich foods (e.g., salmon, eggs). As a result, elevated levels of 5-HIAA are suggestive but not diagnostic of the carcinoid syndrome. Patients with NETs may also experience symptoms of carcinoid syndrome related to other secreted products, including histamine, absent evidence of serotonin secretion. Conversely, patients without NETs may also describe symptoms analogous to carcinoid syndrome but due to other causes. The symptoms associated with systemic mastocytosis, in particular, can be easily confused with carcinoid syndrome.

The symptoms of carcinoid syndrome, including diarrhea, are generally refractory to standard antidiarrheals or other traditional medications but can often be well controlled with somatostatin analogues (Fig. 84-7). Approximately 90% of NETs express somatostatin receptors. The presence of somatostatin receptors on NETs can be easily confirmed with uptake on somatostatin scintigraphy such as ⁶⁸GA dotate PET scan; uptake on somatostatin scintigraphy is predictive of response to treatment with somatostatin analogues. Somatostatin is a 14-amino-acid peptide that inhibits the secretion of a broad range of hormones. Due to its short half-life, administration of somatostatin

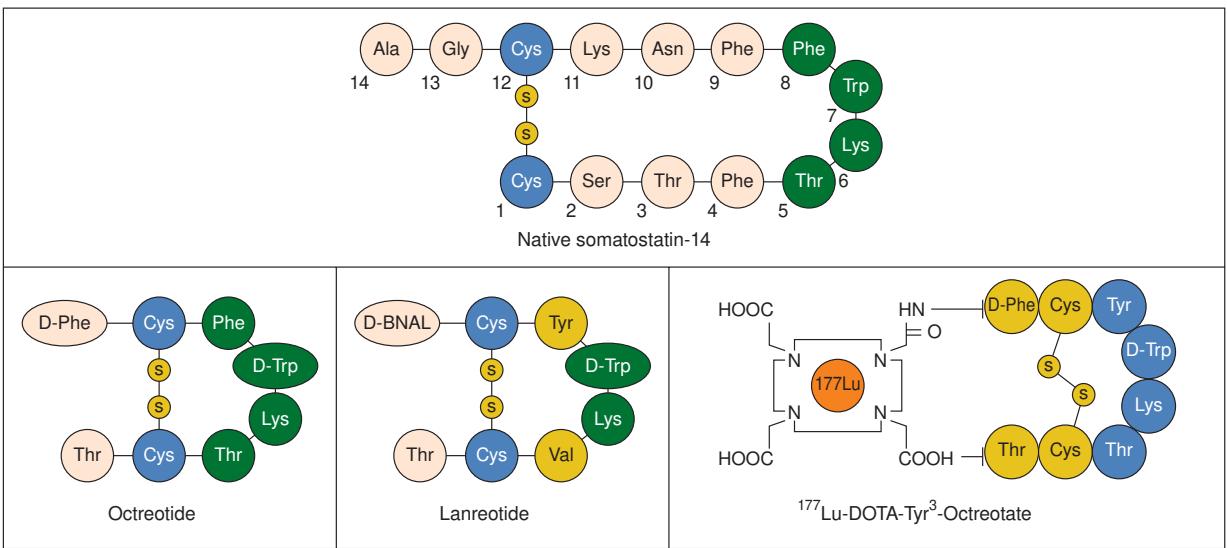


FIGURE 84-7 Somatostatin analogues. Commonly used somatostatin analogues include octreotide and lanreotide, which mirror the molecular structure of human somatostatin and bind to somatostatin receptors on neuroendocrine tumors. Somatostatin analogues inhibit tumoral hormone secretion and also have an antiproliferative effect. Radiolabeled somatostatin analogues such as ^{177}Lu -DOTA-octreotate, shown in the figure, share a similar molecular structure and are used therapeutically.

itself is not therapeutically practical. Longer-acting somatostatin analogues, including octreotide and lanreotide, share an 8-amino-acid binding domain with naturally occurring somatostatin and bind primarily to somatostatin receptor subtypes 2 and 5. Both have been shown to be effective in the treatment of carcinoid syndrome.

In an initial study, treatment of patients with octreotide 150 µg subcutaneously three times daily controlled symptoms of flushing and diarrhea in 88% of patients. A depot preparation (octreotide long-acting release [LAR]) that can be administered monthly has largely eliminated the need for daily octreotide injections and is now considered a standard approach for symptomatic treatment of advanced NETs. Lanreotide, another long-acting somatostatin analogue that is also administered monthly, appears to have similar clinical efficacy to octreotide in the treatment of metastatic NETs and the carcinoid syndrome. As described further below, both octreotide and lanreotide also share the ability to slow tumor growth, providing an additional benefit to patients.

Somatostatin analogue side effects are generally mild. Mild nausea, abdominal discomfort, bloating, and loose stools occur in up to one-third of patients during the first month or two of treatment but usually subsequently subside. Patients with persistent symptoms of bloating or loose stools may be experiencing pancreatic insufficiency associated with use of somatostatin analogues; use of pancreatic enzyme supplements can ameliorate these symptoms. Mild glucose intolerance may also occur due to inhibition of insulin secretion. One of the more significant side effects associated with somatostatin analogues is impaired gallbladder contractility, resulting in delayed gallbladder emptying, and long-term administration of somatostatin analogues has been associated with an increased risk of cholelithiasis. For this reason, patients with advanced NETs in whom surgery is planned and for whom somatostatin analogue therapy is being considered should generally also undergo prophylactic cholecystectomy.

Over time, for reasons that remain uncertain, patients receiving somatostatin analogues for symptoms of hormone secretion may become refractory to treatment. Not uncommonly, such patients experience symptom exacerbation toward the final week of each treatment cycle. Such patients may benefit from an increased frequency of administration (i.e., every 3 weeks) or use of additional short-acting octreotide for breakthrough symptoms.

The association between high levels of circulating serotonin and symptoms of the carcinoid syndrome has also led to a strategy aiming to directly inhibit serotonin synthesis (Fig. 84-6). This approach was

first undertaken in the late 1960s with the drug para-chlorophenylalanine, which was reported to reduce symptoms of carcinoid syndrome but also caused significant central nervous system (CNS) side effects. Telotristat ethyl, a tryptophan hydroxylase inhibitor with minimal CNS penetration, was evaluated in a randomized trial that enrolled 135 patients with persistent carcinoid syndrome-related diarrhea while receiving somatostatin analogues. Treatment with telotristat ethyl was associated with a reduction in bowel movement frequency as well as significant decreases in urinary 5-HIAA compared to placebo. Thus, telotristat is a treatment option for patients with carcinoid syndrome who have persistent diarrhea despite treatment with somatostatin analogues.

■ CARCINOID CRISIS

Carcinoid crisis has been described in the setting of tumor manipulation during surgery and, less commonly, after other interventions such as hepatic artery embolization or radionuclide therapy. It may also occur as a result of exogenous administration of epinephrine or during induction of anesthesia. It is most common in patients who already have significant symptoms of carcinoid syndrome and is thought to be caused by a sudden release of biologically active compounds from the tumor. Carcinoid crisis can be life-threatening and can manifest as either profound hypotension or hypertension. Prospective studies on the prevention and management of carcinoid crisis are limited; however, somatostatin analogues should be readily available during surgical procedures, and in some cases, continuous prophylactic intravenous administration of somatostatin analogues has been utilized as a way to mitigate risk.

■ CARCINOID HEART DISEASE

Carcinoid heart disease occurs in approximately two-thirds of patients with the carcinoid syndrome. Carcinoid heart lesions are characterized by plaque-like, fibrous endocardial thickening that classically involves the right side of the heart and often causes retraction and fixation of the leaflets of the tricuspid and pulmonary valves (Fig. 84-8). The fibrosis in carcinoid heart disease is thought to be directly related to exposure of heart valve fibroblasts to high circulating levels of serotonin. Lesions similar to those observed in carcinoid heart disease were observed in patients receiving fenfluramine, a drug also known to increase serotonin signaling, as well as in patients receiving ergot-containing dopamine receptor agonists for Parkinson's disease. Metabolites of fenfluramine, as well as the dopamine receptor agonists, have high

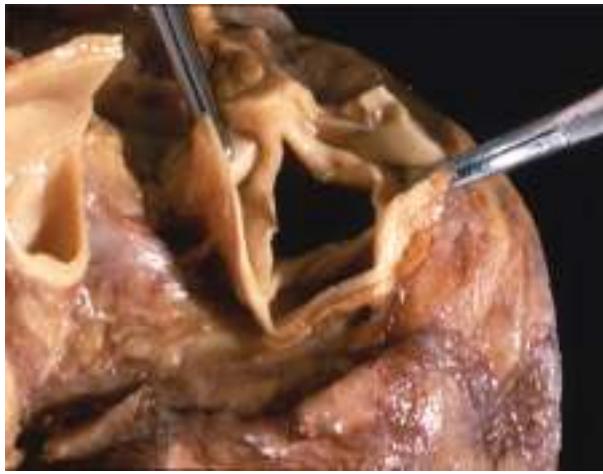


FIGURE 84-8 Carcinoid heart disease. Fibrosis secondary to elevated levels of circulating serotonin classically involves the tricuspid valve, resulting in valve retraction and tricuspid regurgitation.

affinity for serotonin receptor subtype 5-HT_{2B} receptors, whose activation is known to cause fibroblast mitogenesis and which are normally expressed in heart valve fibroblasts. These observations support the hypothesis that serotonin overproduction in patients with carcinoid syndrome mediates the valvular changes by activating 5-HT_{2B} receptors in the endocardium.

Tricuspid regurgitation is a nearly universal feature of carcinoid heart disease; tricuspid stenosis, pulmonary regurgitation, and pulmonary stenosis may also occur. Left-sided heart disease occurs in <10% of patients and has been associated with the presence of a patent foramen ovale. The preponderance of lesions in the right heart is related directly to the fact that serotonin is secreted by liver metastases or retroperitoneal tumor deposits into the venous circulation and subsequently into the right atrium and right ventricle. The lower incidence of heart disease in the left heart is postulated to be due to the fact that serotonin is metabolized in the pulmonary vasculature before entering the left atrium and ventricle. Among patients with carcinoid syndrome, patients with heart disease exhibit higher levels of serum serotonin and urinary 5-HIAA excretion than patients without heart disease. Treatment with somatostatin analogues resulting in decreased serotonin secretion does not result in regression of cardiac lesions. Reduction of serotonin levels as a result of treatment with somatostatin analogues or with the tryptophan hydroxylase inhibitor telotristat ethyl seems likely to slow progression of carcinoid heart disease but has not been formally evaluated in clinical trials.

Right-sided heart failure in patients with carcinoid heart disease may lead to significant morbidity and mortality. The development of multiple new treatments to improve overall disease control in patients with advanced NETs has led to increased interest in valvular replacement, which may result in significant clinical benefit in appropriately selected patients with carcinoid heart disease. The appropriate timing of valve replacement in such patients can be challenging given the competing desires to perform surgery before the onset of severe right-sided heart failure, which can increase surgical morbidity, and the need to achieve adequate overall tumor control. However, advanced and less invasive techniques, including catheter-based valve replacement, have made valve replacement an increasingly attractive option for patients with this condition.

HEPATIC DIRECTED THERAPY FOR METASTATIC NETS

The liver is one of the most common sites for metastases in patients with NETs and, in some cases, is the only site of metastatic disease. Hepatic-directed therapies can often be effective as a means of controlling, if not eliminating, metastases, particularly in patients who have more indolent tumors with well-differentiated histology. Common

approaches for such patients include surgical resection, ablation or embolization, and orthotopic liver transplantation.

For patients with limited hepatic disease whose tumors have well-differentiated histology, surgical resection is generally considered the preferable option. While data are limited to retrospective series with the consequent risk of selection bias, long-term survival durations and symptomatic improvements reported in select populations of patients undergoing hepatic resection of neuroendocrine liver metastases compare favorably with outcomes associated with other management approaches, and 5-year survival rates approach 90% in some series. In patients in whom anatomy precludes resection or in whom a greater number of lesions are present, radiofrequency ablation or cryoablation can also be used, either as a primary treatment modality or as an adjunct to surgical resection. While ablation is considered to be less morbid than hepatic resection, it is generally utilized only in smaller tumors so that zones of ablation are limited.

In most cases, however, liver metastases are large, multiple, and involve both lobes of the liver. In such cases, the benefit of surgical resection and ablation is limited. Hepatic arterial embolization can be considered in these cases, assuming that extrahepatic disease remains relatively limited and that clinical benefit can be achieved by reducing hepatic tumor bulk. Hepatic artery embolization is based on the principle that tumors in the liver derive most of their blood supply from the hepatic artery, whereas healthy hepatocytes derive most of their blood supply from the portal vein. Multiple different embolization techniques have been explored, ranging from the simple infusion of gel foam powder into the hepatic artery (bland embolization) to the administration of chemotherapy or chemotherapy-eluting beads into the hepatic artery (chemoembolization) or the intra-arterial administration of radioisotope-tagged microspheres (radioembolization). Limited data suggest an optimal approach to embolization, and few studies have compared these approaches directly. Tumor response rates with all of these approaches generally exceed 50%. Specific approaches are therefore often tailored to the patient, taking into account tumor location, overall tumor burden, and comorbidities. Bland embolization, for example, may be associated with less morbidity, whereas chemoembolization or radioembolization may result in longer durations of response.

The role of orthotopic liver transplantation for the treatment of NETs remains uncertain. Data from available institutional series suggest that a small number of highly selected patients may achieve long-term survival. However, 5-year overall median survival durations in most series are ~50%, and the majority of patients undergoing hepatic transplantation develop tumor recurrence. Additionally, the widespread utility of hepatic transplantation is limited by organ availability. Decisions regarding proceeding with transplantation in patients with advanced NETs are therefore highly individualized.

SYSTEMIC TREATMENT TO CONTROL TUMOR GROWTH

While hepatic-directed therapies can be effective in the management of patients with liver-predominant disease, a majority of patients will either present with or ultimately develop more widespread metastases. A number of systemic treatment options have been developed and can be effective in treating such patients. These options include treatment with traditional somatostatin analogues, peptide receptor radioligand therapy, traditional cytotoxic chemotherapy, and an increasing array of molecularly targeted therapies targeting the mTOR or vascular endothelial growth factor (VEGF) pathways (Table 84-3). The choice and sequence of therapy depend in part on the type of tumor, the extent of disease, and patient symptoms and comorbidities.

■ SOMATOSTATIN ANALOGUES

While somatostatin analogues were originally developed as a treatment to reduce hormone secretion in NETs, they are also effective in slowing tumor growth. The biologic mechanisms underlying this effect remain uncertain, but clinical studies have been definitive. The first of these studies, the PROMID study, randomized patients with metastatic small-intestinal NET to receive either octreotide LAR at a

TABLE 84-3 Selected Randomized Trials of Therapeutic Agents for the Treatment of Advanced Neuroendocrine Tumors (NETs)		
TUMOR TYPE	NUMBER OF PATIENTS	PROGRESSION-FREE SURVIVAL
Pancreatic and extrapancreatic NET		
Lanreotide vs placebo (CLARINET)	204	65 vs 33% at 2 years ($p < .001$)
Pancreatic NET		
Everolimus vs placebo (RADIANT 3)	410	11 months vs 4.6 months ($p < .001$)
Sunitinib vs placebo	171	11.4 months vs 5.5 months ($p < .001$)
Surufatinib vs placebo	264	10.9 months vs 3.7 months ($p = .001$)
Temozolamide/capecitabine vs temozolamide	144	22.7 months vs 14.4 months ($p = .021$)
Extrapancreatic NET		
Octreotide vs placebo (PROMID)	85	14.3 months vs 6 months ^a
Everolimus + octreotide vs octreotide (RADIANT 2)	429	16.4 months vs 11.3 months
Everolimus vs placebo (RADIANT 4)	302	11 months vs 3.9 months
Surufatinib vs placebo	198	9.2 months vs 3.8 months ($p < .0001$)
Pazopanib vs placebo	171	11.6 months vs 8.5 months ($p < .0005$)
177-Lutetium dotatate vs octreotide (NETTER 1)	230	65.2 vs 10.8% at 20 months ($p < .001$)

^aTime to tumor progression.

dose of 30 mg monthly or placebo. The median time to tumor progression in patients receiving octreotide was 14 months compared to only 6 months for patients receiving placebo. Because the study was limited to patients with small-intestinal NET, the generalizability of these results to patients with NETs of other origins, including pancreatic NET, was initially uncertain. This question was ultimately addressed by the phase 3 CLARINET trial, which compared lanreotide, a somatostatin analogue that is similar to octreotide in its somatostatin receptor-binding affinities, to placebo in 204 patients with a range of advanced well- or moderately differentiated gastroenteropancreatic NETs. Progression-free survival duration at 2 years was 65% in patients receiving lanreotide and 33% in patients receiving placebo, a difference that was statistically significant. One unusual aspect of the PROMID and CLARINET studies is the difference in progression-free survival durations in the placebo arms of the studies, which has been attributed to differences in patient selection. Either octreotide or lanreotide is currently considered an acceptable option for control of tumor growth in patients with advanced NETs.

Whether treatment with somatostatin analogues also increases overall survival in patients with advanced NETs has not been demonstrated, although a correlation between progression-free survival and overall survival in patients with advanced NETs treated with single-agent somatostatin analogue therapy has been shown. The timing of initiation of somatostatin analogues in patients with advanced NETs remains uncertain. The variable clinical course of NETs means that tumors can remain indolent for years even without treatment. For patients with asymptomatic, small-volume disease, observation alone may be an appropriate initial option. However, for patients with a larger disease burden, evidence of disease progression, or symptomatic disease, somatostatin analogues are generally used as an initial systemic treatment due to their ease of use and tolerability.

■ PEPTIDE RECEPTOR RADIOLIGAND THERAPY

Peptide receptor radioligand therapy employs the systemic administration of radiolabeled somatostatin analogues and is a treatment option for patients who require more aggressive treatment due to progression

on traditional somatostatin analogues or other therapies (Fig. 84-7). Peptide receptor radioligand therapy may also be considered as an initial treatment in patients with significant symptoms or tumor burden. With this approach, a radioligand is coupled to a somatostatin analogue, using the somatostatin analogue to target the tumor. When bound to the tumor cell, the radioligand is then internalized, resulting in cell death. Due to its mechanism of action, peptide receptor radioligand therapy is only considered in patients whose tumors demonstrate uptake on somatostatin scintigraphy.

Several different radioligands have been evaluated, the most successful of which have been yttrium (⁹⁰Y) and lutetium (¹⁷⁷Lu). These two ligands differ from one another in terms of their particle energy and tissue penetration; of the two, ⁹⁰Y-DOTA-TOC emits higher-energy β particles and has deeper tissue penetration. ⁹⁰Y-DOTA-TOC (⁹⁰Y-dotatoc) has been evaluated in numerous series with overall tumor responses reported in approximately one-third of patients. Enthusiasm for this approach, however, has been tempered due to concerns about side effects including both renal and hematologic toxicity.

¹⁷⁷Lu-DOTA-octreotate emits both β particles and lower-energy γ particles and, in most studies, has been associated with less toxicity than ⁹⁰Y-DOTA-TOC. Initial single-center studies with ¹⁷⁷Lu-DOTA-octreotate showed promising antitumor activity, and based on these studies, a randomized trial of ¹⁷⁷Lu-dotatate in midgut GI NETs was undertaken. In this study (NETTER-1), 229 patients with inoperable, somatostatin receptor-positive midgut NETs were randomly assigned to receive either four doses of ¹⁷⁷Lu-dotatate administered intravenously every 8 weeks or treatment with high-dose octreotide LAR (60 mg) every 4 weeks. Treatment with ¹⁷⁷Lu-dotatate was associated with objective tumor responses in 18% of patients and also was associated with a significant improvement in progression-free survival: progression-free survival at month 20 was 10.8% for octreotide LAR alone and 65.2% in the ¹⁷⁷Lu-dotatate group. Subsequent analyses have also suggested improved overall survival associated with ¹⁷⁷Lu-dotatate treatment, as well as improvements in quality of life across a number of parameters, including global health status, overall physical functioning, fatigue, pain, and diarrhea.

The renal clearance of radiopeptides, including ¹⁷⁷Lu-DOTA-octreotate, poses a risk of renal toxicity. The renal toxicity can be mitigated with the coadministration of intravenous amino acids during treatment. The most common adverse event among patients receiving ¹⁷⁷Lu-dotatate in the NETTER-1 study was nausea, most likely related to the amino acid infusions rather than to the radioisotope itself. Mild thrombocytopenia and leukopenia were also reported.

One limitation of the NETTER-1 study was its restriction to patients with advanced small intestine NETs. However, longer-term safety data as well as data supporting the efficacy of ¹⁷⁷Lu-dotatate in a broader range of gastroenteropancreatic NETs are available from large institutional series that include >1000 patients. Long-term toxicities from these series have included rare cases of acute leukemia and myelodysplastic syndrome, presumably associated with radiation exposure. Nevertheless, these studies generally support both the efficacy and safety of ¹⁷⁷Lu-dotatate as a treatment for patients with a range of somatostatin receptor-positive NETs.

■ ALKYLATING AGENTS

While the efficacy of traditional cytotoxic chemotherapy appears to be minimal in most extrapancreatic GI NETs, alkylating agents have a clear role in the treatment of advanced pancreatic NETs. Streptozocin-based combination therapy was historically used as treatment standard in such patients but has largely fallen out of favor due to both toxicity concerns and a cumbersome administration schedule. Temozolamide is an orally administered alkylating agent that has largely replaced streptozocin as a backbone in combination regimens used for the treatment of pancreatic NETs.

Initial studies evaluating temozolamide in combination with a range of different agents showed that temozolamide-based combination therapy was associated with tumor responses in 24–70% of patients. One of the most active combination regimens appeared to be temozolamide and capecitabine. This combination was subsequently compared to

temozolomide alone in a prospective randomized study undertaken by the Eastern Cooperative Oncology Group that enrolled 144 patients with advanced pancreatic NETs. The overall response rates in the two arms were relatively similar; 33% of patients who received the combination of temozolomide and capecitabine experienced objective tumor responses as compared to 28% of the patients who received temozolomide as a single agent. However, progression-free survival was significantly longer in the combination arm (22.7 vs 14.4 months). Based on these results, the combination of temozolomide and capecitabine is now the preferred chemotherapy combination for advanced pancreatic NETs.

The reason that some pancreatic NETs respond to alkylating agents while others do not is uncertain. In patients with glioblastoma, methylation of the promoter region for methylguanine DNA methyltransferase (MGMT) is associated with decreased MGMT protein expression and is highly associated with temozolomide responsiveness. MGMT is an enzyme that is responsible for repairing DNA damage induced by alkylating agents. Reduced levels of MGMT presumably impair the ability of tumor cells to repair their DNA in response to treatment and enhance the cytotoxicity of temozolomide. Several retrospective studies have suggested that lack of MGMT expression in pancreatic NET may be associated with responsiveness to temozolomide-based therapy; however, this finding has not yet been prospectively validated.

SMALL MOLECULE TYROSINE KINASE INHIBITORS

The highly vascular nature of NETs combined with observations in preclinical models that disruption of signaling pathways associated with VEGF inhibits neuroendocrine cell growth prompted a number of clinical trials evaluating therapeutic agents that inhibit the VEGF pathway in both pancreatic and extrapancreatic NETs. The VEGF pathway is activated through the binding of VEGF to its cell surface receptor, which initiates an intracellular signaling cascade that promotes angiogenesis as well as cell growth, proliferation, and survival. Clinical trials of VEGF pathway inhibitors in NETs have included a number of small-molecule tyrosine kinase inhibitors that, while they differ to some extent in specificity, all have in common the property targeting VEGFR2, the receptor isoform most strongly implicated in promoting angiogenesis.

Sunitinib, a multitargeted tyrosine kinase inhibitor that inhibits a range of growth factor receptors including VEGFR2, was one of the first agents in this class found to have activity in pancreatic NETs. In an initial phase 2 trial, sunitinib was administered to 109 patients with either pancreatic or extrapancreatic NET. Of 61 patients with pancreatic NET enrolled in the study, 11 had evidence of an objective tumor response. Based on these observations, sunitinib was evaluated in an international, randomized trial in which continuous administration of sunitinib (37.5 mg daily) was compared with placebo in 171 patients with advanced, progressive pancreatic NET. The median progression-free survival was significantly longer in patients treated with sunitinib compared with patients treated with placebo (11.4 vs 5.5 months). Common side effects associated with sunitinib included hypertension, proteinuria, and fatigue.

A second VEGFR-targeted tyrosine kinase inhibitor, surufatinib, has been evaluated in a randomized trial in which 264 patients with advanced pancreatic NETs from 21 centers in China were randomized to receive either surufatinib, administered at a dose of 300 mg daily, or placebo. Patients receiving surufatinib experienced a median progression-free survival duration of 10.9 months, as compared to 3.7 months in patients receiving placebo, closely mirroring the results of the earlier sunitinib study. Other small-molecular tyrosine kinase inhibitors have been evaluated in smaller, single-arm studies and have shown activity in pancreatic NETs, including sorafenib, cabozantinib, pazopanib, and axitinib.

Small-molecule tyrosine kinase inhibitors targeting the VEGF pathway have also been evaluated in patients with advanced nonpancreatic GI NET. In most of these studies, objective tumor response rates are lower than those seen in pancreatic NET, though many of these initial studies also revealed low rates of tumor progression and encouraging

progression-free survival durations, suggesting that these agents had antitumor activity. Pazopanib was compared to placebo in a randomized study undertaken by the ALLIANCE cooperative group, which enrolled 171 patients with nonpancreatic NETs. Patients treated with pazopanib in this study had a superior progression-free survival compared to those who received placebo (11.6 vs 8.5 months), a difference that was statistically significant. Surufatinib was used in a randomized study of 198 patients with extrapancreatic NETs; the median progression-free survival was 9.2 months in patients receiving surufatinib and 3.8 months in those receiving placebo, a statistically significant difference. These studies suggest that VEGF-targeted tyrosine kinase inhibitors have antitumor activity in extrapancreatic and pancreatic NETs.

mTOR INHIBITORS

mTOR is an intracellular protein kinase that has been implicated in the regulation of a number of processes regulating cell growth in both normal and malignant cells. It functions as a downstream component of the PI3-AKT-mTOR pathway. This pathway is negatively regulated by the tuberous sclerosis complex, comprising TSC1 and TSC2. An association between the development of pancreatic NETs and inherited mutations in TSC2 in patients with tuberous sclerosis complex was a contributing factor to initial interest in exploring mTOR inhibition as a therapeutic approach in this setting.

Following initial evidence of antitumor activity associated with everolimus (10 mg daily) in an international, multicenter, phase 2 trial of 160 patients, everolimus monotherapy (10 mg daily) was compared with best supportive care alone in the RADIANT-3 trial that enrolled 410 patients with advanced progressing pancreatic NET. While overall objective responses were uncommon, treatment with everolimus was associated with a significant prolongation in median progression-free survival (11.0 vs 4.6 months) compared to placebo, supporting its use as a standard treatment to control tumor growth in patients with advanced pancreatic NET. Common toxicities associated with everolimus are generally mild and can include stomatitis and rash; a more severe but less common side effect is pneumonitis.

Everolimus was also associated with promising activity in early phase 2 studies enrolling patients extrapancreatic NET. The first large randomized study evaluating everolimus was the RADIANT 2 trial; 429 patients with advanced GI NETs were randomly assigned to receive octreotide LAR (30 mg intramuscularly every 28 days) with or without everolimus (10 mg daily). Treatment with everolimus in this study was associated with an improvement in median progression-free survival (16.4 vs 11.3 months), but the difference in this study was of only borderline statistical significance. A second study, the RADIANT 4 study, enrolled 302 patients with advanced NETs of either GI (excluding pancreatic) or lung origin, randomizing them to receive either everolimus or placebo. In this study, treatment with octreotide was not required. As in the RADIANT 3 study, objective tumor responses were uncommon; however, median progression-free survival in patients who received everolimus was significantly longer than in those who received placebo (11 vs 3.9 months). Based on the results of this study, everolimus is considered a standard treatment for control of tumor growth in extrapancreatic NETs.

OTHER SYSTEMIC TREATMENTS FOR CONTROL OF TUMOR GROWTH

Interferon α has been used as a treatment for advanced NETs for several decades. With the development of newer approaches, its routine use has diminished. The use of interferon α was based primarily on observations in large, retrospective series where low-dose interferon α was reported to both reduce symptoms of hormonal hypersecretion and slow tumor progression. Interferon can be myelosuppressive, requiring dose titration, and in some patients can induce both fatigue and depression. Antitumor activity has also been reported with oxaliplatin-based chemotherapy regimens. A combined analysis of two phase 2 trials examining oxaliplatin-fluoropyrimidine chemotherapy plus bevacizumab in advanced NET suggested antitumor activity for these regimens; the benefit appeared to be greatest in patients with intermediate-grade rather than low-grade tumors.

■ SYSTEMIC THERAPY FOR HIGH GRADE NEUROENDOCRINE CARCINOMA

High-grade NETs are relatively uncommon; their clinical behavior is fundamentally different from well-differentiated NETs in that these tumors pursue an aggressive clinical course. Systemic chemotherapy for advanced high-grade neuroendocrine carcinoma has historically followed a paradigm analogous to that used for small-cell carcinoma of the lung, with combinations of either cisplatin or carboplatin administered together with etoposide generally considered the preferred first-line approach. One of the most important elements in determining the optimal chemotherapeutic approach is assessing the Ki-67 proliferative index. A large retrospective series that evaluated 252 patients with high-grade neuroendocrine carcinoma found that the activity of platinum-based therapy was greatest in patients who had a Ki-67 proliferative index of 55% or higher; in these patients, the overall tumor response rate was 42%. In contrast, the overall response rate in patients in whom the Ki-67 proliferative index was <55% was only 15%. As in small-cell carcinoma of the lung, immune checkpoint inhibitors also appear to have some activity in high-grade neuroendocrine carcinoma. While minimal activity has been noted in well-differentiated NETs, a combination of ipilimumab and nivolumab was associated with an overall tumor response rate of 42% in an initial phase 2 trial enrolling 19 patients with high-grade neuroendocrine carcinoma.

A

Dr. Robert Jensen contributed this chapter in previous editions, and some material from his chapter is retained here.

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■ EPIDEMIOLOGY

The incidence of cancers of the kidney and renal pelvis rose for three decades, reached a plateau of approximately 64,000 cases annually in the United States between 2012 and 2018, but has since increased to approximately 76,000 cases annually, resulting in close to 14,000 deaths per year. It is the eighth most common cancer overall in the United States, the sixth most common in males, and the eighth most common in females; the male-to-female ratio is 2:1. Although this malignancy may be diagnosed at any age, it is uncommon in those under 45 years, and incidence peaks between the ages of 55 and 75 years. Many factors have been investigated as possible contributing causes; associations include cigarette smoking, obesity, and hypertension. Risk is also increased for patients with polycystic kidney disease that has been complicated by chronic renal failure.

Most cases of renal cell carcinoma (RCC) are sporadic, although familial forms have been reported (Table 85-1). One well established example includes clear cell RCC arising in the context of von Hippel-Lindau (VHL) syndrome, an autosomal dominant disorder. Genetic studies identified the *VHL* gene on the short arm of chromosome 3. Individuals with VHL syndrome have an estimated lifetime risk of developing clear cell RCC of around 70%. Other *VHL*-associated neoplasms include retinal hemangioma, hemangioblastoma of the spinal cord and cerebellum, pheochromocytoma, and neuroendocrine tumors and cysts. Birt-Hogg-Dubé syndrome is a rare human autosomal dominant genetic disorder characterized by fibrofolliculomas (benign tumors arising in hair follicles), pulmonary cysts, and renal cell carcinomas of varying histologies, most commonly the chromophobe type, occurring in about a third of patients. This disorder is associated with mutations in the *FLCN* gene, which codes for folliculin. Other hereditary syndromes are summarized in Table 85-1.

■ PATHOLOGY AND GENETICS

Renal cell malignancies represent a heterogeneous group of tumors with distinct histopathologic, genetic, and clinical features (Table 85-2). Categories include clear cell carcinoma (70% of cases), papillary tumors (10–15%), chromophobe tumors (<5%), renal medullary carcinoma (<1%), translocation carcinoma (<5%), and other less common variants. Papillary tumors can be bilateral and multifocal. Chromophobe tumors tend to have a more indolent clinical course. Translocation-associated RCC, rare in adult patients, is the predominant histology in children. Renal medullary carcinoma is rare, very aggressive, and associated with sickle cell trait. Tumors that do not meet criteria for defined variants are generally referred to as “unclassified” with variable clinical courses.

Clear cell tumors, the predominant histology, are found in >80% of patients who develop metastases and arise from the epithelial cells of the proximal tubules. Loss of chromosome 3p is uniformly seen as the earliest event in the development of these cancers. This leads to loss of heterozygosity for a number of relevant 3p genes, including *VHL*, *PBRM1*, *BAP1*, and *SETD2*, which can be functionally lost through secondary events in the remaining allele. *VHL* encodes a tumor suppressor protein that is involved in regulating the transcription of vascular endothelial growth factor (VEGF) and a number of other effectors through ubiquitination of hypoxia-inducible factors (HIF). Inactivation of *VHL*, through upregulation of VEGF signaling, promotes tumor angiogenesis and growth, ultimately rendering clear cell RCC cells susceptible to antiangiogenesis therapy.

Large-scale sequencing efforts have helped elucidate recurrent patterns of genomic evolution that correlate with distinct clinical phenotypes, e.g., varying levels of aggressiveness or specific patterns of metastatic spread. For example, early loss of chromosome 9p appears to confer a high risk for early metastatic dissemination and correlates with poor cancer-specific survival.

A growing number of other RCC variants are well defined (see Table 85-2 for examples). For instance, up to 15% of RCCs are of the papillary subtype, with several subtypes that can be distinguished either by light microscopy or tumor genomics. For example, activating mutations in the *MET* oncogene or gain of chromosome 7 (where *MET* is located) are hallmark events of type 1 papillary RCC and considered

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Renal Cell Carcinoma

Robert J. Motzer, Martin H. Voss



Renal cell carcinomas account for 90–95% of malignant neoplasms arising from the kidney. Notable features include frequent diagnosis without symptoms, resistance to cytotoxic agents, robust activity of angiogenesis-targeted agents, immune infiltration commonly rendering tumors susceptible to checkpoint-directed immunotherapy, and a variable clinical course for patients with metastatic disease, including anecdotal reports of spontaneous regression. Most of the remaining 5–10% of malignant neoplasms arising from the kidney are transitional cell carcinomas (urothelial carcinomas) originating in the lining of the renal pelvis. See Chap. 86 for transitional cell carcinomas.

TABLE 85-1 Hereditary Renal Cell Tumors

SYNDROME	CHROMOSOME(S)	GENE	PROTEIN	KIDNEY TUMOR TYPE	ADDITIONAL CLINICAL FINDINGS
von Hippel-Lindau syndrome	3p25	VHL	von Hippel-Lindau protein	Clear cell	Hemangioblastoma of the retina and central nervous system; pheochromocytoma; pancreatic and renal cysts; neuroendocrine tumors
Hereditary papillary RCC	7p31	MET	MET	Papillary (type I)	Bilateral and multifocal kidney tumors
Hereditary leiomyomatosis and RCC (HLRCC syndrome)	1q42	FH	Fumarate hydratase	Papillary (non-type I)	Leiomyoma; uterine leiomyoma/leiomyosarcoma
Birt-Hogg-Dubé syndrome	17p11	FLCN	Folliculin	Chromophobe; oncocytoma	Facial fibrofolliculoma; pulmonary cysts
Tuberous sclerosis	9q34 16p13	TSC1 TSC2	Hamartin Tuberin	Angiomyolipomas; lymphangiomyomatosis; rare RCC with variety of histologic appearances	Angiofibroma, subungual fibroma; cardiac rhabdomyoma; adenomatous small intestine polyps; pulmonary and renal cysts; cortical tuber; subependymal giant cell astrocytomas
BAP1 tumor predisposition syndrome	3p21	BAP1	BAP1	Clear cell	Atypical Spitz tumors; uveal melanoma; cutaneous melanoma; basal cell carcinoma; malignant mesothelioma

Abbreviation: RCC, renal cell carcinoma.

actionable via targeted MET inhibitors. Tumors of the less common chromophobe subtype originate from the distal nephron. They are in part driven by changes in mitochondrial gene function and typically characterized by aneuploidy with common loss of an entire chromosome copy for chromosomes 1, 2, 6, 10, 13, and 17.

CLINICAL PRESENTATION

Presenting signs and symptoms may include hematuria, flank or abdominal pain, and a palpable mass. Other symptoms are fever, weight loss, anemia, and a varicocele. Tumors are, however, commonly detected as an incidental finding on a radiograph. Widespread use of radiologic cross-sectional imaging (computed tomography [CT], magnetic resonance imaging [MRI]) contributes to earlier detection of renal masses during evaluation for other medical conditions. The increasing number of incidentally discovered low-stage tumors has contributed to an improved 5-year survival for patients with RCC and increased use of nephron-sparing surgery (partial nephrectomy). A spectrum of paraneoplastic syndromes has been associated with these malignancies, including erythrocytosis, hypercalcemia, nonmetastatic hepatic dysfunction (Stauffer's syndrome), and acquired dysfibrinogenemia. Erythrocytosis is noted at presentation in only about 3% of patients. Anemia, commonly a sign of more advanced disease, is more common. Kidney cancer was called the "internist's tumor" since it was often discovered from the initial presentation of a paraneoplastic syndrome. This was more common before the era of modern imaging, as was initial presentation by the classic triad of hematuria, flank pain, and a palpable abdominal mass.

The standard evaluation of patients with suspected renal tumors includes a CT scan of the abdomen and pelvis, chest radiograph, and urine analysis. If metastatic disease is suspected from the chest radiograph, a CT of the chest is warranted. MRI is useful in evaluating the

inferior vena cava in cases of suspected tumor involvement or invasion by thrombus, or when intravenous contrast administration given with CT is prohibited by impaired renal function. In clinical practice, any solid renal masses should be considered malignant until proven otherwise; a definitive diagnosis is required. If no metastases are demonstrated, surgery is indicated, even if the renal vein or inferior vena cava is invaded. In small tumors (particularly those of clear cell variant) the risk of impending metastatic spread is lower and surgery can potentially be delayed. In that setting, a needle biopsy should be performed to confirm the underlying histology, and radiographic surveillance is indicated until the time of surgery. The differential diagnosis of a renal mass includes cysts, benign neoplasms (adenoma, angiomyolipoma, oncocytoma), inflammatory lesions (pyelonephritis or abscesses), and other malignancies originating in the kidney such as transitional cell carcinoma of the renal pelvis, sarcoma, lymphoma, and Wilms' tumor or metastases from cancers originating in other organs. All of these are less common causes of renal masses than is RCC. The most common sites of distant metastases are the lungs, lymph nodes, liver, bone, and brain. These tumors may follow an unpredictable and protracted clinical course.

STAGING AND PROGNOSIS

Staging is based on the American Joint Committee on Cancer (AJCC) staging system (Fig. 85-1). Stage I tumors are ≤ 7 cm in greatest diameter and confined to the kidney, stage II tumors are >7 cm and confined to the kidney, stage III tumors extend through the renal capsule but are confined to Gerota's fascia, grossly infiltrate the renal vein, or involve regional lymph nodes (N1), and stage IV disease includes tumors that have invaded adjacent organs or involve nonregional lymph nodes or distant metastases. Sixty-five percent of patients present with stage I or II disease, 15–20% with stage III, and 15–20% with stage IV. The 5-year survival rate is currently 75% across all RCCs, but varies greatly by stage.

TABLE 85-2 Classification of Malignant Epithelial Neoplasms Arising from the Kidney

CARCINOMA TYPE	CHARACTERISTIC GROWTH PATTERN	CHROMOSOMAL EVENTS	GENES WITH RECURRENT SOMATIC ALTERATIONS
Clear cell	Acinar or sarcomatoid	3p-, 5q+, 14q-, 9p-	VHL, PBRM1, BAP1, SETD2
Papillary	Papillary or sarcomatoid	+7, +17, 9p-	MET, FH, CDKN2A (focal deletions)
Chromophobe	Solid, tubular, or sarcomatoid	Whole arm losses (1, 2, 6, 10, 13, 17, and 21)	TP53, PTEN, TERT promotor
Renal medullary carcinoma	Varying growth patterns, including cribriform, reticular, sarcomatoid, adenoid, and microcystic	+8q, 22q-, 22q translocations	SMARCB1 (focal deletions, mutations, gene fusions), SETD2
MITF translocation ^a	Mimicking clear cell and papillary variants	Xp11.2 translocations; t(6;11) translocations	TFE3 gene fusions, TFEB gene fusions

^aMicrophthalmia transcription factor gene family.

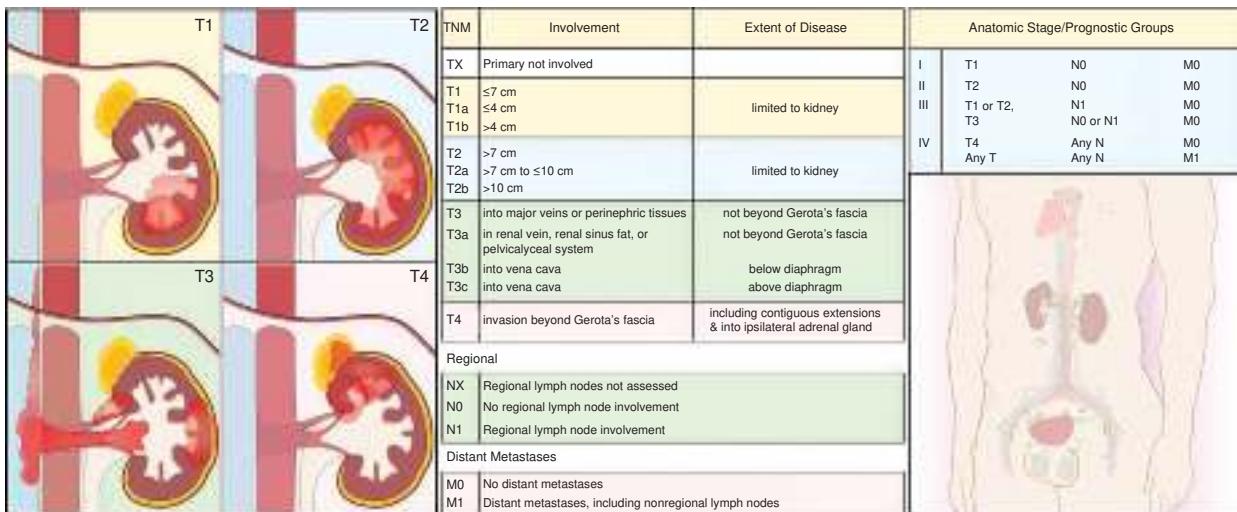


FIGURE 85-1 Renal cell carcinoma staging. TNM, tumor-node-metastasis.

Prognostic risk models are helpful for counseling patients diagnosed with metastatic disease and for anticipating survival rates when designing a clinical trial. A widely used prognostic model for advanced disease, the International Metastatic RCC Database Consortium (IMDC) risk model, incorporates six factors shown to correlate with worse survival: poor performance status, low hemoglobin concentration, high serum calcium, high neutrophil levels, high platelet levels, and <1-year interval from diagnosis to systemic treatment. Patients with zero risk factors had significantly longer median survival (43 months) than patients with one or two risk factors (22.5 months) and those with three to six risk factors (8 months) when treated with first-line angiogenesis inhibitors (see below).

TREATMENT

Renal Cell Carcinoma

LOCALIZED TUMOR

The standard management for stage I or II tumors and selected cases of stage III disease is radical or partial nephrectomy. A radical nephrectomy involves en bloc removal of Gerota's fascia and its contents, including the kidney, and commonly the ipsilateral adrenal gland and regional lymph nodes that appear abnormal on imaging or intraoperatively. Open, laparoscopic, or robotic surgical techniques may be used. The role of a template lymphadenectomy in patients without apparent lymphadenopathy is controversial. Extension into the renal vein or inferior vena cava (stage III disease) does not preclude resection, which would then include thrombectomy.

Nephron-sparing approaches, i.e., open or laparoscopic partial nephrectomy, may be appropriate depending on the size and location of the tumor. This approach is particularly relevant for patients with solitary kidneys, bilateral tumors, or chronic renal insufficiency but can also be applied electively to resect small masses for patients with normal kidney function. Radical nephrectomy carries a greater risk for chronic kidney disease and cardiovascular morbidity and mortality.

Adjuvant systemic therapies, including cytokines and targeted agents, have been studied in randomized clinical trials, largely with negative results, and the standard of care remains active surveillance after nephrectomy.

METASTATIC DISEASE

Surgery has a limited role for patients with metastatic disease. Long-term survival may occur in patients who relapse with a solitary site

that is removed (metastasectomy). Nephrectomy despite presence of metastases (cytoreductive nephrectomy) is considered for carefully selected patients with stage IV disease. One indication for this approach can be to alleviate pain or hemorrhage of a primary tumor.

Radiation therapy is used for palliation of bone or brain metastases. The type of radiotherapy most commonly used is external-beam therapy, including stereotactic radiosurgery and other forms of image-guided radiotherapy.

Systemic therapy is the mainstay of care for metastatic disease. The timing of initiating such treatment should be carefully considered; some patients are asymptomatic at diagnosis, and with indolent behavior, it may be best to document progression before initiating treatment.

Metastatic RCC is refractory to cytotoxic chemotherapy. Patients are treated with molecularly targeted agents, including targeted immunotherapies. Treatments are continued with noncurative intent while tolerated and until disease progression is evident on cross-sectional imaging. Outcomes for patients with metastatic disease improved when increased understanding of underlying biology led to the successful development of several tyrosine kinase inhibitors (TKIs) targeting proangiogenic signaling through the VEGF receptors as well as allosteric inhibitors of mammalian target of rapamycin (mTOR) signaling. Serial large-scale randomized trials demonstrated that such agents, typically orally available, could be administered sequentially and in combination. Pivotal studies, by design, defined a dedicated space for each regimen in treatment-naïve or pretreated patients (Table 85-3).

Targeted immunotherapies were introduced after VEGF- and mTOR-directed agents had established standards of care in the first- and second-line setting. Nivolumab, a checkpoint inhibitor targeting PD-1, was compared to the mTOR inhibitor everolimus in a randomized trial in patients who had progressed on prior TKI therapy, challenging the standard approach in pretreated patients. Nivolumab demonstrated superior overall survival, positioning it as the new second-line agent of choice. Subsequently, immunotherapy combination regimens demonstrated efficacy in randomized trials conducted in treatment-naïve patients. In separate studies, two doublets demonstrated survival benefit over standard sunitinib therapy and changed the standard of care for untreated metastatic clear cell RCC: nivolumab in combination with the CTLA-4-directed checkpoint inhibitor ipilimumab proved superior to sunitinib in patients with high-risk features per the IMDC model, achieving complete radiographic disappearance of cancer in >10% of patients treated with the combination. In a second trial, the combination of the

TABLE 85-3 Commonly Used Systemic Regimens for Metastatic Renal Cell Carcinoma

CLASS	DRUG	FIRST FDA APPROVAL FOR RCC	CURRENTLY USED FOR
Antiangiogenic: TKIs	Sunitinib	2006	Advanced RCC, first line
	Pazopanib	2009	Advanced RCC, first line
	Axitinib	2012	Advanced RCC, pretreated
	Cabozantinib	2016 2017	Advanced RCC, pretreated with antiangiogenic therapy Advanced RCC, first line
Immunotherapy: checkpoint inhibitor	Nivolumab	2015	Advanced RCC, pretreated with antiangiogenic therapy
Combination therapies			
TKI + mTOR inhibitor	Lenvatinib + everolimus	2016	Advanced RCC, pretreated with one antiangiogenic therapy
PD-1 inhibitor + CTLA-4 inhibitor	Nivolumab + ipilimumab	2018	Advanced intermediate- or poor-risk RCC, first line
PD-1 inhibitor + TKI	Pembrolizumab + axitinib	2019	Advanced RCC, first line

Abbreviations: CTLA-4, cytotoxic T-lymphocyte-associated protein; FDA, U.S. Food and Drug Administration; mTOR, mammalian target of rapamycin; PD-1, programmed cell death-1; RCC, renal cell carcinoma; TKI, tyrosine kinase inhibitor.

TKI axitinib together with the PD-1 inhibitor pembrolizumab was superior to sunitinib alone in all-comers with untreated metastatic RCC, again with high response rates across all IMDC risk groups. In both trials, responses were long-lasting, with improved time to disease progression and longer overall survival for combination regimens. Additional trials are ongoing to fortify the new standard of combination therapy in the first line.

With an ever-growing number of approved options directed toward different molecular targets, biomarkers are urgently needed to help individualize therapeutic choices and to gain insight as to whether and why treatments are working. Although a multitude of candidate biomarkers have been investigated for their predictive value in metastatic RCC, none have been validated for clinical use to date.

Projected overall survival in patients starting systemic therapies for newly diagnosed metastatic disease has tripled over the past 15–20 years; this can largely be attributed to the successful drug developments discussed here.

■ GLOBAL CONSIDERATIONS

Worldwide, over 400,000 patients are diagnosed each year with malignant tumors arising from the kidney, resulting in >175,000 deaths annually. Kidney cancer is the 10th most common cancer in men and the 15th most common cancer in women. Higher incidence is observed in developed countries, including the United States, Canada, Europe, Australia, New Zealand, and Uruguay. Relatively low rates are reported in Southeast Asia and Africa. The incidence of kidney cancer has been steadily increasing over the past four decades. Mortality trends have stabilized in Europe and the United States, but not in less developed countries. This is likely related to differences in access to optimal therapies. Treatment guidelines for both localized and metastatic renal cancer are similar between U.S. and European documents and contingent on the access to adequate health care and availability of targeted drugs to treat metastases.

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Cancer of the Bladder and Urinary Tract

Noah M. Hahn



GLOBAL CONSIDERATIONS

Within the United States, urothelial carcinomas of the bladder and urinary tract are most closely related to tobacco smoking history. However, within developing countries, water supplies contaminated with arsenic or schistosomiasis parasites also are major carcinogenic contributors.

INTRODUCTION

Cancers of the urinary tract including the bladder, renal pelvis, ureter, and urethra occur frequently, and they represent the second most common class of genitourinary cancers. Bladder cancer alone represents the sixth most common cancer diagnosis annually in the United States with 81,400 new cases and 17,980 deaths every year. Because cancers of the renal pelvis are often lumped in with all kidney cancers, the true incidence and mortality from nonbladder urinary tract cancers are less precise. While less frequent than bladder cancer, an additional 20,000 new cases and 5000 deaths are estimated every year. An accelerated understanding of the molecular underpinnings of bladder and urinary tract cancer biology has led to a significant increase in urothelial cancer clinical trials resulting in U.S. Food and Drug Administration (FDA) approval of multiple new therapeutic agents since 2016 with more expected to follow. This chapter reviews the established, current, and emerging evidence that serves as the basis for the rapidly evolving standards of care for patients with bladder and urinary tract cancers.

■ CLINICAL EPIDEMIOLOGY AND RISK FACTORS

Bladder cancer typically affects older patients with a median age at diagnosis of 73 years. Males are four times more frequently affected than females. Similarly, bladder cancer is more common in Caucasians than in Asian patients. Inheritable germline genetic risk factors have been identified in up to one-seventh of patients with bladder or urinary tract cancers. However, a singular germline genetic alteration has not

been observed in a majority of these cases, and the impact of germline genetic alterations on family members of urothelial cancer patients is uncertain. Patients with defects in mismatch repair genes leading to microsatellite instability (*MLH1*, *MSH2*, *MSH6*, etc.) as part of the familial cancer Lynch syndrome are at particular risk of upper urinary tract cancers of the renal pelvis and ureter. Additionally, patients with Cowden disease (*PTEN* mutations) or retinoblastoma (*RBL* mutations) are at increased risk for developing bladder cancer.

Historically, associations have existed between environmental toxic exposures and higher rates of developing bladder cancer. Carcinogenic agents associated with increased risk of bladder cancer have included the aromatic amines benzidine and beta-naphthylamine that can be present in industrial dyes as well as arsenic that can be found in some drinking water supplies in underdeveloped countries. Other chemicals in the leather, paint, rubber, textiles, and printing industries have been associated with bladder cancer. Associations with exposures to hair dyes and hair sprays in workers in the hairstyling field have been suggested. Additionally, concern has been raised regarding use of the antidiabetic medication, pioglitazone, and bladder cancer risk. Extensive reviews and meta-analyses have produced differing conclusions. The data suggest a small risk of bladder cancer from long-term pioglitazone use, which has led to inclusion of bladder cancer risk within the pioglitazone prescribing information. An association between chronic inflammatory states and the development of squamous bladder cancer clearly exists in underdeveloped countries in patients chronically infected with the parasitic disease schistosomiasis and in paraplegic patients with chronic indwelling catheters. Above and beyond each of these associations, however, smoking of tobacco products (cigarettes, cigars, pipes, etc.) remains the overwhelming leading risk factor for development of bladder cancer. Among new bladder cancer diagnoses, 90% of cases occur in current or former smokers. Toxicologists have estimated that >70 confirmed carcinogenic toxins are present within tobacco smoke. It is estimated that one-third of bladder cancer cases could be prevented through simple modification of lifestyle choices, in particular cessation of smoking.

■ CLINICAL PRESENTATION AND DIAGNOSTIC WORKUP

Occasionally, patients will present with flank pain in association with an upper tract renal pelvis or ureter cancer or due to hydronephrosis in association with a bladder tumor obstructing the orifice of the ureter within the bladder. Only in rare cases do patients present with significant cachexia and widespread metastatic disease. For most patients, painless hematuria (either gross or microscopic) represents the initial manifestation of an underlying urinary tract cancer. In females, hematuria due to malignancy can often be mistaken for a urinary tract infection or menstrual bleeding. While treatment with antibiotics is warranted if a concurrent urinary tract infection is noted on initial urinalysis, persistent hematuria requires further workup. Painless hematuria in males is almost always abnormal and should be worked up. Initial investigations in patients of either sex should include urine cytology and visual examination of the bladder by cystoscopy. Cytology is successful in identifying cancer in only 50% of individuals with high-grade bladder cancers. In addition to urine cytology, radiographic evaluation of the kidneys and upper urinary tract by CT urogram should be performed. Because of the increased sensitivity and reduced IV contrast loads, CT uograms have largely replaced IV pyelograms as the preferred upper urinary tract imaging modality. A magnetic resonance (MR) urogram may be substituted in patients with poor renal function. Additional diagnostic testing of the urine to assess for cancer-associated chromosomal changes by fluorescent *in situ* hybridization, increased levels of nuclear mitotic proteins, increased bladder tumor-associated antigens, or higher levels of staining on cells shed by the bladder may identify some cancers missed by traditional cytology testing. However, they may also produce abnormal results in patients who do not have cancer. For now, these adjunct molecular tests are primarily utilized in detecting recurrent cancer in patients with a prior diagnosis of urinary tract cancer. Small tumors, particularly flat noninvasive tumors of the bladder, may be detected at higher rates with

the use of blue light cystoscopy or narrow-band imaging cystoscopy. Both blue light and narrow-band imaging cystoscopies are now used routinely in the monitoring of patients with bladder cancer. For patients with no bladder abnormalities in whom upper tract tumors are suspected, visualization of the upper urinary tracts and renal pelvices should be performed by ureteroscopy or retrograde pyelography.

In all patients with abnormalities noted in the bladder or upper urinary tracts, complete endoscopic resection for histologic diagnosis and staging should be performed when possible via either transurethral resection of bladder tumor (TURBT) or endoscopic resection of upper tract tumors.

■ HISTOLOGY

Urothelial carcinoma, often called transitional cell carcinoma in the past, is the most common urinary tract cancer histology and is observed in ~90% of cases. Squamous, glandular, micropapillary, plasmacytoid, sarcomatoid, and other variant features can often be found in portions of urothelial carcinoma tumors; however, pure variant histologies are rare. The presence of some variant histologies including micropapillary and plasmacytoid has been associated with worse surgical outcomes compared to urothelial carcinoma. Nonurothelial variant histologies including squamous cell carcinoma, adenocarcinoma, small-cell carcinoma, and carcinosarcoma collectively account for $\leq 10\%$ of urinary tract tumors. Examples of traditional urothelial carcinoma and some of the variant histologies are shown in Fig. 86-1.

■ MOLECULAR BIOLOGY

Clinically, urothelial carcinoma of the bladder displays a biphasic phenotype characterized by (1) low-grade papillary tumors that frequently recur but rarely invade or metastasize and (2) high-grade sometimes flat tumors that invade early leading to lethal metastatic disease. In both of these phenotypes, loss of portions of chromosomes 9q and 9p by loss of heterozygosity is an early molecular event, whose exact significance is not clear. Potential candidate regulatory genes in these genomic regions include *CDKN2A*, a cyclin-dependent kinase inhibitor, and *TSC1*, a gene encoding hamartin mutated in tuberous sclerosis. Early investigations have demonstrated that low-grade tumors are characterized by alterations in the *RAS/RAF* signaling pathway with activating *FGFR3* mutations or gene fusions present in 60–80% of patients. In contrast, the high-grade invasive phenotype is notable for early deleterious mutations in *TP53* and *RBL*, alterations in *CDH1* (E-cadherin), and increased expression of *VEGFR2*. In urothelial carcinoma of the renal pelvis and ureter, 10–20% of cases may be associated with Lynch syndrome hereditary defects in the *MLH1*, *MSH2*, or *MSH6* mismatch repair genes, leading to microsatellite instability and frequent DNA mutations. Testing for germline mutations in these genes is recommended in patients with upper urinary tract urothelial carcinoma under the age of 60 at diagnosis, with a first-degree relative with a Lynch syndrome-associated cancer diagnosed under the age of 50, or with two first-degree relatives with a Lynch syndrome-associated cancer regardless of the age at diagnosis.

As genomic analysis technologies have improved, so has our understanding of the molecular biology unique to urothelial carcinoma. In 2017, the full bladder cancer results of The Cancer Genome Atlas (TCGA) project were published. This effort comprehensively analyzed gene mutations, fusions, expression, copy number variations, methylation, and microRNA across the genome of patients with bladder urothelial carcinoma treated with surgery. Key findings from this effort include (1) genomic alterations in genes (e.g., *FGFR3*, *EGFR*, *ERBB2*, *ERBB3*, *PIK3CA*, *TSC1*, etc.) targetable by currently approved drugs or drugs in development in 71% of patients; (2) genomic alterations in chromatin-modifying genes (*KMT2D*, *KDM6A*, *KMT2C*, *EP300*, *CREBBP*, etc.) in the majority of patients; (3) hypermethylation with epigenetic silencing of gene expression in one-fourth of patients; and (4) the identification by RNA sequencing of five distinct intrinsic molecular subtypes (luminal papillary, luminal infiltrated, luminal, basal squamous, and neuronal) closely resembling luminal and basal subclassifications of breast cancers. These bladder TCGA findings have led to clinical trial designs enriching for patients with specific

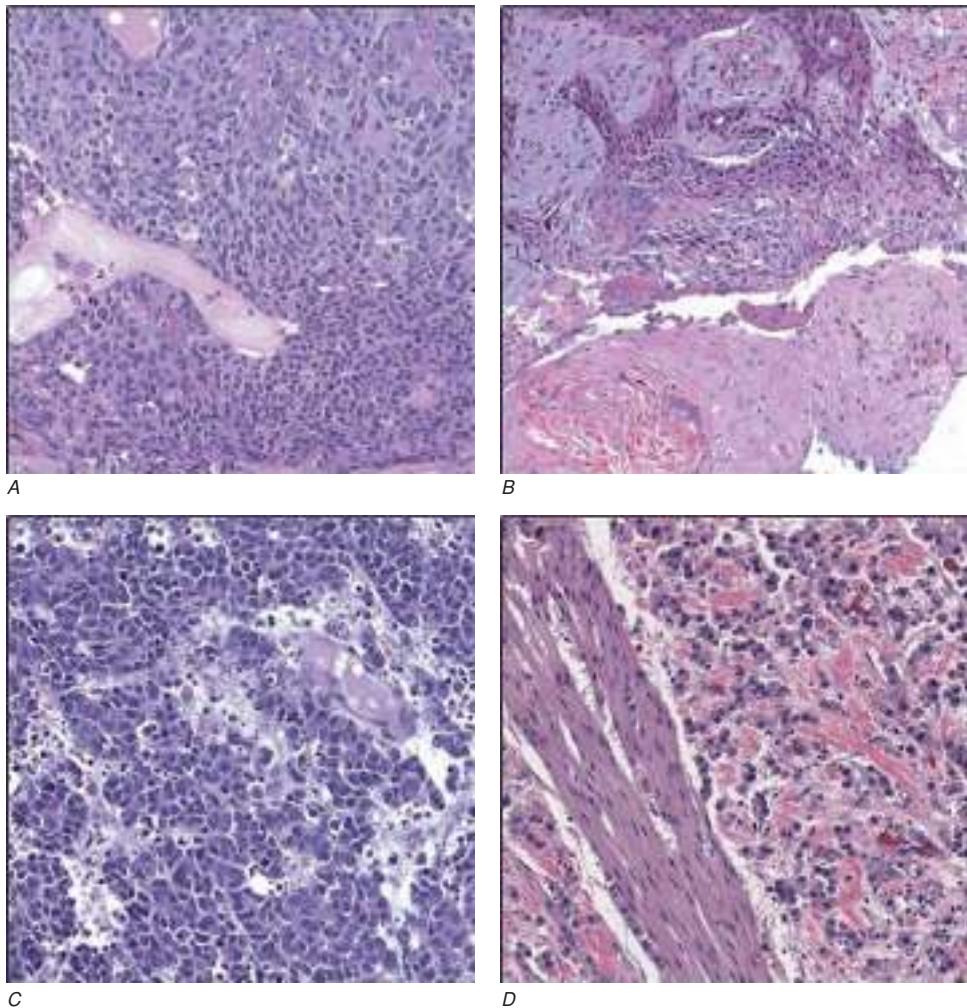


FIGURE 86-1 Bladder and urinary tract cancer histologies. *A*. Urothelial carcinoma. *B*. Squamous cell carcinoma. *C*. Small-cell carcinoma. *D*. Plasmacytoid variant. (Courtesy of Alex Baras, MD, PhD, Johns Hopkins University Department of Pathology.)

gene mutation profiles as well as interrogation of candidate biomarkers according to intrinsic molecular subtypes.

■ STAGING AND OUTCOMES BY STAGE

The staging of bladder cancer is dependent on the depth of invasion within the bladder wall, involvement of lymph nodes, and spread to surrounding and distant organs as depicted in Fig. 86-2. Approximately 75% of bladder cancer presents with non-muscle-invasive bladder cancer (NMIBC), 18% with disease invading into or through the muscular wall of the bladder, and only 3% presenting with metastatic spread to distant organs. NMIBC is defined by tumors that involve only the immediate epithelial layer of cells (carcinoma *in situ* [CIS] and Ta) or that only penetrate into the connective tissue below the urothelium (T1) but not into the muscular layer known as the *muscularis propria*. Muscle-invasive bladder cancer (MIBC) is defined by tumors that invade into the muscularis propria (T2), through the muscularis propria to involve the surrounding serosa (T3), or into immediately adjacent pelvic organs such as the rectum, prostate, vagina, or cervix (T4). Lymph node staging is classified according to involvement of a solitary node within the true pelvis (N1), two nodes involved in the true pelvis (N2), or involvement of the common iliac nodes (N3). Any disease that has spread beyond the common iliac nodes is considered metastatic (M1). The staging of bladder cancer is driven primarily by the T stage of the tumor, with stages 0a-II defined entirely by the T stage in the absence of nodal or metastatic disease. Involvement of

regional lymph nodes in the true pelvis or along the common iliac artery qualifies as stage III disease, whereas involvement of any distant metastases qualifies as stage IV disease. Clinical outcomes of patients with bladder cancer correlate closely with staging at diagnosis with 5-year overall survival rates of 70–90% for disease confined to the bladder (stage I-II), 36–50% for disease that penetrates through the bladder or has spread to regional lymph nodes (stage III), and only 5% for disease extending to metastatic sites (stage IV).

■ TREATMENT APPROACHES

Early-Stage Disease For NMIBC, removal of all visible tumors by TURBT in the operating room is considered the mainstay of surgical treatment. Risk of recurrence can be classified as low, intermediate, or high depending on the presence of features summarized in Table 86-1. For patients with low-risk disease, meta-analyses have demonstrated a 12% reduction in early relapses when a single chemotherapy treatment of mitomycin C, epirubicin, or gemcitabine was instilled directly into the bladder (intravesical therapy) within 24 hours of the TURBT. For patients with intermediate- or high-risk tumors, weekly intravesical instillations for 6 consecutive weeks of the attenuated mycobacterium strain known as *Bacille Calmette-Guérin* (BCG) reduce the risk of recurrence at 12 months from 56 to 29%. In addition, BCG treatment has been shown to decrease the rate of progression to MIBC by 27%. Intravesical

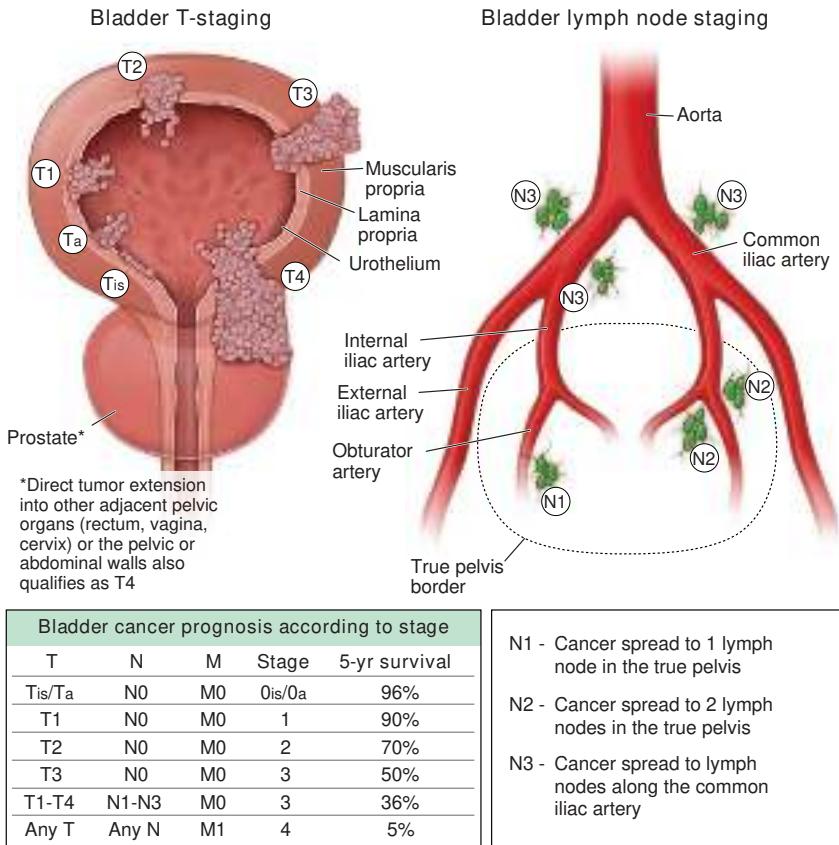


FIGURE 86-2 Bladder cancer staging and prognosis. TNM, tumor-node-metastasis.

BCG is generally well tolerated. Side effects can include dysuria, urinary frequency, bladder spasms, hematuria, and, in rare cases (<5%), a systemic inflammatory response that can mimic disseminated BCG infection. Following a 6-week induction BCG schedule, additional maintenance BCG treatments given according to the Southwest Oncology Group schedule further reduce the risk of recurrent NMIBC compared to induction BCG alone. In patients with NMIBC that recurs long after initial BCG treatment, a repeat course of BCG can be considered. For patients with recurrence after a second adequate course of BCG or with relapsed NMIBC within 6 months of initial BCG exposure, surgical removal of the entire bladder by cystectomy is recommended due to the high risk of progression to MIBC and potentially metastatic disease. For patients who are not fit enough for or who refuse cystectomy, non-BCG alternative intravesical agents (mitomycin C, gemcitabine, docetaxel, valrubicin) or systemically

administered agents that inhibit the PD-1/PD-L1 immune checkpoint pathway (pembrolizumab) can achieve durable tumor responses in a small fraction of patients.

Upper Tract Disease In patients with urothelial carcinoma of the renal pelvis or ureter, endoscopic tissue acquisition and staging are more challenging than primary tumors located in the bladder. Tumors possessing all of the following are considered low risk: solitary tumor, low grade, size <1 cm, and no invasive component on imaging. Low-risk tumors can successfully be treated by laser ureteroscopic ablation or surgical resection and reanastomosis of the remaining ureter ends in tumors that cannot be successfully eradicated endoscopically.

Muscle-Invasive Disease In patients with urothelial carcinoma of the bladder that invades into or through the muscularis propria but with no evidence of metastatic spread, more aggressive therapy options summarized in Table 86-2 are required to achieve cure. In carefully selected patients with no evidence of CIS or hydronephrosis, bladder-sparing combined-modality therapy with concurrent chemotherapy and radiation can achieve cure in ~65% of patients. Various chemotherapy regimens have been utilized in combination with radiation including cisplatin, carboplatin, 5-fluorouracil, mitomycin C, paclitaxel, and gemcitabine. It is important to note that a maximal debulking of all visible

tumor by TURBT is required prior to initiation of combined-modality therapy. In patients who achieve a complete response to combined-modality therapy, regular cystoscopic monitoring of the bladder is required with salvage cystectomy offered to patients who develop MBC in follow-up.

In a similar fashion, bladder-sparing partial cystectomy can be performed in a very small subset of MIBC patients. The ideal patient for partial cystectomy is the patient with a solitary, clinical T2 urothelial carcinoma in the dome of the bladder. In such patients, the tumor and immediate surrounding urothelium can be resected with reconstruction of the remaining bladder to maintain near physiologic urinary function.

In the majority of patients, however, resection of the entire bladder is required. In males, a cystoprostatectomy with removal of the bladder, prostate, and pelvic lymph nodes is performed, whereas in females, an anterior exenteration with removal of the bladder, uterus, ovaries, cervix, and pelvic lymph nodes is performed. With the bladder removed, three options exist to reroute the urine outflow. In an ileostomy, the bilateral ureters are connected to a portion of ileum that is brought through an incision in the abdominal wall to create a stoma that drains urine into an affixed bag outside of the body. In a continent urinary reservoir or "Indiana pouch," the ureters are connected to a portion of ileum that has been separated from both ends from the rest of the small-bowel transit to form a urinary reservoir. The remaining small bowel is reanastomosed, and the urinary reservoir is brought up just beneath the abdominal wall muscles with patients catheterizing the urinary reservoir several times per day via a small stoma tract. Last, in a neobladder, the same urinary reservoir described previously is brought down into the pelvis and is anastomosed to the remaining urethra to provide the opportunity to the patient to void urine through the urethra. The choice of which urinary reconstruction to perform is affected not only by patient choice but also by anatomic tumor considerations and

TABLE 86-1 Non-Muscle-Invasive Bladder Cancer Recurrence Risk Groups

RISK GROUP	CHARACTERISTICS
Low risk	Initial tumor, solitary tumor, low grade, <3 cm, no CIS
Intermediate risk	All tumors not defined in the two adjacent categories (between the category of low and high risk)
High risk	Any of the following: <ul style="list-style-type: none"> • T1 tumor • High-grade • CIS • Multiple and recurrent and large (>3 cm) Ta low-grade tumors (all conditions must be met for this point on Ta low-grade tumors)

Abbreviation: CIS, carcinoma in situ.

TABLE 86-2 Treatment Approaches to MIBC Patients

TREATMENT	PATIENT SELECTION	CLINICAL OUTCOMES
Bladder-sparing chemoradiation	No CIS, no hydronephrosis, maximal TURBT required	65% cure, 55% bladder intact, highly dependent on patient selection
Bladder-sparing partial cystectomy	Solitary tumors in dome of bladder are ideal	Variable, highly dependent on patient selection
Cystectomy	Any MIBC patient	50% cure with surgery alone, highly dependent on pathologic stage
Neoadjuvant cisplatin-based chemotherapy	Cisplatin-eligible MIBC patients	5–10% improvement in overall survival compared to cystectomy alone
Adjuvant cisplatin-based chemotherapy	Cisplatin-eligible high-risk postcystectomy MIBC patients (pT3-4, N+)	Similar improvement as neoadjuvant treatment, data less robust, many patients not suitable for adjuvant treatment

Abbreviations: CIS, carcinoma in situ; MIBC, muscle-invasive bladder cancer; TURBT, transurethral resection of bladder tumor.

urologist experience with each procedure. Regardless of the type of surgery performed, all patients undergo a significant catabolic change in their metabolism following removal of the bladder. While many MIBC patients are affected by weight loss preoperatively, it is not uncommon for postcystectomy patients to lose an additional 10–15 lb in the first month postoperatively. In addition, patients can experience long-term nutritional changes such as low B_{12} levels due to alterations in small-bowel physiology caused by all of the urinary diversion options.

Despite aggressive surgery, only half of patients undergoing cystectomy are cured by surgery alone. Therefore, many clinical trials have investigated the role of systemic chemotherapy before (neoadjuvant) or after (adjuvant) surgery. Meta-analyses have shown a 5–10% absolute overall survival advantage when combination chemotherapy regimens utilizing cisplatin have been used before surgery. A similar benefit exists with cisplatin-based combination chemotherapy given after surgery. However, the data in the adjuvant setting are based on smaller, older trials. Furthermore, in the postoperative setting, some patients may not recover sufficiently from their surgery within a time frame optimal for chemotherapy administration. Importantly, non-cisplatin-containing chemotherapy regimens have proven inferior to cisplatin-containing regimens. Therefore, if patients are not suitable candidates for cisplatin administration due to poor functional status or comorbidities (e.g., poor renal function), patients should proceed directly to surgery and forego neoadjuvant therapy.

For patients with high-risk urothelial carcinoma of the upper urinary tract, resection of the kidney and ureter (including the ureter bladder cuff) by nephroureterectomy is preferred. Segmental ureterectomy may be appropriate in patients with decreased renal function in which nephron-sparing outcomes are critical to prevent the need for dialysis. Similarly, in CIS patients, administration of BCG therapy via a nephrostomy tube can be considered to preserve intact renal function. The use of cisplatin-based neoadjuvant chemotherapy has been associated with a pathologic complete response at surgery of 14% in upper tract urothelial carcinoma patients. Similarly, in the post-nephroureterectomy setting, adjuvant platinum-based chemotherapy (carboplatin or cisplatin) reduced recurrence rates by 55% compared to surgery alone. The use of perioperative chemotherapy either before or after surgery is now recommended for upper tract urothelial carcinoma patients in national guidelines.

Metastatic Disease For patients with metastatic urothelial carcinoma regardless of primary tumor origin, systemic chemotherapy is the most established standard of care. In a randomized phase 3 clinical

trial, the combination of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) demonstrated an improvement in median overall survival from 8.2 to 12.5 months compared to single-agent cisplatin. In a head-to-head randomized phase 3 clinical trial, the combination of cisplatin and gemcitabine (CG) demonstrated similar overall survival compared to MVAC with a more favorable side effect profile. Since 2000, treatment with either MVAC or CG has remained a standard first-line treatment of patients with metastatic urothelial carcinoma with adequate renal function and functional status suitable for cisplatin therapy. For patients with lymph node-only metastases and good functional status, cure is achieved in 15–20% of such patients. Unfortunately, only ~5% of metastatic patients fulfill both these criteria. Furthermore, approximately half of patients with urothelial carcinoma have renal insufficiency, comorbidities, or frail functional status, and are not candidates for cisplatin treatment. In cisplatin-ineligible patients, carboplatin-based chemotherapy regimens have historically been used with median overall survival rates decreased to 9.3 months. Agents inhibiting the immune checkpoint programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) pathways have become additional standard options for both front-line chemotherapy-naïve (atezolizumab, pembrolizumab), front-line maintenance (avelumab), and second-line postplatinum (pembrolizumab, nivolumab, and avelumab) metastatic urothelial carcinoma patients. Although these agents only result in tumor responses in 10–30% of patients, they have been approved due to their improved safety profiles compared to traditional chemotherapy options and the prolonged durability of some tumor responses. These agents aim to reactivate a patient's own immune system to recognize and eliminate their cancer. As such, their unique side effect profile is characterized by immune-related toxicities that are rare but can be severe and may include colitis, pneumonitis, hepatitis, nephritis, myocarditis, rash, hypothyroidism, Guillain-Barré syndrome, idiopathic thrombocytopenia purpura, and adrenal insufficiency.

In patients with activating tumor fibroblast growth factor 2 or 3 (*FGFR2/3*) mutations or fusions with progressive disease following platinum-based therapy, the oral FGFR tyrosine kinase inhibitor erdafitinib is another standard option resulting in tumor responses in 32% of patients with a median duration of response of 5.4 months. Additionally, thenectin-4-targeting antibody-drug conjugate enfortumab vedotin provides an additional standard option for patients with progression after both platinum-based therapy and PD-1/PD-L1 immune checkpoint therapy independent of tumor mutation status. Tumor responses are observed in 44% of patients, including patients with liver metastases, with a median response duration of 7.6 months. Additional novel urothelial carcinoma therapeutics are under ongoing investigation.

FURTHER READING

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Benign and malignant changes in the prostate increase with age. Autopsies of men in the eighth decade of life show hyperplastic changes in >90% and malignant changes in >70% of individuals. The high prevalence of these diseases among the elderly, who often have competing causes of morbidity and mortality, mandates a risk-adapted approach to diagnosis and treatment. This can be achieved by considering these diseases as a series of states. Each state represents a distinct clinical milestone for which therapy(ies) may be recommended based on disease extent, current symptoms, the risk of developing symptoms, or the risk of death from disease in relation to death from other causes within a given time frame. For benign proliferative disorders, symptoms of bladder outlet obstruction and potential complications including urinary retention and urinary tract infection are weighed against the side effects and complications of medical or surgical intervention. For prostate malignancies, the likelihood that a clinically significant cancer is present in the gland and the concomitant risk of symptoms or death from cancer are balanced against the morbidities of the recommended treatments and preexisting comorbidities.

ANATOMY AND PATHOLOGY

The prostate is in the pelvis and is adjacent to the rectum, the bladder, the periprostatic and dorsal vein complexes, the neurovascular bundles that are responsible for erectile function, and the urinary sphincter that is responsible for passive urinary control. The prostate is composed of branching tubuloalveolar glands arranged in lobules surrounded by fibromuscular stroma. The acinar unit includes an epithelial compartment made up of epithelial, basal, and neuroendocrine cells separated by a basement membrane and a stromal compartment that includes fibroblasts and smooth-muscle cells. Prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) are produced in the epithelial cells. Both prostate epithelial cells and stromal cells express androgen receptors (ARs) and depend on androgens for growth. Testosterone, the major circulating androgen, is converted by the enzyme 5 α -reductase to dihydrotestosterone in the gland.

The perirethral portion of the gland increases in size during puberty and after the age of 55 years due to the growth of nonmalignant cells in the transition zone of the prostate that surrounds the urethra. Most cancers develop in the peripheral zone, and cancers in this location may be palpated during a digital rectal examination (DRE).

PROSTATE CANCER

The American Cancer Society's estimates for prostate cancer in the United States for 2021 are ~248,530 new prostate cancer cases and ~34,130 deaths from prostate cancer. The absolute number of prostate cancer deaths has decreased in the past 10 years, attributed by some to the widespread use of PSA-based detection strategies. However, the paradox of management is that although 1 in 8 men will eventually be diagnosed with prostate cancer and the disease remains the second leading cause of cancer deaths in men, only 1 man in 41 with prostate cancer will die of his disease.

EPIDEMIOLOGY

Epidemiologic studies show that the risk of being diagnosed with prostate cancer increases 2.5-fold if one first-degree relative is affected and fivefold if two or more are affected. Current estimates are that 40% of early-onset and 5–10% of all prostate cancers are hereditary. Prostate cancer affects ethnic groups differently. Matched for age, African-American males have a higher incidence and present at a more advanced stage with higher-grade, more aggressive cancers. Genome-wide association studies (GWAS) have identified >40 prostate cancer susceptibility loci that are estimated to explain up to 25% of prostate

cancer risk. Among the genes implicated in variations in incidence and outcome are single-nucleotide polymorphisms (SNPs) in the vitamin D receptor in African Americans and variants in the AR, CYP3A4, both involved in the deactivation of testosterone, as well as CYP17, which is involved in steroid biosynthesis. One early change is hypermethylation of the GSTP1 gene promoter, which leads to loss of function of a gene that detoxifies carcinogens. The finding that many prostate cancers develop adjacent to a lesion termed *proliferative inflammatory atrophy* (PIA) suggests a role for inflammation.

The prevalence of autopsy-detected cancers is similar around the world, while the incidence of clinical disease varies. Thus, environmental and dietary factors may play a role in prostate cancer growth and progression. High consumption of dietary fats, such as α -linoleic acid or polycyclic aromatic hydrocarbons that form when red meats are cooked, is believed to increase risk. Like breast cancer in Asian women, the risk of prostate cancer in Asian men increases when they move to Western environments. Protective factors include consumption of the isoflavonoid genistein (which inhibits 5 α -reductase), cruciferous vegetables with isothiocyanate sulforaphane, lycopene found in tomatoes, and inhibitors of cholesterol biosynthesis (e.g., statin drugs). Not smoking, regular exercise, and maintaining a healthy body weight may reduce the risk of progression.

DIAGNOSIS AND TREATMENT BY CLINICAL STATE

The prostate cancer continuum—from the appearance of a preneoplastic and invasive lesion that is localized to the gland, to a metastatic lesion causing symptoms and, ultimately, mortality—can span decades. To limit overdiagnosis of clinically insignificant cancers and for disease management in general, competing risks are considered in the context of a series of clinical states (Fig. 87-1). The states are defined operationally based on whether or not a cancer diagnosis has been established and, for those with a diagnosis, the state of the primary tumor (treated vs untreated), whether or not metastases are detectable on imaging studies, and the measured level of testosterone in the blood. With this approach, an individual resides in only one state and remains in that state until he has progressed. At each assessment, the decision to offer treatment and the specific form of treatment are based on the presence or absence of cancer-related symptoms, and if absent, the risk posed by the cancer relative to competing causes of morbidity and mortality that may be present in that individual. It follows that the more advanced the disease, the greater is the need for treatment.

For those without a cancer diagnosis, the decision to undergo testing to detect a cancer is based on the individual's estimated life expectancy and, separately, the probability that a clinically significant cancer may be present. For those with a prostate cancer diagnosis, the clinical states model considers the probability of developing symptoms or dying from the disease. Thus, a patient with a localized tumor that has been surgically removed remains in the state of localized disease if the PSA remains undetectable. The time within a state then becomes a measure of the efficacy of an intervention, though the effect may not be assessable for years. Because many men with active cancer are not at risk for developing metastases, symptoms, or death, the clinical states model allows a distinction between *cure*—the elimination of all cancer cells, the primary therapeutic objective of treatment for most cancers—and *cancer control*, by which the tempo of the illness is determined to be so slow or has been altered by treatment to the point where it is unlikely to cause symptoms, to metastasize, or to shorten a patient's life expectancy. Importantly, from a patient standpoint, both outcomes can be considered equivalent therapeutically assuming the patient has not experienced symptoms of the disease or the treatment needed to control it. Even when a recurrence is documented, immediate therapy is not always necessary. Rather, as at the time of diagnosis, the need for intervention is based on the tempo of the illness as it unfolds in the individual, relative to the risk-to-benefit ratio of the intervention being considered.

NO CANCER DIAGNOSIS

Prevention No agent is currently approved for the prevention of prostate cancer. The results from several large double-blind,

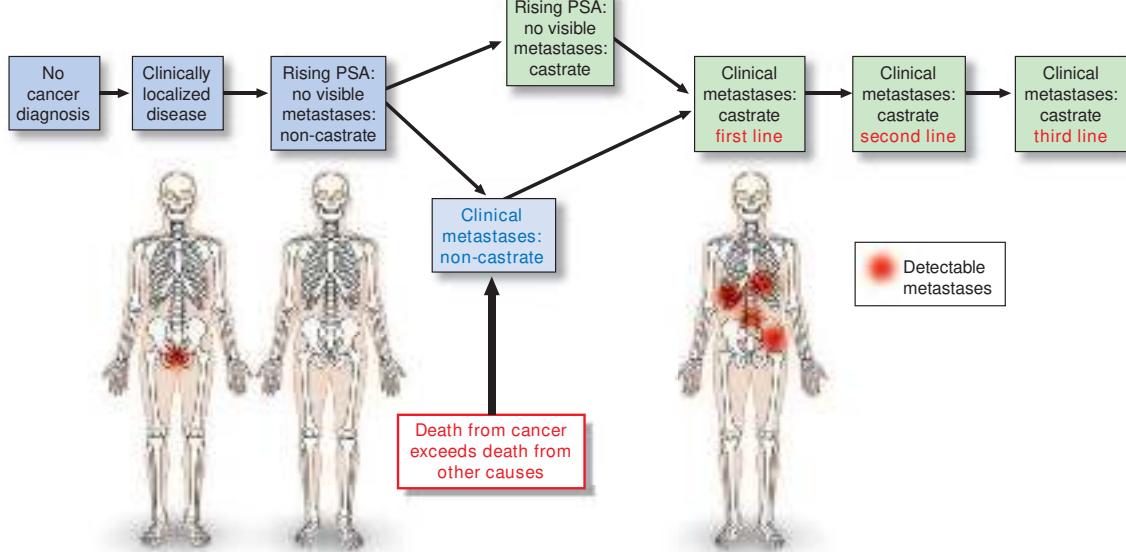


FIGURE 87-1 Clinical states of prostate cancer. PSA, prostate-specific antigen.

randomized chemoprevention trials have established 5 α -reductase inhibitors (5ARIs) as the predominant therapy to reduce the future risk of a prostate cancer diagnosis. The Prostate Cancer Prevention Trial (PCPT), in which men aged >55 years received placebo or the 5ARI finasteride, which inhibits the type 1 isoform, showed a 25% (95% confidence interval 19–31%) reduction in prostate cancer incidence from 24% with placebo to 18% with finasteride. In REDUCE (Reduction by Dutasteride of Prostate Cancer Events trial), a reduction in incidence from 25% with placebo to 20% with dutasteride was found ($p = .001$). Dutasteride inhibits both the type 1 and type 2 5ARI isoforms. While both studies met their endpoint, there was concern that most of the cancers that were “prevented” were low risk. Neither drug is approved for prostate cancer prevention. In comparison, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), which enrolled African-American men aged ≥ 50 years and others aged ≥ 55 years, showed no difference in cancer incidence in patients receiving vitamin E (4.6%) or selenium (4.9%) alone or in combination (4.6%) relative to placebo (4.4%). A similar lack of benefit for vitamin E, vitamin C, and selenium was seen in the Physicians Health Study II.

Early Detection and Diagnosis The decision to pursue a diagnosis of prostate cancer must balance the benefit from detecting and treating clinically significant cancers that, left untreated, would adversely affect a patient's quality and duration of life, against the morbidity associated with the overdiagnosis and overtreatment of clinically insignificant cancers that are highly prevalent in the general population. The balance is best approached through shared decision-making between the patient and physician. Considerations for whether to pursue a diagnosis include symptoms, an abnormal DRE, or more typically, a change in or an elevated serum PSA. Genetic risk is also considered.

PHYSICAL EXAMINATION The DRE focuses on prostate size, consistency, and abnormalities within or beyond the gland. Many cancers occur in the peripheral zone and may be palpated on DRE. Carcinomas are characteristically hard, nodular, and irregular, while induration may also be due to benign prostatic hyperplasia (BPH) or calculi. Overall, 20–25% of men with an abnormal DRE have prostate cancer.

PROSTATE SPECIFIC ANTIGEN PSA (kallikrein-related peptidase 3; *KLK3*) is a kallikrein-related serine protease that causes liquefaction of seminal coagulum. It is produced by both nonmalignant and malignant epithelial cells and, as such, is prostate-specific, not prostate cancer-specific. Serum levels of PSA may increase from prostatitis, BPH, or

prostate cancer. Serum levels are not significantly affected by the DRE. PSA circulating in the blood is inactive and mainly occurs as a complex with the protease inhibitor α_1 -antichymotrypsin and as free (unbound) PSA forms. The formation of complexes between PSA, α_1 -macroglobulin, or other protease inhibitors is less significant. Free PSA is rapidly eliminated from the blood by glomerular filtration with an estimated half-life of 12–18 h. Elimination of PSA bound to α_1 -antichymotrypsin is slow (estimated half-life of 1–2 weeks) as it, too, is largely cleared by the kidneys. Levels should be undetectable after about 6 weeks if the prostate has been completely removed (radical prostatectomy).

PSA testing was approved by the U.S. Food and Drug Administration (FDA) in 1994 for early detection of prostate cancer, and the widespread use of the test has played a significant role in the proportion of men diagnosed with early-stage cancers: >70–80% of newly diagnosed cancers are clinically organ confined. The level of PSA in blood is strongly associated with the risk and outcome of prostate cancer. A single PSA measured at age 60 is associated (area under the curve [AUC] of 0.90) with lifetime risk of death from prostate cancer. Most (90%) prostate cancer deaths occur among men with PSA levels in the top quartile (>2 ng/mL), although only a minority of men with PSA >2 ng/mL will develop lethal prostate cancer. Despite this and mortality rate reductions reported from large randomized prostate cancer screening trials, routine use of the test remains controversial.

In 2012, the U.S. Preventive Services Task Force (USPSTF) published a review of the evidence for PSA-based screening for prostate cancer and made a clear recommendation against screening. By giving a grade of “D” in the recommendation statement that was based on this review, the USPSTF concluded that “there is moderate or high certainty that this service has no net benefit or that the harms outweigh the benefits.” In 2013, the American Urological Association (AUA) updated their consensus statement regarding prostate cancer screening. They concluded that the quality of evidence for the benefits of screening was moderate for men aged 55–69 years. For men outside this age range, evidence was lacking for benefit, but the harms of screening, including overdiagnosis and overtreatment, remained. The AUA recommends shared decision-making for men aged 55–69 years considering PSA-based screening, a target age group for whom benefits may outweigh harms. Outside this age range, PSA-based screening as a routine was not recommended. The entire guideline is available at [http://www.auanet.org/guidelines/early-detection-of-prostate-cancer-\(2013-reviewed-and-validity-confirmed-2015\)](http://www.auanet.org/guidelines/early-detection-of-prostate-cancer-(2013-reviewed-and-validity-confirmed-2015).). As of 2017, the USPSTF has issued a revised recommendation with a grade of “C” for PSA-based

prostate cancer screening for men aged 55–69. Now they recommend shared decision-making for men between the ages of 55 and 69 and do not recommend screening for men aged 70 or greater, roughly in agreement with the 2013 AUA guideline. The USPSTF also notes that the increased use of active surveillance (observation with selective delayed treatment) for low-risk prostate cancer has reduced the risks of screening.

We believe that implementation of the following three guidelines will further improve PSA screening outcomes in the United States and will have a greater practical impact on men's health than the USPSTF and AUA recommendations that are based almost solely on age. First, avoid PSA tests in men with little to gain. There is no rationale for recommending PSA screening in asymptomatic men with a short life expectancy. Hence, men over the age of 75 should only be tested in special circumstances, such as a higher than median PSA measured before age 70 or excellent overall health. In addition, because a baseline PSA is a strong predictor of the future risk of lethal prostate cancer, men with low PSAs, for example <1 ng/mL, can undergo testing less frequently, perhaps every 5 years, with screening possibly ending at age 60 if the PSA remains at ≤ 1 ng/mL. Men with PSAs that are above an age median but below biopsy thresholds can be counseled about their elevated risk and actively encouraged to return for regular screening and more comprehensive risk assessment. Second, do not treat those who do not need treatment. High proportions of men with screen-detected prostate cancer do not need immediate treatment and can be managed by active surveillance. Third, refer men who do need treatment to high-volume centers. Although it is clearly not feasible to restrict treatment exclusively to high-volume centers, shifting treatment trends so that more patients are treated at such centers by high-volume providers will improve cancer control and decrease complications. The goal of prostate cancer screening should be to maximize the benefits of PSA testing and minimize its harms. Following the three rules outlined here should continue to improve the ratio of harms to benefits from PSA screening.

The PSA criteria used to recommend a diagnostic prostate biopsy have evolved over time. However, based on the commonly used cut point for prostate biopsy (a total PSA ≥ 4 ng/mL), most men with a PSA elevation do not have histologic evidence of prostate cancer at biopsy. In addition, many men with PSA levels below this cut point harbor cancer cells in their prostate. Information from the Prostate Cancer Prevention Trial demonstrates that there is no PSA below which the risk of prostate cancer is zero. Thus, the PSA level establishes the likelihood that a man will harbor cancer if he undergoes a prostate biopsy. The goal is to increase the sensitivity of the test for younger men harboring clinically significant cancers that may cause symptoms and shorten survival and to reduce the frequency of detecting cancers of low malignant potential in elderly men more likely to die of other causes. Patients with symptomatic bacterial prostatitis should have a course of antibiotics before biopsy. However, the routine use of antibiotics in an asymptomatic man with an elevated PSA level is strongly discouraged.

SECOND LINE SCREENING TESTS Several tests have been developed to better stratify men with an elevated PSA test into those more or less likely to have clinically significant prostate cancer. The 4Kscore Test (OPKO Lab, Nashville, TN) measures four prostate-specific kallikreins (total PSA, free PSA, intact PSA, and human kallikrein 2). The results are combined with clinical information in an algorithm that estimates an individual's percent risk of having an aggressive prostate cancer should that individual opt for a prostate biopsy. The 4Kscore test has also been shown to identify the likelihood that an individual will develop aggressive prostate cancer, defined as high-grade prostate cancer pathology and/or poor prostate cancer clinical outcomes, within 20 years.

The Prostate Health Index (PHI™, Innovative Diagnostic Laboratory, Richmond, VA) is a blood test that estimates the risk of having prostate cancer. The PHI test is a combination of free PSA, total PSA, and the [-2]proPSA isoform of free PSA. These three tests are combined in a formula that calculates the PHI score. The PHI score is a better predictor of prostate cancer than the total PSA test alone or the free PSA test alone. Urine-based testing measuring exosomes (ExoDx Prostate Test) or mRNA levels of prostate cancer-related genes (Select-MDx) is also available.

PROSTATE BIOPSY A diagnosis of cancer is established by an image-guided needle biopsy. Direct visualization by transrectal ultrasound (TRUS), magnetic resonance imaging (MRI), or fusion of the ultrasound and MRI images ensures that all areas of the gland, including suspicious areas, are sampled. Contemporary schemas advise an extended-pattern 12-core biopsy that includes sampling from the peripheral zone as well as a lesion-directed palpable nodule or suspicious image-guided sampling. Because a prostate biopsy is subject to sampling error, men with an abnormal PSA and negative biopsy are frequently advised to undergo additional testing, which may include a 4Kscore test, PHI, prostate MRI, and/or repeat biopsy.

PATHOLOGY Each core of the biopsy is examined for the presence of cancer, and the amount of cancer is quantified based on the length of the cancer within the core and the percentage of the core involved. Of the cancers identified, >95% are adenocarcinomas; the rest are squamous or transitional cell tumors or, rarely, carcinosarcomas or small-cell histologies. Metastases to the prostate are rare, but in some cases, colon cancers or transitional cell tumors of the bladder invade the gland by direct extension.

When prostate cancer is diagnosed, a measure of histologic aggressiveness is assigned using the *Gleason grading system*, in which the dominant and secondary glandular histologic patterns are scored from 1 (well differentiated) to 5 (undifferentiated) and summed to give a total score of 2–10 for each tumor. The most poorly differentiated area of tumor (i.e., the area with the highest histologic grade) often determines biologic behavior. The presence or absence of perineural invasion and extracapsular spread is also recorded.

Over the years, the Gleason grading system has undergone several changes. Currently, Gleason total scores of 2–5 are no longer assigned, and in practice, the lowest total score is now assigned a 6, although the scale continues to range from 2 to 10. This leads to a logical yet incorrect assumption on the part of patients that their Gleason 6 cancer is in the middle of the scale, triggering the fear that their cancer is serious and the assumption that treatment is necessary despite Gleason score 6 being favorable risk. To address these issues, a new five-grade group system has been developed:

- Grade group 1 (Gleason score 6)
- Grade group 2 (Gleason score 3+4 = 7)
- Grade group 3 (Gleason score 4+3 = 7)
- Grade group 4 (Gleason score 4+4 = 8)
- Grade group 5 (Gleason scores 9 and 10)

The new system simplifies the grading of prostate cancer, appropriately classifying the lowest risk as grade group 1 (rather than Gleason score 6), and accurately predicts prognosis.

PROSTATE CANCER STAGING The TNM (tumor, node, metastasis) staging system includes categories for cancers that are identified solely on the basis of an abnormal PSA (T1c), those that are palpable but clinically confined to the gland (T2), and those that have extended outside the gland (T3 and T4) (Table 87-1, Fig. 87-2). DRE alone is inaccurate in determining the extent of disease within the gland, the presence or absence of capsular invasion, involvement of seminal vesicles, and extension of disease to lymph nodes. Because of the inadequacy of DRE for staging, the TNM staging system was modified to include the results of imaging. Unfortunately, no single test has been proven to accurately indicate the stage or the presence of organ-confined disease, seminal vesicle involvement, or lymph node spread.

TRUS is the imaging technique most frequently used to assess the primary tumor, but its chief use is directing prostate biopsies, not staging. No TRUS finding consistently indicates cancer with certainty. Computed tomography (CT) lacks sensitivity and specificity to detect extraprostatic extension and is inferior to MRI in visualization of lymph nodes. In general, MRI is superior to CT to detect cancers in the prostate, to assess local disease extent, and fused with ultrasound imaging, to guide sites to biopsy within the gland. MRI is also useful for the planning of surgery and radiation therapy.

Radiouclide bone scans (bone scintigraphy) are used to evaluate spread to osseous sites. This test is sensitive but relatively nonspecific

TABLE 87-1 TNM Classification

TNM (tumor, node, metastasis) Staging System for Prostate Cancer ^a	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Localized Disease	
T1	Clinically inapparent tumor, neither palpable nor visible by imaging
T1a	Tumor incidental histologic finding in ≤5% of resected tissue; not palpable
T1b	Tumor incidental histologic finding in >5% of resected tissue
T1c	Tumor identified by needle biopsy (e.g., because of elevated PSA)
T2	Tumor confined within prostate ^b
T2a	Tumor involves half of one lobe or less
T2b	Tumor involves more than one half of one lobe, not both lobes
T2c	Tumor involves both lobes
Local Extension	
T3	Tumor extends through the prostate capsule ^c
T3a	Extracapsular extension (unilateral or bilateral)
T3b	Tumor invades seminal vesicles
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall
Metastatic Disease	
N1	Positive regional lymph nodes
M1	Distant metastases

^aRevised from SB Edge et al (eds): *AJCC Cancer Staging Manual*, 7th ed. New York, Springer, 2010. ^bTumor found in one or both lobes by needle biopsy, but not palpable or reliably visible by imaging, is classified as T1c. ^cInvasion into the prostatic apex or into (but not beyond) the prostatic capsule is classified not as T3 but as T2.

Abbreviation: PSA, prostate-specific antigen.

because it does not detect the cancer itself, only reaction of the bone to the presence of the cancer itself. Consequently, areas of increased uptake are not always related to metastatic disease. Healing fractures, arthritis, Paget's disease, and other conditions will also cause abnormal uptake. True-positive bone scans are uncommon when the PSA is <10 ng/mL unless the tumor is high-grade.

TREATMENT

Prostate Cancer

CLINICALLY LOCALIZED PROSTATE CANCER

Patients with clinically localized disease are managed by radical prostatectomy, radiation therapy, or active surveillance. Choice of therapy requires the consideration of several factors: the presence of symptoms, the probability that the untreated tumor will adversely affect the quality or duration of survival and thus require treatment,

and the probability that the tumor can be cured by single-modality therapy directed to the prostate versus requiring both local and systemic therapy to achieve cure.

There is no clear evidence for the superiority of any one form of local therapy relative to another. This is due to the lack of prospective randomized trials, referral bias and physician bias, variation in the experience of the treating teams, and differences in trial endpoints and the definitions of cancer control. Often, PSA relapse-free survival is used because an effect on metastatic progression or survival may not be apparent for years. For many patients, however, a PSA recurrence does not necessarily mean that the disease will cause symptoms or shorten survival. After radical surgery to remove all prostate tissue, PSA should become undetectable in the blood within 6 weeks. If PSA remains or becomes detectable after radical prostatectomy, the patient is considered to have persistent or recurrent disease. After radiation therapy, in contrast, PSA does not become undetectable because the remaining nonmalignant elements of the gland continue to produce PSA even if all cancer cells have been eliminated. Similarly, cancer control is not well defined for a patient managed by active surveillance because PSA levels may continue to rise in the absence of therapy. Other outcomes are time to objective progression (local or systemic), cancer-specific survival, and overall survival; however, these outcomes may take years to assess.

The more extensive the local disease, the higher the probability of regional lymph node involvement (even when imaging studies are normal), the lower the probability of local control, and the higher the probability of relapse and the development of metastases. More important is that within the categories of clinical stage T1, T2, and T3 disease are cancers with a range of prognoses. Some T3 tumors are curable with therapy directed solely at the prostate, and some T1 lesions have a high probability of systemic relapse that requires the integration of local and systemic therapy to achieve cure. For T1c cancers, stage alone is inadequate to predict outcome and select treatment; other factors must be considered.

To better assess risk and guide treatment selection, many groups have developed prognostic models or nomograms that use a combination of the initial clinical T stage, biopsy Gleason score, the number of biopsy cores in which cancer is detected, and baseline PSA. Some use discrete cut points (PSA <10 or ≥10 ng/mL; Gleason score of 6, 7, or ≥8); others employ nomograms that use PSA and Gleason score as continuous variables. More than 100 nomograms have been reported to predict (1) the probability that a clinically significant cancer is present, (2) disease extent (organ-confined vs non-organ-confined, node-negative or -positive), or (3) the probability of treatment success for specific local therapies using pretreatment variables. Considerable controversy exists over what constitutes "high risk" based on a predicted probability of success or failure. In these situations, nomograms and predictive models can only go so far. Exactly what probability of success or failure would lead a physician to recommend and a patient to seek alternative

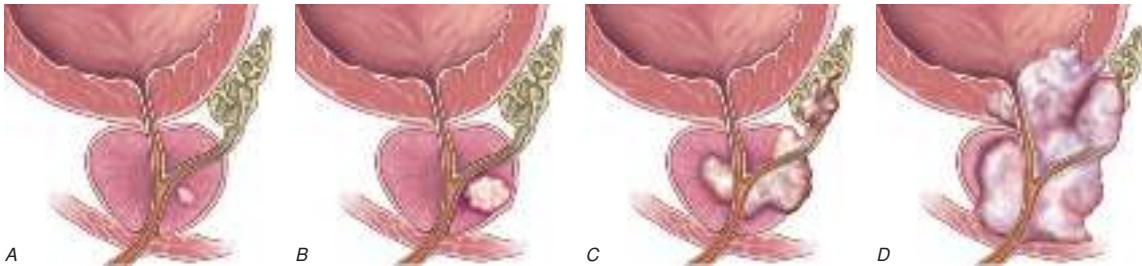


FIGURE 87-2 T stages of prostate cancer. A. T1—Clinically inapparent tumor, neither palpable nor visible by imaging. B. T2—Tumor confined within prostate. C. T3—Tumor extends through prostate capsule and may invade the seminal vesicles. D. T4—Tumor is fixed or invades adjacent structures. Eighty percent of patients present with local disease (T1 and T2), which is associated with a 5-year survival rate of 100%. An additional 12% of patients present with regional disease (T3 and T4 without metastases), which is also associated with a 100% survival rate after 5 years. Four percent of patients present with distant disease (T4 with metastases), which is associated with a 30% 5-year survival rate. (Three percent of patients are ungraded.) (Reproduced with permission from MSKCC, data from AJCC, <http://seer.cancer.gov/statfacts/html/prost.html>. © 2010 Memorial Sloan-Kettering Cancer Center Medical Graphics.)

approaches is controversial. As an example, it may be appropriate to recommend radical surgery for a younger patient with a low probability of cure. Nomograms are being refined continually to incorporate additional clinical parameters, biologic determinants, and year of treatment, which can also affect outcomes, making treatment decisions a dynamic process.

Radical Prostatectomy The goal of radical prostatectomy is to excise the cancer completely with a clear margin, to maintain continence by preserving the external sphincter, and to preserve potency by sparing the autonomic nerves in the neurovascular bundle. The procedure is advised for patients with a life expectancy of 10 years or more and is performed via a retropubic or perineal approach or via a minimally invasive robotic-assisted or hand-held laparoscopic approach. Outcomes can be predicted using postoperative nomograms that consider pretreatment factors and the pathologic findings at surgery. PSA failure is usually defined as a value >0.1 or 0.2 ng/mL . Specific criteria to guide the choice of one approach over another are lacking. Minimally invasive approaches offer the advantage of a shorter hospital stay and reduced blood loss. Rates of cancer control, recovery of continence, and recovery of erectile function are comparable. The individual surgeon, rather than the surgical approach used, is most important in determining outcomes after surgery.

Neoadjuvant hormonal treatment with gonadotropin-releasing hormone (GnRH) agonists/antagonists alone has also been explored to improve the outcomes of surgery for high-risk patients using a variety of definitions. The results of several large trials testing 3 or 8 months of androgen depletion before surgery showed that serum PSA levels decreased by 96%, prostate volumes decreased by 34%, and margin positivity rates decreased from 41–17%. Unfortunately, these findings have not been shown to improve PSA relapse-free survival.

Factors associated with incontinence following radical prostatectomy include older age and urethral length, which impacts the ability to preserve the urethra beyond the apex and the distal sphincter. The skill and experience of the surgeon are also factors.

The likelihood of recovery of erectile function is associated with younger age, quality of erections before surgery, and the absence of damage to the neurovascular bundles. In general, erectile function begins to return ~6 months after surgery if neurovascular tissue has been preserved. Potency is reduced by half if at least one neurovascular bundle is sacrificed. Overall, with the availability of drugs such as sildenafil, intraurethral inserts of alprostadil, and intracavernosal injections of vasodilators, many patients recover satisfactory sexual function.

Radiation Therapy Radiation therapy is given by external beam, by radioactive sources implanted into the gland, or by a combination of the two techniques.

External beam radiation therapy Contemporary external beam intensity-modulated radiation therapy (IMRT) permits shaping of the dose and allows the delivery of higher doses to the prostate and a dramatic reduction in normal tissue exposure compared with three-dimensional conformal treatment alone. These advances have enabled the safe administration of doses $>80 \text{ Gy}$ and resulted in higher local control rates and fewer side effects.

Cancer control after radiation therapy has been defined by various criteria, including a decline in PSA to <0.5 or 1 ng/mL , “nonrising” PSA values, and a negative biopsy of the prostate 2 years after completion of treatment. The current standard definition of biochemical failure (the Phoenix definition) is a rise in PSA by $\geq 2 \text{ ng/mL}$ higher than the lowest PSA achieved. Radiation dose is critical to the eradication of prostate cancer. In a representative study, a PSA nadir of $<1.0 \text{ ng/mL}$ was achieved in 90% of patients receiving 81.0 Gy versus 76% and 56% of those receiving 70.2 and 64.8 Gy, respectively. Positive biopsy rates at 2.5 years were 4% for those treated with 81 Gy versus 27% and 36% for those receiving 75.6 and 70.2 Gy, respectively.

Hypofractionation schedules, utilizing fewer treatments of higher radiation doses, have been evaluated and shown to provide good cancer control rates based on posttreatment biopsies showing no evidence of cancer, with no apparent increase in treatment-related morbidity. Hypofractionated treatments can range from as few as 5 treatments to upward of 26 treatments, both regimens representing substantial reductions in treatment length.

Multiple clinical trials have evaluated the use of androgen deprivation therapy (ADT) in combination with radiation. In patients with unfavorable intermediate-risk prostate cancer, short-course ADT (6 months), when combined with external beam radiotherapy, has demonstrated significant improvements in overall survival. In patients with high-risk disease, longer courses of ADT (18–36 months) have proven superior to shorter courses and represent the current standard of care when combined with radiotherapy.

The Prostate Testing for Cancer and Treatment (ProtecT) trial investigated the effects of active monitoring, radical prostatectomy, and radical radiotherapy with hormones on patient-reported outcomes in men diagnosed with low- and intermediate-risk prostate cancer (~75% with Gleason score 6 or grade group 1 cancer). Patient-reported outcomes among 1643 men who completed questionnaires before diagnosis, at 6 and 12 months, and annually thereafter were compared. Of the three treatments, prostatectomy had the greatest negative effect on sexual function and urinary continence, and although there was some recovery, these outcomes remained worse in the prostatectomy group than in the other groups throughout the trial. The negative effect of radiotherapy on sexual function was greatest at 6 months, but sexual function then recovered somewhat and was stable thereafter; radiotherapy had little effect on urinary continence. Sexual and urinary function declined gradually in the active-monitoring group. Bowel function was worse in the radiotherapy group at 6 months than in the other groups but then recovered somewhat, except for the increasing frequency of bloody stools; bowel function was unchanged in the other groups. Urinary voiding and nocturia were worse in the radiotherapy group at 6 months but then mostly recovered and were like the other groups after 12 months. Effects on quality of life mirrored the reported changes in function. No significant differences were observed among the groups in measures of anxiety, depression, or general health-related or cancer-related quality of life.

Brachytherapy Brachytherapy is the direct implantation of radioactive sources (seeds) into the prostate. It is based on the principle that the deposition of radiation energy in tissues decreases as a function of the square of the distance from the source (Chap. 73). The goal is to deliver intensive irradiation to the prostate, minimizing the exposure of the surrounding tissues. The current standard technique achieves a more homogeneous dose distribution by placing seeds according to a customized template based on imaging assessment of the cancer and computer-optimized dosimetry. The implantation is performed transperineally as an outpatient procedure with real-time imaging.

Improvements in brachytherapy techniques have resulted in fewer complications and a marked reduction in local failure rates. In a series of 197 patients followed for a median of 3 years, 5-year actuarial PSA relapse-free survival for patients with pretherapy PSA levels of 0–4, 4–10, and $>10 \text{ ng/mL}$ were 98, 90, and 89%, respectively. In a separate report of 201 patients who underwent posttreatment biopsies, 80% were negative, 17% were indeterminate, and 3% were positive. The results did not change with longer follow-up. Brachytherapy is well tolerated, although most patients experience urinary frequency and urgency that can persist for several months. Higher complication rates are observed in patients who have undergone a prior transurethral resection of the prostate (TURP), while those with obstructive symptoms at baseline are at a higher risk for retention and persistent voiding symptoms. Proctitis has been reported in <2% of patients.

Active surveillance With the advent of PSA testing, many patients are diagnosed with low-risk prostate cancers that may

not pose a threat to either the quantity or quality of man's life. Active surveillance, described previously as *watchful waiting* or *deferred therapy*, evolved from (1) studies that evaluated predominantly elderly men with well-differentiated tumors who remained untreated and demonstrated no clinically significant progression for protracted periods, (2) recognition of the contrast between incidence and disease-specific mortality, (3) the high prevalence of autopsy cancers, and (4) an effort to reduce overtreatment and treatment-related side effects. In practice, active surveillance is the treatment recommended to patients with cancers of low aggressiveness that can be safely monitored at fixed intervals with DREs, PSA measurements, imaging (usually prostate MRI), and repeat prostate biopsies as indicated until histopathologic or serologic changes correlative of progression warrant treatment with curative intent.

Case selection is critical, and determining the clinical parameters predictive of cancer aggressiveness that can be used to reliably select men most likely to benefit from active surveillance is an area of intense study. One set of criteria includes men with clinical T1c tumors that are biopsy Gleason grade 6 (grade group 1) involving three or fewer cores, with each core having <50% involvement by tumor, and a PSA density of <0.15. Nomograms to help predict which patients can safely be managed by active surveillance continue to be refined, and as their predictive accuracy improves, it can be anticipated that more patients will be candidates.

RISING PSA AFTER DEFINITIVE LOCAL THERAPY

Patients in this state include those in whom the sole manifestation of disease is a rising PSA after surgery and/or radiation therapy. There is no evidence of disease on imaging studies. For these patients, the central issue is whether the rise in PSA results from persistent disease in the primary site, systemic disease, or both. In theory, disease in the primary site may still be curable by additional local treatment.

The decision to recommend radiation therapy after prostatectomy is guided by the pathologic findings at surgery, the timing of PSA failure, and the PSA level at the time of failure. Traditional imaging (MRI, CT, and radionuclide bone scans), especially at low levels of PSA, are typically uninformative. New positron emission tomography (PET) tracers such as C-11 choline, F-18 fluciclovine, and F-18 or Ga-68 prostate-specific membrane antigen (PSMA) that directly image the cancer are more sensitive and can detect low-volume disease in the prostate bed or other sites to better inform the decision to recommend additional local therapies. All are FDA approved. Detection rates, both in and outside the prostate bed, correlate with the absolute level of PSA. Factors that predict for response to salvage radiation therapy are a positive surgical margin, lower Gleason score in the radical prostatectomy specimen, long interval from surgery to PSA failure, slow PSA doubling time, and low (<0.5 ng/mL) PSA value at the time of radiation treatment. For patients with a rising PSA after radiation therapy, salvage local therapy can be considered if the disease was "curable" at the outset, if persistent disease has been documented by a biopsy of the prostate, and if no disease is detectable outside of the prostate bed or regional lymph nodes by imaging. Unfortunately, case selection is poorly defined in most series, and morbidities are significant. Options include salvage radical prostatectomy, salvage cryotherapy, salvage radiation therapy, and salvage high-intensity focused ultrasound.

The rise in PSA after surgery or radiation therapy may indicate subclinical or micrometastatic disease with or without local recurrence. In these cases, the need for treatment depends, in part, on the estimated probability that the patient will show evidence of metastatic disease on a scan and in what time frame. That immediate therapy is not always required was shown in a series where patients who developed a rising PSA after radical prostatectomy received no systemic therapy until metastatic disease was documented. Overall, the median time to metastatic progression was 8 years, and 63% of the patients with rising PSA values remained free of metastases at 5 years. Factors associated with progression included the Gleason score of the radical prostatectomy specimen, time to recurrence after surgery, and PSA doubling time. For those with Gleason

score 28, the probability of metastatic progression was 37, 51, and 71% at 3, 5, and 7 years, respectively. If the time to recurrence was <2 years and PSA doubling time was long (>10 months), the proportions with metastatic disease at the same time intervals were 23, 32, and 53%, versus 47, 69, and 79% if the doubling time was short (<10 months). PSA doubling times are also prognostic for survival. In one series, all patients who succumbed to disease had PSA doubling times of ≥ 3 months. Most physicians advise treatment when PSA doubling times are ≤ 2 months. A difficulty with predicting the risk of metastatic spread, symptoms, or death from disease in the rising PSA state is that most patients receive some form of therapy before the development of metastases. Nevertheless, predictive models continue to be refined.

METASTATIC DISEASE: NONCASTRATE

The state of *noncastrate metastatic disease* includes men with metastases visible on an imaging study at the time of diagnosis or after local therapy(ies) who have testosterone levels >150 ng/dL. Symptoms of metastatic disease include pain from osseous spread, although many patients are asymptomatic despite extensive spread. Less common are symptoms related to marrow infiltration by tumor (myelophthisis), coagulopathy, or spinal cord compression. Standard treatment is to deplete or lower androgens via ADT by medical or surgical means, the latter being the least acceptable to patients. A less frequently used approach is to block androgen binding to the AR with antiandrogens. More than 90% of male hormones originate in the testes; <10% are synthesized in the adrenal gland (Fig. 87-3).

Testosterone-Lowering Agents Medical therapies that lower testosterone levels include the GnRH agonists/antagonists, pure GnRH antagonists, 17,20-lyase inhibitors, CYP17 inhibitors, and estrogens such as diethylstilbestrol (DES). The latter are rarely utilized due to the risk of vascular complications that include fluid retention, phlebitis, emboli, and stroke. GnRH agonists/antagonists, such as leuprolide acetate and goserelin acetate, initially produce a rise in luteinizing hormone and follicle-stimulating hormone followed by a downregulation of receptors in the pituitary gland, which effects a chemical castration. Regulatory approval was based on randomized trials showing reduced cardiovascular toxicities relative to DES, with equivalent potency. The initial rise in testosterone may result in a clinical flare of the disease, and as such, these agents are relatively contraindicated in men with significant obstructive symptoms, cancer-related pain, or spinal cord compromise, events that do not occur with GnRH antagonists such as degarelix, given by injection, or relugolix, given orally, that rapidly achieve castrate levels of testosterone. AR antagonists that block testosterone binding to the receptor are also used to prevent flare.

Agents that lower testosterone are associated with an androgen-deprivation syndrome that includes hot flushes, weakness, fatigue, loss of muscle mass, anemia, changes in cognition and personality, and depression. Changes in lipids, obesity, insulin resistance, and an increased risk of diabetes and cardiovascular disease are also seen, along with a decrease in bone density that worsens over time and results in an increased risk of clinical fractures. This is a particular concern in men with preexisting osteopenia that results from hypogonadism that may be worsened with steroid or alcohol use and significantly underappreciated. Baseline fracture risk can be assessed using the FRAX scale, and to minimize fracture risk, patients are advised to take calcium and vitamin D supplementation, along with a bisphosphonate, RANK-ligand inhibitor (denosumab), or toremifene.

Antiandrogens Nonsteroidal first-generation antiandrogens such as bicalutamide and nilutamide have largely been replaced by the more potent next-generation agents (enzalutamide, apalutamide, and darolutamide) that do not lower serum androgen levels and result in fewer hot flushes, less of an effect on libido, less muscle wasting, fewer personality changes, and less bone loss relative to testosterone-lowering therapies. However, over time, testosterone

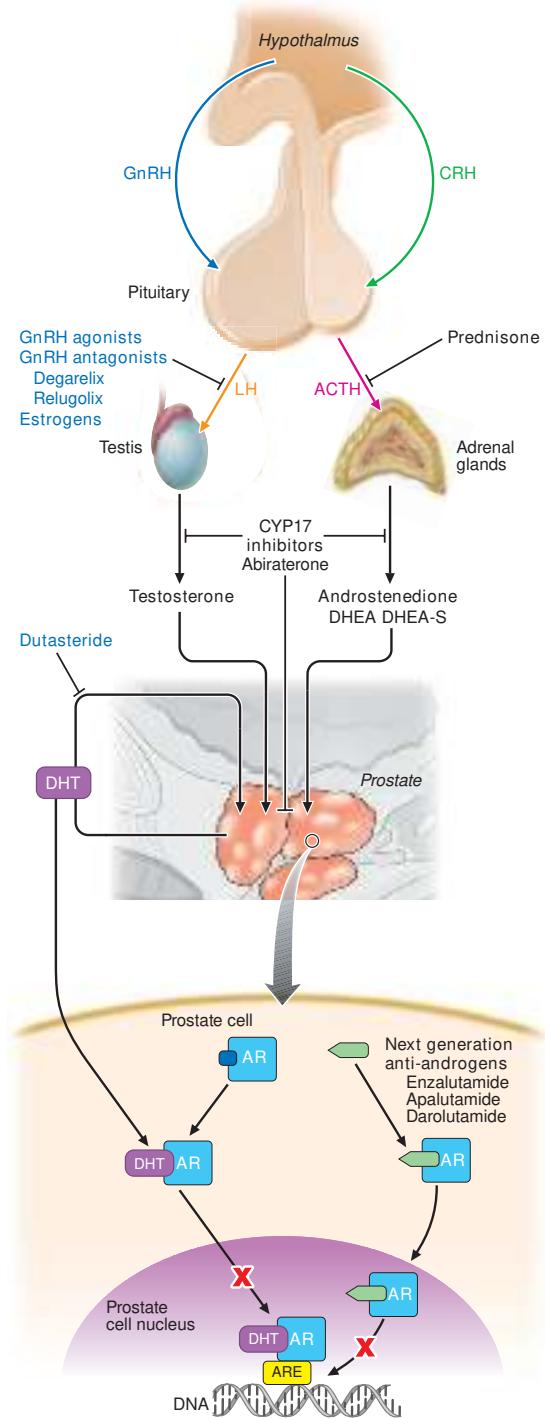


FIGURE 87-3 Sites of action of different hormone therapies.

levels increase and are converted to estrogen, which can result in mastalgia and gynecomastia that limits long-term use but can be prevented in part by tamoxifen or prophylactic breast irradiation.

Most reported randomized trials suggest that the cancer-specific outcomes are inferior when antiandrogens are used alone. Bicalutamide, even at a dose of 150 mg (three times the approved dose for use in combination in GnRH agonists), resulted in a shorter time to progression and inferior survival compared with surgical castration for patients with established metastatic disease.

Improving on the outcomes with ADT alone was a focus of the field for decades. One approach was to combine a first-generation antiandrogen (flutamide, bicalutamide, or nilutamide) with a GnRH analogue or surgical orchectomy; however, this approach did not improve outcomes, and current use is largely limited to the first 2–4 weeks of treatment to protect against flare.

Practice standards changed when an improvement in time to progression and overall survival was shown when ADT was combined with docetaxel, the first systemic therapy shown to prolong life in metastatic castration-resistant prostate cancer (mCRPC) approved in 2004, relative to ADT alone. The greatest benefit was seen for patients with “high-volume” disease defined as the presence of ≥4 lesions on radionuclide bone scan or visceral disease. For abiraterone acetate and prednisone, benefit was seen across disease states ranging from high-risk localized to metastatic disease. Longer progression-free and overall survival times have been noted in separate phase 3 trials comparing ADT with abiraterone, a CYP17 inhibitor that blocks androgen synthesis, and ADT with the AR antagonists enzalutamide and apalutamide versus the ADT standard, further changing the standards of care.

Intermittent Androgen Deprivation Therapy (IADT) One way to reduce the side effects of androgen depletion is to administer antiandrogens on an intermittent basis. This was proposed as a way to prevent the selection of cells that are resistant to androgen depletion. The hypothesis is that by allowing endogenous testosterone levels to rise, the cells that survive androgen depletion will induce a normal differentiation pathway. In this way, the surviving cells that are allowed to proliferate in the presence of androgen will retain sensitivity to subsequent androgen depletion. Applied in the clinic, androgen depletion is continued for 2–6 months beyond the point of maximal response. Once treatment is stopped, endogenous testosterone levels increase, and the symptoms associated with hormone treatment abate. PSA levels also begin to rise, and at some level, treatment is restarted. With this approach, multiple cycles of regression and proliferation have been documented in individual patients. Unknown is whether the intermittent approach increases, decreases, or does not change the overall duration of sensitivity to androgen depletion. The approach is safe, but long-term data are needed to assess the course in men with low PSA levels. A trial to address this question is ongoing.

Outcomes of Androgen Deprivation The anti-prostate cancer effects of the various androgen depletion strategies are similar, and the clinical course is predictable: an initial response, a period of stability in which tumor cells are dormant and nonproliferative, followed after a variable period of time by a rise in PSA and regrowth that is visible on a scan as a castration-resistant lesion. Androgen depletion is not curative because cells that survive castration are present when the disease is first diagnosed. Considered by disease manifestation, PSA levels return to normal in 60–70% of patients, and measurable disease regression occurs in 50%; improvements in bone scan occur in 25% of cases, but the majority remain stable. Duration of survival is inversely proportional to disease extent at the time androgen depletion is first started and the nadir level of PSA at 6 months. Patients with nadir values above a certain threshold have markedly inferior survival times and should be considered for alternative approaches.

An unresolved question remains on how early systemic therapies should be offered to patients: in the adjuvant setting after surgery or radiation treatment of the primary tumor; at the time that a PSA recurrence is documented; or wait until metastatic disease or symptoms of disease are manifest? Trials in support of early therapy have been largely underpowered relative to the reported benefit or have been criticized on methodologic grounds. One that showed a survival benefit for patients treated with radiation therapy and 3 years of ADT, relative to radiation alone, was criticized for the poor outcomes of the control group. Another showing a survival benefit for patients with positive lymph nodes who were randomized to immediate medical or surgical castration compared with

observation ($p = .02$) was criticized because the confidence intervals around the 5- and 8-year survival distributions for the two groups overlapped.

METASTATIC DISEASE: CASTRATE

Castration-resistant prostate cancer (CRPC), disease that progresses while the measured levels of testosterone in the blood are 50 ng/mL or lower, can produce some of the most feared complications of the disease and is lethal for most men. The most common manifestation is a rising PSA, frequently co-occurring with progression in bone. Nodal and/or visceral spread is less frequent. Symptoms may or may not be present. The bone- and PSA-dominant pattern limits the ability to assess treatment effects reliably because traditional bone imaging is inaccurate and no PSA-based outcome has been shown to be a true surrogate for survival, and accordingly, favorable changes with either can be used to support regulatory approvals. Critical for management is that therapeutic objectives be based on the manifestations of the disease in the individual at the time a change in therapy is being considered. As such, for the patient with symptomatic bone disease, relief of pain can be more clinically relevant than lowering the PSA. Naturally, for all patients, the central focus is delaying or preventing disease progression, symptoms, and death from disease.

Through 2010, docetaxel was the only FDA-approved life-prolonging therapy for CRPC. Since then, our understanding of the biology of the disease has increased significantly, which in turn has led to improved therapies. In particular, it is now recognized that the majority of mCRPCs continue to express the AR and remain AR signaling dependent, and upward of 50% of cases harbor a series of oncogenic changes including overexpression, splice variants lacking the ligand binding domain and that stimulate growth independent of the ligand, and upregulation of the enzymes in the androgen biosynthesis pathway, leading to an increase in intratumoral androgens. These oncogenic changes have been successfully targeted with the next-generation antiandrogens enzalutamide, apalutamide, and darolutamide and the CYP17 inhibitor abiraterone acetate (given in combination with prednisone), all of which have been proven to prolong life and are FDA approved for use in CRPC in both the pre- and postchemotherapy setting.

Large-scale molecular profiling efforts have led to a biologically based disease taxonomy that continues to evolve and showed a markedly higher than expected frequency of germline and somatic *BRCA2* alterations, along with other genes in the DNA damage repair pathway that have been targeted successfully with poly-ADP ribose polymerase (PARP) inhibitors of which two, olaparib and rucaparib, are FDA approved, and one, niraparib, has achieved a breakthrough designation. Also approved is the checkpoint inhibitor pembrolizumab for tumors with high microsatellite instability (MSI) scores, an alteration found in 2–3% of prostate cancers for which a dedicated prostate cancer trial would never have been conducted.

Other classes of therapy are approved based on a demonstrated survival benefit include the biologic agent sipuleucel-T, the second-generation taxane cabazitaxel, and the α -emitting bone-targeting radiopharmaceutical radium-223. Approval is also anticipated for PSMA-directed radionuclide therapy based on the survival benefit of Lu-177 PSMA in the phase 3 VISION trial relative to best supportive care alone. Overall, an intense focus of current CRPC research is to understand the optimal sequence in which to utilize these agents to maximize benefit for the individual patient. Most of these agents are also being tested earlier in the course of the disease when tumor burdens are lower and the disease less heterogeneous. The result has been an increase in the frequency of late-state tumors that have undergone a lineage transformation from epithelial to neuroendocrine phenotypes and are highly resistant to available therapies.

Pain Management Pain secondary to osseous metastases is one of the most feared complications of the disease and a major cause of morbidity, worsened by the narcotics needed to control symptoms.

Management requires accurate diagnoses because noncancer etiologies including degenerative disease, spinal stenosis, and vertebral collapse secondary to bone loss are common. Neurologic symptoms, including those suggestive of base of skull disease or spinal cord compromise, require emergency evaluation because loss of function may be permanent if not addressed quickly. Neurologic symptoms and loss of function are best treated with external beam radiation, as are single sites of pain. Diffuse symptoms in the absence of neurologic deficits can be treated with bone-seeking radioisotopes, such as radium-223 or the β emitter ^{153}Sm -EDTMP; mitoxantrone; or other systemic therapies, such as abiraterone acetate, enzalutamide, and docetaxel. Radium-223 is indicated for patients with symptoms, whereas ^{153}Sm -EDTMP and mitoxantrone are approved for the palliation of pain but have not been shown to prolong life. Abiraterone, enzalutamide, and docetaxel do not have a formal indication for pain but were shown to palliate pain in the registration trials that led to their approval by showing a survival benefit.

Other bone-targeting agents, including bisphosphonates such as zoledronic acid and the RANK-ligand inhibitor denosumab, have been shown to reduce the frequency and development of skeletal complications including pain requiring analgesia, neurologic compromise from epidural extension of tumor, and/or the need for surgery or radiation therapy to treat symptomatic osseous disease. It is important to note that, for all of these agents, the direct effect on the tumor is modest, and benefits are seen without declines in PSA or improvements on imaging.

BENIGN DISEASE

■ BENIGN PROSTATIC HYPERPLASIA

BPH is a pathologic process that contributes to the development of lower urinary tract symptoms (LUTS) in men. LUTS, arising from lower urinary tract dysfunction, are further subdivided into obstructive symptoms (urinary hesitancy, straining, weak stream, terminal dribbling, prolonged voiding, incomplete emptying) and irritative symptoms (urinary frequency, urgency, urge incontinence, small voided volumes). LUTS and other sequelae of BPH are not just due to a mass effect but are also likely due to a combination of the prostatic enlargement and age-related detrusor dysfunction.

Diagnostic Procedures and Treatment LUTS are generally measured using a validated, reproducible index that is designed to determine disease severity and response to therapy—the AUA’s Symptom Index (AUASI), also adopted as the International Prostate Symptom Score (IPSS) (Table 87-2). Serial AUASI is particularly useful in following patients as they are treated with various forms of therapy. Asymptomatic patients do not require treatment regardless of the size of the gland, while those with an inability to urinate, gross hematuria, recurrent infection, or bladder stones require evaluation and treatment. In patients with symptoms, uroflowmetry can identify those with normal flow rates who are unlikely to benefit from treatment, and bladder ultrasound can identify those with high postvoid residuals who may need intervention. Pressure-flow (urodynamic) studies detect primary bladder dysfunction. Cystoscopy is recommended if hematuria is documented and to assess the urinary outflow tract before surgery. Imaging of the upper tracts is advised for patients with hematuria, a history of calculi, or prior urinary tract problems.

Symptomatic relief is the most common reason men seek treatment for BPH, and therefore, symptomatic relief is usually the goal of therapy for BPH. α -Adrenergic receptor antagonists are thought to treat the dynamic aspect of BPH by reducing sympathetic tone of the bladder outlet, thereby decreasing resistance and improving urinary flow. 5ARIs are thought to treat the static aspect of BPH by reducing prostate volume and having a similar, albeit delayed effect. 5ARIs have also proven beneficial in the prevention of BPH progression, as measured by prostate volume, the risk of developing acute urinary retention, and the risk of having BPH-related surgery. The use of an alpha-adrenergic

TABLE 87-2 AUA Symptom Index

QUESTIONS TO BE ANSWERED	AUA SYMPTOM SCORE (CIRCLE 1 NUMBER ON EACH LINE)					
	NOT AT ALL	LESS THAN 1 TIME IN 5	LESS THAN HALF THE TIME	ABOUT HALF THE TIME	MORE THAN HALF THE TIME	ALMOST ALWAYS
Over the past month, how often have you had a sensation of not emptying your bladder completely after you finished urinating?	0+	1	2	3	4	5
Over the past month, how often have you had to urinate again less than 2 h after you finished urinating?	0	1	2	3	4	5
Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5
Over the past month, how often have you found it difficult to postpone urination?	0	1	2	3	4	5
Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5
Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5
Over the past month, how many times did you most typically get up to urinate from the time you went to bed at night until the time you got up in the morning?	(None)	(1 time)	(2 times)	(3 times)	(4 times)	(5 times)
Sum of 7 circled numbers (AUA Symptom Score): _____						

Abbreviation: AUA, American Urological Association.

Source: Reproduced with permission from MJ Barry et al: The American Urological Association symptom index for benign prostatic hyperplasia. The Measurement Committee of the American Urological Association. *J Urol* 148:1549, 1992.

receptor antagonist and a 5ARI as combination therapy seeks to provide symptomatic relief while preventing progression of BPH.

Another class of medications that has shown improvement in LUTS secondary to BPH is phosphodiesterase-5 (PDE5) inhibitors, used currently in the treatment of erectile dysfunction. All four of the PDE5 inhibitors available in the United States—sildenafil, vardenafil, tadalafil, and avanafil—appear to be effective in the treatment of LUTS secondary to BPH. The use of PDE5 inhibitors is not without controversy, however, given the fact that short-acting phosphodiesterase inhibitors such as sildenafil need to be dosed separately from alpha blockers such as tamsulosin because of potential hypotensive effects.

Symptoms due to BPH often coexist with symptoms due to overactive bladder, and the most common pharmacologic agents for the treatment of overactive bladder symptoms are anticholinergics. This has led to multiple studies evaluating the efficacy of anticholinergics for the treatment of LUTS secondary to BPH.

Surgical therapy is now considered second-line therapy and is usually reserved for patients after a trial of medical therapy. The goal of surgical therapy is to reduce the size of the prostate, effectively reducing resistance to urine flow. Surgical approaches include TURP, transurethral incision, or removal of the gland via a retropubic, suprapubic, or perineal approach. Also used are transurethral ultrasound-guided laser-induced prostatectomy (TULIP), stents, and hyperthermia.

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Testicular Cancer

David J. Vaughn



Testicular germ cell tumors (GCTs) represent 95% of all testicular neoplasms. Non-GCTs of the testis are much less common. Approximately 5% of GCTs arise in extragonadal locations including the mediastinum, retroperitoneum, and pineal gland. Treatment for testicular GCTs is determined by pathology and stage. The development of effective chemotherapy for this disease represents a landmark achievement in oncology. About 95% of newly diagnosed patients with testicular GCTs will be cured. For this reason, testicular cancer has been called “a model for a curable neoplasm.”

■ INCIDENCE

In 2021, 9500 cases of testicular GCTs will be diagnosed in the United States, with <450 deaths. These tumors are diagnosed most commonly in men between 20 and 40 years. The incidence of GCTs is increasing in men age 50 years and older.

■ GLOBAL CONSIDERATIONS

The incidence of testicular GCTs appears to be increasing worldwide. The disease has the highest incidence in Scandinavia, Western Europe, and Australia/New Zealand. Africa and Asia have the lowest incidence. The incidence in the United States and the United Kingdom is intermediate. While there does not appear to be a distinct biology related to geography, several countries have reported a migration to earlier stage disease in part related to public awareness and earlier diagnosis.

■ EPIDEMIOLOGY

GCTs are predominantly seen in young Caucasian men. The disease is much less commonly seen in African Americans. Testicular GCTs have an estimated heritability of almost 50%. Interestingly, the risk of GCT is higher in male siblings than in offspring of the patient. Although epidemiologic studies have been performed attempting to identify a relationship with environmental exposures, no conclusive causal links have been established.

Risk Factors The strongest risk factors for testicular GCT include a prior history of the disease, cryptorchidism, and a history of testicular germ cell neoplasia in situ (GCNIS). Patients with a prior history of testicular GCT have a 2% risk of developing a contralateral GCT. These are more commonly metachronous than synchronous. Men with cryptorchidism have approximately a four- to sixfold increased risk of developing testicular GCT. Orchidopexy before puberty decreases but does not eliminate this risk. Interestingly, the contralateral descended testis is also at risk for this disease. Men undergoing infertility evaluation in which a testicular biopsy demonstrates GCNIS have a significant risk of developing GCT. Although scrotal ultrasound of patients with testicular GCT may demonstrate testicular microcalcifications that may be related to GCNIS, the significance of testicular microcalcifications in the general population is unclear.

■ BIOLOGY

The primordial germ cell is the cell of origin for GCTs. Most malignant GCTs arise from GCNIS. The molecular events that result in the development of GCNIS and subsequent malignant GCT have not been fully determined. However, genetic analysis of GCTs has demonstrated an excess copy number of isochromosome 12p ([12p]) in most cases. Several genome-wide association studies have identified multiple independent loci associated with testicular GCT risk. The strongest of these is the *KITLG* (KIT ligand) locus on chromosome 12. These loci contribute significantly to the heritable risk of this disease.

■ PATHOLOGY

GCTs are either seminomas or nonseminomas. For a tumor to be considered a seminoma, it must be 100% seminoma. Any mixed GCT is best approached as a nonseminomatous GCT (NSGCT). Seminomas represent 50% of cases. Seminomas arise most commonly in patients in the fourth decade of life. Seminomas may contain syncytiotrophoblastic cells, which may secrete β human chorionic gonadotropin (hCG). Seminomas do not secrete α fetoprotein (AFP). Seminomas are exquisitely sensitive to both chemotherapy and radiation therapy. NSGCTs are most commonly diagnosed in the third decade of life. The histologic subtypes include embryonal carcinoma, yolk sac tumor, choriocarcinoma, and teratoma. Embryonal carcinoma is the most undifferentiated NSGCT subtype with the potential to differentiate into the other subtypes. Embryonal carcinoma may secrete AFP, hCG, both, or neither. Yolk sac tumor often secretes AFP. Choriocarcinoma is an aggressive subtype, often secreting hCG at very high levels. These NSGCT subtypes are all considered chemotherapy sensitive. Teratoma is composed of somatic cell types that are derived from two or more germinal layers (endoderm, mesoderm, and ectoderm). Teratomas are classified as mature, in which cell types resemble normal adult somatic

tissue; immature, in which cell types resemble fetal somatic tissue; and malignant, in which the cell types have undergone malignant transformation into the malignant counterpart of the somatic tissue. Teratomas are chemotherapy resistant and must be approached surgically.

■ INITIAL PRESENTATION

Signs and Symptoms Although a painless testicular mass is pathognomonic of a GCT, most patients present with testicular swelling, firmness, discomfort, or a combination of these. The differential diagnosis may include epididymitis or orchitis and a trial of antibacterials may be considered. Patients with retroperitoneal metastases may complain of back or flank pain. Patients may have cough, shortness of breath, or hemoptysis as a result of lung metastases. In patients with elevation of serum hCG, gynecomastia may be present. Diagnostic delay is not uncommon and may be associated with a more advanced stage at diagnosis.

Physical Examination Careful examination of the affected testis and the contralateral normal testis should be performed. Many tumors will have a hard consistency to palpation. Some patients may show testicular atrophy. Evaluation for supraclavicular lymphadenopathy, gynecomastia, and abdominal mass should be performed. Inguinal lymphadenopathy is rare. Most patients with lung metastases will have normal auscultation of the lungs.

Diagnostic Testing If a firm testicular mass is identified, a scrotal ultrasound should be performed. Patients with suspected epididymitis or orchitis who do not respond to antibiotics should also undergo scrotal ultrasound. Scrotal ultrasound should include both testicles. On ultrasound, a testicular GCT is hypoechoic and may be multifocal. A solid mass identified on ultrasound should be considered malignant until otherwise proven. Transscrotal aspiration or biopsy of a testicular mass should never be performed. Such scrotal violation may result in tumor seeding of the scrotum or inguinal lymph nodes.

Serum Tumor Markers Serum AFP, hCG, and lactate dehydrogenase (LDH) should be measured in patients suspected of testicular GCT. AFP is elevated in 60–70% of patients who present with NSGCTs. Seminomas never secrete AFP. A patient with a seminoma with elevation of AFP should be approached as having an NSGCT. The half-life of AFP is 5–7 days. A falsely elevated AFP may be seen in patients with hepatic disease or a condition called hereditary persistence of AFP, in which patients may have baseline AFP levels that are mildly elevated. hCG may be elevated in both NSGCTs as well as seminomas. Patients with choriocarcinoma may have markedly elevated levels of hCG. The half-life for hCG is 24–36 h. False-positive elevation of hCG may be seen secondary to hypogonadism, marijuana use, or as a result of interfering substances measured by the assay. LDH is a non-specific marker for GCT. Its principal use is to help in the assessment of the risk classification of a patient with metastatic disease. Although elevation of serum tumor markers supports the diagnosis of a testicular GCT, it should be remembered that most patients with seminoma and up to a third of patients with NSGCTs do not have elevated levels. Serum microRNA (miR)-371a-3 has been identified as a promising biomarker for GCT, and validation studies are ongoing.

■ INITIAL MANAGEMENT

Inguinal Orchiectomy Prompt referral to urology should be performed if a testicular GCT is suspected. The initial treatment for most patients suspected of having a testicular GCT is radical inguinal orchiectomy with removal of the testicle and spermatic cord to the level of the internal inguinal ring. In patients who present with metastatic disease and the diagnosis of GCT is certain, orchiectomy may be deferred until completion of chemotherapy. Although some institutions perform testis-sparing surgery in select patients, the gold standard remains radical inguinal orchiectomy. Pathologic examination of the entire testicle is important, since testicular GCTs may be multifocal. Given the rarity of this cancer, review by an experienced pathologist is essential for accurate tumor classification. Serum tumor markers should be obtained before and after orchiectomy.

Staging The staging of testicular GCT is based on an understanding of the pattern of spread. The initial spread is by the lymphatic route to the retroperitoneal lymph nodes. A left-sided testicular GCT spreads first to the primary landing zone of left paraaortic lymph nodes inferior to the left renal vessels. A right-sided testicular GCT spreads first to the primary landing zone of the aortocaval nodes inferior to the right renal vessels. Nodal metastases may extend into the iliac regions. If scrotal violation occurred, inguinal lymph node metastases may be seen. Subsequent lymphatic spread is to the retrocrural, mediastinal, and supraclavicular lymph nodes. Hematogenous spread to the lung is the next most common site of metastasis. Metastases to the liver, bone, and brain are less commonly seen. Patients with newly diagnosed testicular GCTs should undergo computed tomography (CT) scan of the abdomen and pelvis. Chest x-ray should be performed. CT scan of the chest is performed if retroperitoneal metastases are present or if lung nodules are identified on chest x-ray. Bone scan and magnetic resonance imaging (MRI) of the brain are not routinely performed unless clinically indicated. Positron emission tomography (PET) has little role in the initial staging of testicular GCTs.

The American Joint Committee on Cancer tumor-node-metastasis (TNM) staging classification is used. There are three main stages of testicular GCT. Stage I is limited to the testis; stage II involves the retroperitoneal lymph nodes; and stage III includes lymph node involvement beyond the retroperitoneum and/or distant metastatic disease.

■ STAGE BASED MANAGEMENT

Treatment of testicular GCT is based on two factors: (1) whether the tumor is seminoma or NSGCT and (2) the stage of the patient. This is summarized in Fig. 88-1.

Stage I • SEMINOMA About 70% of newly diagnosed patients with seminoma present with stage I disease. This is defined as no evidence of metastatic disease on imaging of the chest, abdomen, and pelvis. Approximately 15% of patients with stage I seminoma have metastatic disease at the microscopic level, usually in the retroperitoneum. Historically, patients with stage I seminoma were treated with a course of adjuvant radiation therapy to the paraaortic lymph nodes. While still an option, this is not usually performed because of concerns for late radiation-induced secondary malignancies. Active surveillance is the most common approach elected by these patients following orchectomy. With active surveillance, interval physical examination and CT scan of the abdomen are performed. For the 15% of patients who develop metastatic disease during active surveillance, treatment with definitive radiation therapy or chemotherapy is curative in nearly all. A third option for clinical stage I seminoma is adjuvant chemotherapy with carboplatin monotherapy for one or two cycles. While effective in decreasing the risk of recurrence, it should be remembered that most patients are cured by orchectomy alone, and therefore, the additional treatment is unnecessary. In addition, long-term data on toxicity are not available.

NSGCTs About 40% of newly diagnosed patients with NSGCTs present with stage I disease. Because NSGCTs have an increased potential for invasion and metastasis, spread to the retroperitoneum and beyond is more common than with seminoma. If pre-orchectomy serum tumor markers are elevated, these must normalize after orchectomy to be considered stage I. Patients with persistently elevated or rising serum tumor markers after orchectomy have stage IS disease and should be treated with cisplatin-based chemotherapy. If the tumor is limited to testis without lymphovascular invasion, the risk of recurrence is approximately 20%. However, if the tumor has high-risk features including lymphovascular invasion, invasion of the spermatic cord, or invasion of the scrotum, the risk of recurrence is 50% or higher. Historically, a prophylactic retroperitoneal lymph node dissection (RPLND) was performed. This surgery is not only diagnostic but also therapeutic. In fact, most patients who undergo prophylactic RPLND will never require chemotherapy. While still an option, this approach subjects many patients to unnecessary major abdominal surgery. RPLND is also associated with a small risk of retrograde ejaculation due to nerve injury, and nerve-sparing techniques have been developed. Active surveillance is frequently performed especially for

patients without lymphovascular invasion. Most patients who relapse will be treated with cisplatin-based chemotherapy and achieve cure rates approaching 100%. Active surveillance can also be employed for patients with higher risk features, although the risk of progression is significantly higher. For this reason, some advocate adjuvant cisplatin-based chemotherapy with BEP (bleomycin, etoposide, cisplatin) for one cycle for these patients. Other centers favor a prophylactic RPLND. Almost all patients who present with stage I NSGCTs will achieve cure.

Stage II • SEMINOMA Approximately 15–20% of newly diagnosed patients with seminoma present with stage II disease. Patients are subgrouped into IIA, IIB, or IIC based on the size of the retroperitoneal nodes (\leq cm, >2 to 5 cm, or >5 cm, respectively). Patients with stage IIA disease are usually treated with “dogleg” radiation therapy (referring to the shape of the radiation field), which includes the paraaortic and ipsilateral iliac nodes. Cisplatin-based chemotherapy may also be considered. Stage IIB disease is treated with cisplatin-based chemotherapy or, in select patients, radiation therapy. Most patients treated with radiation therapy who relapse will subsequently be cured with cisplatin-based chemotherapy. For patients with stage IIC disease, cisplatin-based chemotherapy should be used.

NSGCTs Approximately 15% of newly diagnosed patients with NSGCTs present with clinical stage II disease. Patients with stage IIA disease may be treated with primary RPLND. Alternatively, these patients may be treated with cisplatin-based chemotherapy. Patients with stage IIB and IIC disease are best initially managed with cisplatin-based chemotherapy.

Stage III Patients who present with stage III GCT (seminoma or NSGCT) are treated with cisplatin-based chemotherapy. These patients are classified into good-, intermediate-, or poor-risk categories using the International Germ Cell Consensus Classification system, which is based on clinical factors including histology, site of primary, the presence of nonpulmonary visceral metastatic disease, and the level of postorchectomy serum tumor markers (Table 88-1). Most patients with stage III GCT present with good-risk disease; >90% will be cured. The remainder present with intermediate-risk or poor-risk disease, associated with 5-year survival rates of 80% and 50%, respectively. Select patients with rapidly progressive metastatic disease and life-threatening symptoms such as hemoptysis in whom there is a high clinical suspicion of GCT should emergently initiate cisplatin-based chemotherapy, even without a tissue diagnosis.

Chemotherapy The development of cisplatin-based chemotherapy represents an important advance in cancer medicine. Through a series of carefully performed clinical trials with the aim of maximizing cure while minimizing the extent of treatment, the chemotherapy approach to the treatment of these patients has been standardized. Patients with good-risk metastatic GCT are treated with either three cycles of BEP or four cycles of etoposide and cisplatin (EP). Patients with intermediate- and poor-risk metastatic disease are treated with either four cycles of BEP or four cycles of etoposide, ifosfamide, and cisplatin (VIP). Maintaining dose and schedule is important, as dose modifications and delays have been associated with inferior outcomes. Serum tumor markers should be monitored throughout treatment and should normalize during or after treatment. Cisplatin-based chemotherapy is associated with myelosuppression, nausea and vomiting, and alopecia. Cisplatin may result in nephrotoxicity, ototoxicity, and peripheral neuropathy. Bleomycin may result in pulmonary toxicity, and risk factors for this include age >40, renal failure, tobacco use, and the cumulative dose of bleomycin received. For patients at increased risk of bleomycin-induced pneumonitis, non-bleomycin-containing regimens as noted above may be given. Cisplatin-based chemotherapy is also associated with sterility. Approximately 30% of newly diagnosed testicular GCT patients have severe oligospermia or azoospermia. For the remainder with normal baseline spermatogenesis who receive cisplatin-based chemotherapy, all will be azoospermic at the completion of therapy. Approximately 80% of these patients will recover spermatogenesis over a period of several years. For this reason, prechemotherapy sperm banking should be offered to all patients treated with chemotherapy.

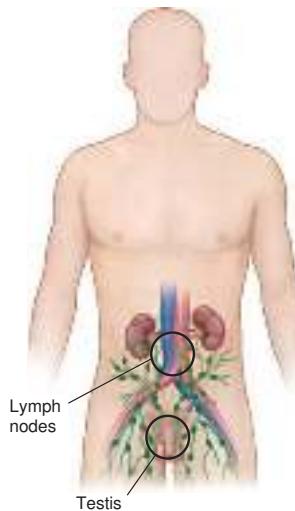
Stage 1



	Seminoma	NSGCT
Stage IA Testis only, no lymphovascular invasion	Active surveillance; or, Adjuvant carboplatin x 1 or 2 cycles; or, Adjuvant para-aortic RT	Active surveillance; or, Nerve-sparing RPLND; or Adjuvant BEP x 1 cycle
Stage IB Testis only, with lymphovascular invasion or invasion of spermatic cord or scrotum	Active surveillance; or, Adjuvant carboplatin x 1 or 2 cycles; or, Adjuvant para-aortic RT	Active surveillance; or, Adjuvant BEP x 1 cycle; or Nerve-sparing RPLND
Stage IS Elevated serum tumor markers post-orchiectomy	BEP x 3 cycles; or, EP x 4 cycles	BEP x 3 cycles; or, EP x 4 cycles

A

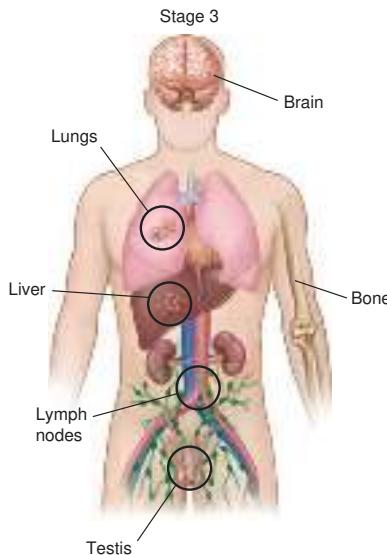
Stage 2



	Seminoma	NSGCT
Stage IIA N1: nodes ≤ 2 cm	Para-aortic and ipsilateral iliac RT; or, BEP x 3 cycles or EP x 4 cycles	Nerve-sparing RPLND; or, BEP x 3 cycles or EP x 4 cycles
Stage IIB N2: nodes > 2 to 5 cm	BEP x 3 cycles or EP x 4 cycles; or, Para-aortic and ipsilateral iliac RT	BEP x 3 cycles or EP x 4 cycles +/- postchemotherapy RPLND
Stage IIC N3: nodes > 5 cm	BEP x 3 cycles or EP x 4 cycles	BEP x 3 cycles or EP x 4 cycles +/- postchemotherapy RPLND

B

FIGURE 88-1 Stage-based management of testicular germ cell tumor.



	Seminoma	NSGCT
Stage IIIA (good-risk)	BEP x 3 cycles; or, EP x 4 cycles	BEP x 3 cycles; or, EP x 4 cycles; +/- Postchemotherapy surgery
Stage IIIB (intermediate-risk)	BEP x 4 cycles; or, VIP x 4 cycles	BEP x 4 cycles; or, VIP x 4 cycles +/- Postchemotherapy surgery
Stage IIIC (poor-risk)	N/A	BEP x 4 cycles; or, VIP x 4 cycles +/- Postchemotherapy surgery

Abbreviations: BEP, bleomycin, etoposide, cisplatin; EP, etoposide, cisplatin; N/A, not applicable; NSGCT, nonseminomatous germ cell tumor; RPLND, retroperitoneal lymph node dissection; RT, radiation therapy; VIP, etoposide, ifosfamide, cisplatin.

C

FIGURE 88-1 (Continued)

TABLE 88-1 International Germ Cell Consensus Classification System

RISK GROUP	SEMINOMA	NSGCT
Good	Any primary site; and normal AFP, any hCG, any LDH; and nonpulmonary visceral metastases absent	Gonadal or retroperitoneal primary; and nonpulmonary visceral metastases absent; and AFP <1000 ng/mL; and hCG <5000 mIU/mL; and LDH <1.5 × ULN
Intermediate	Any primary site; and normal AFP, any hCG, any LDH; and nonpulmonary visceral metastases present	Gonadal or retroperitoneal primary; and nonpulmonary visceral metastases absent; and one of the following: AFP 1000–10,000 ng/mL hCG 5000–50,000 mIU/mL LDH 1.5–10 × ULN
Poor	N/A	Mediastinal primary; or nonpulmonary visceral metastases present; or one of the following: AFP >10,000 ng/mL hCG >50,000 mIU/mL LDH >10 × ULN

Abbreviations: AFP, α fetoprotein; hCG, human chorionic gonadotropin; LDH, lactate dehydrogenase; N/A, not applicable; NSGCT, nonseminomatous germ cell tumor; ULN, upper limit normal. Nonpulmonary visceral metastases include liver, bone, and brain.

Source: Reproduced with permission from International Germ Cell Cancer Collaborative Group: International Germ-Cell Consensus Classification: A prognostic factor based staging system for metastatic germ cell tumors. *J Clin Oncol* 15:594, 1997.

Postchemotherapy Surgery Upon completion of cisplatin-based chemotherapy, many patients with normalized serum tumor markers will have radiographic evidence of residual masses. In approximately half of patients with NSGCT, the residual mass is composed of necrosis and/or fibrosis. About 40% will have residual teratoma and only 10% will have residual viable nonteratomatous GCT. Unfortunately, radiographic imaging cannot accurately differentiate between these entities. For this reason, NSGCT patients with residual masses after chemotherapy undergo resection of all sites of disease. This most commonly includes a postchemotherapy RPLND. However, thoracotomy and neck dissection are required in some patients. If the patients are found to have residual necrosis or teratoma, no additional therapy is required. However, for patients with residual viable nonteratomatous GCT, two additional cycles of chemotherapy are frequently administered. It should be noted that in most centers, patients with minimal residual tumors defined as retroperitoneal lymph nodes of ≤ 1 cm will forego postchemotherapy RPLND. Patients who experience normalization of serum tumor markers with first-line chemotherapy but have enlarging tumors, most often cystic masses in the retroperitoneum, may have “growing teratoma syndrome.” These patients are best approached with surgery.

For patients with metastatic seminoma, most residual masses are necrotic and do not harbor viable tumor. Patients with residual masses of 3 cm or less may be observed without surgery. For patients with residual masses >3 cm, fluorodeoxyglucose (FDG)-PET may be used to distinguish necrosis from viable seminoma and identify patients who should be considered for postchemotherapy surgery or short interval imaging.

■ RELAPSED DISEASE

Approximately 20–30% of patients with metastatic GCTs treated with cisplatin-based chemotherapy will not achieve durable disease control. Most of these patients will experience disease progression within 2 years following completion of chemotherapy. The International Prognostic Factors Study Group developed a risk stratification classification system for patients in first relapse. Contributors to a worsened prognosis include NSGCT histology, extragonadal primary, incomplete response to first-line chemotherapy, time to relapse of 3 months or less, level of serum tumor markers at relapse, and the presence of nonpulmonary visceral metastatic disease.

Patients in first relapse may be treated with either conventional-dose salvage chemotherapy or high-dose salvage chemotherapy with autologous stem cell rescue. There is controversy concerning which approach is optimal. Some institutions advocate for risk stratification, with more favorable prognosis patients receiving conventional-dose chemotherapy and worse prognosis patients receiving high-dose chemotherapy. The most commonly utilized conventional-dose regimen includes paclitaxel, ifosfamide, and cisplatin (TIP). In one study of TIP in patients with more favorable-risk disease, approximately two-thirds experienced 2-year progression-free survival. High-dose chemotherapy consists of initial salvage therapy followed by stem cell harvest and then two or three cycles of high-dose carboplatin and etoposide (CE) with stem cell rescue. The largest series of patients treated with high-dose chemotherapy was reported by researchers at Indiana University where this approach is considered standard for most patients in first relapse regardless of risk classification. In their study, 70% of patients in first relapse achieved durable progression-free survival. A large retrospective analysis has compared conventional-dose salvage chemotherapy to high-dose salvage chemotherapy in patients in first relapse. This study reports a more favorable outcome with high-dose salvage chemotherapy across nearly all risk groups. However, given the retrospective nature of this study and the controversy concerning optimal approaches, an international randomized trial comparing conventional-dose chemotherapy (TIP) to high-dose chemotherapy with autologous stem cell rescue (TI-CE) is underway.

Some patients who experience disease progression after conventional-dose salvage chemotherapy may successfully be treated with high-dose salvage chemotherapy with autologous stem cell rescue. Patients with disease progression after high-dose salvage chemotherapy may be treated with subsequent chemotherapy regimens that include gemcitabine/oxaliplatin, gemcitabine/paclitaxel, epirubicin/cisplatin, and oral etoposide. While these patients may benefit from third-line chemotherapy, few will achieve durable disease control. Select patients with relapsed but resectable disease may be candidates for salvage or so-called “desperation” surgery. Studies of molecularly targeted agents and immune checkpoint inhibitors in this population have to date been generally disappointing.

Patients who experience disease progression >2 years after chemotherapy are considered to have “late relapse.” Late relapse appears to have a different biology than early relapse. These patients tend to have more chemotherapy-resistant disease. Patients with late relapse usually have NSGCT with elevation of serum AFP. Many of these patients experience recurrence in the retroperitoneum many years after first-line chemotherapy, and this likely represents residual retroperitoneal disease that was not controlled after first-line therapy. These patients are best approached with salvage surgery.

■ EXTRAGONADAL GCTS

Approximately 5% of patients who present with GCTs have extragonadal primaries. These mainly originate in the mediastinum or retroperitoneum. Patients suspected of extragonadal GCT should undergo scrotal ultrasound to exclude a gonadal primary. Extragonadal seminomas have a similar excellent prognosis as their gonadal counterparts and are approached the same. Mediastinal NSGCTs are classified as poor risk and are treated with either four cycles of BEP or four cycles of VIP. These patients frequently require postchemotherapy thoracic surgery for residual disease. For this reason, some advocate avoiding bleomycin in this patient population. Klinefelter’s syndrome is associated with an increased risk of mediastinal NSGCTs. Rarely, mediastinal

NSGCTs are associated with hematologic disorders including acute myelogenous leukemia. NSGCTs arising in the retroperitoneum do not have a worse prognosis than their gonadal counterparts. Many patients who present with extragonadal GCTs will undergo core needle biopsy for diagnosis. However, select patients with extragonadal tumors and definitive elevation of serum tumor markers may initiate chemotherapy without a tissue diagnosis.

Cancers of unknown primary are defined as histologically proven metastatic malignancy in which the primary site is not obvious. A subgroup of patients with cancer of unknown primary have occult GCTs. Male gender, age <65 years, midline tumors, and nonsmoking status increase the likelihood of this presentation. Pathology may demonstrate a poorly differentiated malignant neoplasm. Immunohistochemical staining is used to exclude lymphoma. Tumor may be analyzed by fluorescence in situ hybridization for *i*(12p), which confirms the diagnosis. Even if the diagnosis is not certain, patients should be treated with cisplatin-based chemotherapy, which will cure up to 20% of this patient group.

■ TESTICULAR NON GERM CELL TUMORS

Rarely, patients may develop testicular non-GCTs. These include non-Hodgkin’s lymphoma, most commonly occurring in men over the age of 50; sex cord stromal tumors including Leydig cell tumors and Sertoli cell tumors; mesothelioma of the tunica vaginalis; and paratesticular sarcoma. Metastasis to the testis is rare, most commonly occurring in patients with advanced prostate cancer and melanoma.

■ SURVIVORSHIP AND LATE EFFECTS

Because most patients with testicular GCT will experience long-term survival, survivorship care is important. Since many of these patients will be followed by primary care physicians, an understanding of the physical, psychological, and social late effects is important. Late effects are defined as health problems that occur months or years after a disease is diagnosed or after treatment has ended. Late effects may be related to the underlying cancer or to the treatment the patient received. In long-term survivors of testicular GCT, increased cardiovascular risk and increased secondary malignancies have been reported. Patients treated with cisplatin-based chemotherapy have an increased risk of hypertension, hyperlipidemia, metabolic syndrome, and cardiovascular events. Patients treated with high cumulative doses of etoposide (e.g., patients who receive standard chemotherapy, relapse, and then receive salvage high-dose chemotherapy) may experience up to a 1–2% risk of developing acute myelogenous leukemia, typically 2–3 years after completing therapy and associated with an 11q23 translocation. Patients treated with radiation therapy, cisplatin-based chemotherapy, or both have an increased risk of developing secondary solid malignancies.

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OVARIAN CANCER

■ INCIDENCE AND PATHOLOGY

Ovarian cancer remains a leading cause of cancer deaths in American women, ranking behind lung, breast, colon, and pancreatic cancers. The ovary is responsible for hormone and egg production. Between menarche (11–13 years) and menopause (45–55 years), the ovary is responsible for follicle maturation associated with egg maturation, ovulation, and cyclical sex steroid hormone production. These complex biologic functions are linked to stromal and germ cells within the ovary. Cells of the ovary can be broadly grouped into stromal cells and ovarian germ cells and the enveloping epithelial cells. Malignancies arising in each group include multiple histologic variants, each with unique neoplastic behaviors. Epithelial tumors are the most common histologic variant of ovarian neoplasms; they may be benign (50%), frankly malignant (33%), or of borderline malignancy of low malignant potential (16%). In adnexal masses detected by imaging or physical exam, age influences risk of malignancy; tumors in younger women are more likely benign. In the malignant group, the most common tumors are epithelial. In the group of the ovarian epithelial malignancies are the serous tumors (60–70%), mucinous tumors (10%), endometrioid tumors (10–15%), and clear cell tumors (10–15%) tumors. The distribution of histologic types varies in different parts of the world. The less common stromal tumors arise from the ancillary, supportive cells such as steroid hormone-producing cells and likewise have different phenotypes and clinical presentations. Most stromal tumors do not produce estrogen, but ectopic hormone production can be seen in certain subtypes. Tumors arising in the ovarian germ cell lineage are generally similar in biology and behavior to testicular tumors in males, although their intraperitoneal location alters some metastatic behaviors (Chap. 88). Ovarian tissue may also host metastatic tumors arising from breast, colon, gastric, and pancreatic primaries. Bilateral ovarian masses from metastatic mucin-secreting gastrointestinal cancers are termed *Krukenberg tumors*. A survey of other potential primaries is commonly required during the diagnostic workup of ovarian masses.

■ OVARIAN CANCER OF EPITHELIAL ORIGIN

Epidemiology An American woman has approximately a 1 in 72 lifetime risk (1.6%) of developing ovarian cancer, with the majority of affected women developing epithelial tumors. In 2021 in the United States, ~21,500 cases of ovarian cancer are expected to be diagnosed, with >14,000 deaths. Sporadic (not familial) epithelial tumors of the ovary have a peak incidence in women in their fifties and sixties, although age at presentation ranges from the third decade to the eighties and nineties. Ovarian cancer risk has been linked to an interactive mixture of epidemiologic, environmental, and genetic factors. Nulliparity, obesity, diet, infertility treatments, and possibly hormone replacement therapy have all been linked to an increase in risk. Protective factors include the use of oral contraceptives, multiparity, tubal ligation, aspirin use, and breast-feeding. Other epidemiologic factors such as the historical use of perineal talc agents remain controversial. The mechanisms underlying the various protective factors are largely unknown, but theories include suppression of ovulation, modulation of gonadotropins and progestins, and perhaps reduction of ovarian inflammation and damage associated with the repair of the ovarian cortex associated with ovulation.

Genetics and Pathogenesis Ovarian cancers are divided into type 1 cancers and the more aggressive type 2 variant. The type 1 cancers are characterized by low-grade histology and generally indolent behavior. These tumors include the low malignant potential tumors,

low-grade endometrial and mucinous histologies, and clear cell cancers (which are more aggressive). Genetic alterations in type 1 cancers include mutations in *KRAS*, *BRAF*, *PTEN*, and *PIK3CA*. In contrast, studies have implicated serial genetic changes in the fallopian tube as the actual site of origin for most type 2, high-grade serous epithelial ovarian cancers. These aggressive tumors are more common and linked to losses in *TP53* and defective DNA repair. Carcinoma in situ has been identified in the tubal epithelium with early losses in *TP53* and the *BRCA1/BRCA2* gene function characterizing early tubal intraepithelial cancers. Following these early genetic events, additional mutations in these transformed cells lead to tumor cell shedding, metastasis, and invasion. These type 2, poorly differentiated, serous cancer cells can then spread to the ovaries and the peritoneal cavity, aided by the ovarian cancer cell's affinity for mesothelin-expressing cells.

Type 2 serous ovarian cancer is classically a disease characterized by widespread amplifications and deletions rather than single-gene point mutations or common gene fusions. In the Tumor Genome Atlas, loss of tumor-suppressor gene *TP53* function is present in >95% of serous ovarian cancers. Damage to homologous DNA repair genes, especially *BRCA1* and *BRCA2*, is also common in these tumors. Low prevalence but statistically recurrent somatic mutations in seven other genes including *NFI*, *RBL*, and *CDK12* were also seen. The most common heritable abnormality linked to ovarian cancer is a germline mutation in either *BRCA1* (chromosome 17q12–21) or *BRCA2* (chromosome 13q12–13). These genes are essential parts of the homologous DNA repair machinery for double-stranded DNA break repair. Individuals inheriting a single copy of a mutant allele have an increased lifetime risk of breast (46–87% for *BRCA1*; 38–84% for *BRCA2*) and ovarian cancer (39–63% for *BRCA1*; 16.5–27% for *BRCA2*). Many of these women have a family history that includes multiple cases of breast and/or ovarian cancer of at an early age. Male breast cancer, pancreatic cancer, and prostate cancer are also linked to familial *BRCA2* mutations. The most common malignancy in women carrying germline *BRCA1/2* mutations is breast carcinoma, although women harboring germline *BRCA1* mutations also have a marked increased risk of developing ovarian malignancies in their forties and fifties. Women harboring a mutation in *BRCA2* have a lower penetrance of ovarian cancer with onset typically in their fifties or sixties. Other uncommon germline mutation of other genes encoding proteins linked to homologous DNA repair (e.g., *PALB2*) can also contribute to cancer risk, although the frequency of mutation and magnitude of risk increment are much lower and not well defined. Screening studies, even in the mutated *BRCA1/2* families, suggest that any of the available screening techniques, including structured, serial evaluation of the CA-125 serum marker and transvaginal ultrasound, remain insufficient to reliably detect early-stage ovarian cancer in prospective testing. Germline *BRCA1/2* testing is recommended for all incident epithelial ovarian cancers to detect probands for therapeutic intervention and identify relatives at risk. Women with these high-risk germline mutations are advised to undergo prophylactic removal of fallopian tubes and ovaries after completing childbearing, ideally before age 40. Early prophylactic salpingo-oophorectomy is highly protective. Salpingo-oophorectomy also appears to protect these women from subsequent breast cancer (risk reduction 50%). Prophylactic salpingectomy is almost certainly a key part of any surgical prophylaxis strategy for ovarian cancer prevention, but the benefits of isolated oophorectomy on either ovarian or breast cancer risk have not yet been clearly defined. Although less common, ovarian cancer is also another familial form of cancer (along with colorectal and endometrial cancer) that may develop in women with type II Lynch syndrome caused by mutations in one of the DNA mismatch repair genes (*MSH2*, *MLH1*, *MLH6*, *PMS1*, *PMS2*). Ovarian cancer may appear in women younger than 50 years of age in this syndrome.

Neoplasms of the ovary tend to be painless unless they undergo torsion. Nonspecific gastrointestinal symptoms like bloating and early satiety are common at presentation, probably related to compression of local organs or due to symptoms from metastatic disease. Women with ovarian tumors also may have an increased incidence of symptoms including pelvic discomfort, bloating, and perhaps changes in urinary or bowel pattern. Unfortunately, all of these symptoms are common in

TABLE 89-1 Staging and Survival in Gynecologic Malignancies

STAGE	OVARIAN	5-YEAR SURVIVAL, %	ENDOMETRIAL	5-YEAR SURVIVAL, %	CERVIX	5-YEAR SURVIVAL, %
0	—		—		Carcinoma in situ	100
I	Confined to ovary	88–95	Confined to corpus	>90	Confined to uterus	85
II	Confined to pelvic organs	70–80	Involves corpus and cervix	~75	Invades beyond uterus but not to pelvic wall	65
III	Intra-abdominal spread to omentum, diaphragm, or lymph nodes	20–40	Extends outside the uterus but not outside the true pelvis	45–60	Extends to pelvic wall and/or lower third of vagina, or hydronephrosis	35
IV	Spread outside abdominal cavity, parenchymal spread, and pleural effusion cytology	17	Extends outside the true pelvis or involves the bladder or rectum	~20	Invades mucosa of bladder or rectum or extends beyond the true pelvis	7

primary care and are frequently dismissed by either the woman or her health care team until later stages of disease. The pathogenic factors and timing of spread beyond the ovary are still not well understood. The most common symptoms at presentation of advanced disease include a period of progressive complaints of nausea, early satiety, bloating, indigestion, constipation, and abdominal pain. Signs include the rapid increase in abdominal girth due to the accumulation of ascites that typically alerts the patient and her physician that the concurrent gastrointestinal symptoms are likely associated with malignant pathology. Radiologic evaluation typically demonstrates a complex adnexal mass with ascites, carcinomatosis, and pelvic, para-aortic and mesenteric adenopathy in advanced disease. Positron emission tomography (PET) scans are generally not required. Laboratory evaluation often demonstrates a markedly elevated CA-125, a shed mucin component (MUC16) associated with, but not specific for, ovarian cancer. Ovarian cancers are divided into four stages, with stage I tumors confined to the ovary, stage II malignancies confined to the pelvis, and stage III confined to the peritoneal cavity and retroperitoneal nodes (Table 89-1). These three stages are subdivided, with the most common presentation, stage IIIC, defined as tumors with bulky intraperitoneal disease or positive lymph node involvement. About 70% of women present with stage III disease. Stage IV disease includes women with parenchymal metastases (liver, lung, spleen) or, alternatively, abdominal wall or pleural disease. The 30% of patients not presenting with stage III disease are roughly evenly distributed among the other stages.

Screening Ovarian cancer is a highly lethal condition. It is curable in early stages but seldom curable in advanced stages; hence, screening continues to be of considerable interest. Early-stage tumors often secrete excessive amounts of normal proteins that can be measured in the serum such as CA-125, mesothelin, and HE-4. Nevertheless, the incidence of ovarian cancer in the middle-aged female population is very low, with only ~1 in 2000 women between the ages of 50 and 60 carrying an asymptomatic and undetected tumor. Thus, effective screening techniques must be both sensitive and highly specific to minimize the number of false positives. Panels of serum markers have not improved on CA-125 alone, nor have risk assessment strategies using algorithms with multiple CA-125 measurements over time. No other screening strategies have been any more successful to date. Some large studies have suggested that low-specificity screening might even worsen mortality in the screened population. Screening for ovarian cancer is currently not recommended outside of a clinical trial, but large ongoing clinical trials are studying algorithmic detection by serial sampling strategies.

TREATMENT

Ovarian Cancer

Epithelial ovarian cancer can be divided into distinct ‘disease states’ for the purpose of treatment selection, as shown in Fig. 89-1. Surgery by a skilled gynecologic oncologist remains the preferred initial therapy for ovarian cancer. However, the amount of residual visible cancer at the end of a primary operation is

strongly predictive of outcome and is paired with histology, grade, and stage to determine prognosis and treatment. In women presenting with a localized ovarian mass, the principal diagnostic and therapeutic maneuver is abdominal surgery to determine if the tumor is benign or malignant. In the event that the tumor is malignant, the surgical specimen will determine if the tumor arises in the ovary or is a site of metastatic disease. Metastatic disease to the ovary can be seen from primary tumors of the colon, appendix, stomach (Krukenberg tumors), and breast. Needle biopsy is contraindicated to avoid malignant contamination of the peritoneal cavity with malignant cells. Typically, women undergo laparoscopic evaluation and unilateral salpingo-oophorectomy for diagnostic purposes. If pathology reveals a primary ovarian malignancy or the laparoscopy proves disseminated disease is present, then the procedure should be followed by a total hysterectomy, removal of the remaining tube and ovary, omentectomy, and pelvic node sampling along with biopsies of the peritoneal cavity and diaphragms. This extensive surgical procedure is performed because ~30% of tumors that, by visual inspection, appear to be confined to the ovary have already disseminated to the peritoneal cavity and/or surrounding lymph nodes. As with axillary dissections in breast cancer, node sampling is diagnostic, but full lymphadenectomy appears to provide little or no additional therapeutic advantage over nodal sampling. The target outcome of an ovarian cancer surgery is always an R0 resection, with no visible residual cancer. The less favorable ‘optimal resection’ (no disease >1 cm in size) is still clinically useful, and the prognosis of those patients is much better than that of patients who are left with >1 cm of disease at the end of surgery. These ‘suboptimally debulked’ patients derive very little benefit from their surgery. If a suboptimal debulking is anticipated, the surgery should be delayed until after several cycles of neoadjuvant chemotherapy. Such ‘interval debulking’ surgery achieves similar results to primary surgery with diminished surgical morbidity and more timely chemotherapy. Patients without gross residual disease after resection have a median survival in excess of 60 months, compared to 28–42 months for those left with macroscopic tumor or those undergoing interval debulking, regardless of treatment strategy.

After appropriate surgical treatment, primary chemotherapy will consist of combination treatment with paclitaxel and carboplatin. Primary chemotherapy can be delivered intravenously, or alternatively, some therapy can be directly administered into the peritoneal cavity via an indwelling catheter. Some, but not all, randomized studies have demonstrated improved survival with intraperitoneal (IP) therapy. The IP approach is technically more difficult and is increasingly replaced by carboplatin and paclitaxel, which appears to offer similar results.

With optimal debulking surgery and platinum-based chemotherapy (usually carboplatin dosed to an area under the curve [AUC] of 6.0 plus paclitaxel 175 mg/m² by 3-h infusion in monthly cycles), 70% of women who present with advanced-stage tumors show tumor reduction, and 40–50% experience a complete remission with normalization of their CA-125, CT scans, and physical

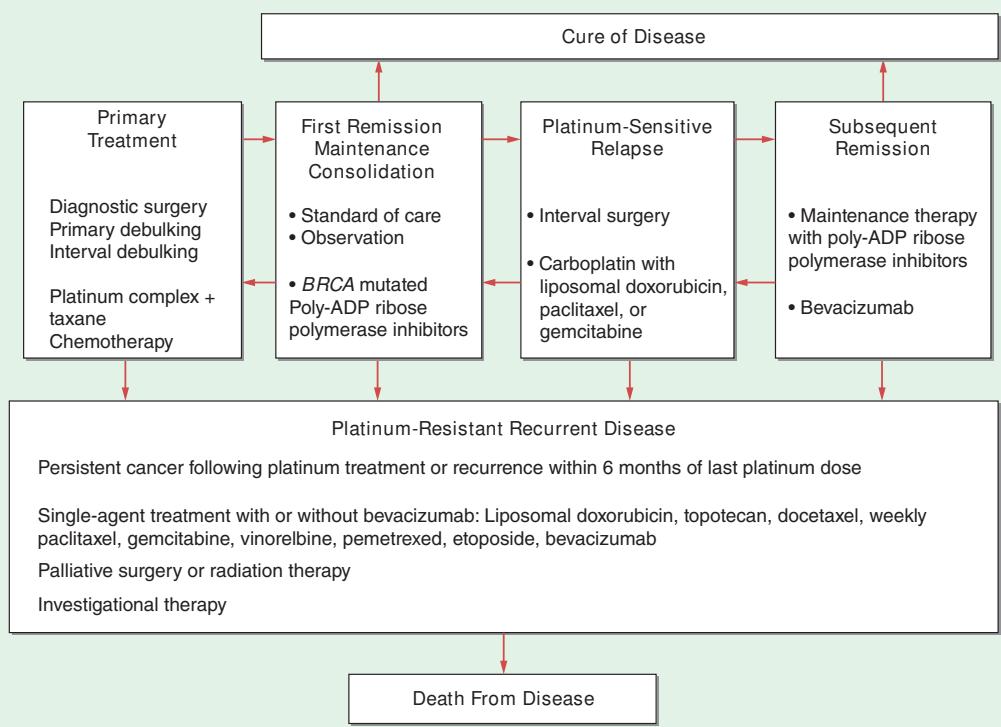


FIGURE 89-1 Disease states model of epithelial ovarian cancer and its treatment. Each box represents a relatively homogenous group of patients who share a palette of potential treatment choices and have a similar prognosis. The arrows indicate that a single patient may move from one state to another during the course of her illness, and the choice of treatments will become different in her new disease state.

examination. Poly-ADP ribose polymerase inhibitors (PARPi) such as niraparib or olaparib, when administered at the completion of intravenous chemotherapy, appear to substantially delay recurrence and probably provide survival advantages as well. In the majority of patients, disease still recurs within 1–4 years from the completion of their primary therapy. CA-125 levels often increase as a first sign of relapse, and CT scan findings are confirmatory. Recurrent disease is often successfully managed for years, but rarely cured, with a variety of chemotherapeutic agents. Additional surgical therapy does not appear to extend survival in randomized trials. Patients with a treatment-free interval are often best treated with additional platinum doublets, combining carboplatin with liposomal doxorubicin, gemcitabine, or a taxane. Eventually all women who experience relapse develop chemotherapy-refractory disease. Refractory ascites, poor bowel motility, and obstruction or tumor-infiltrated aperistaltic bowel are all common premorbid events. Limited surgery to relieve intestinal obstruction, localized radiation therapy to relieve pressure or pain from masses, or palliative chemotherapy may be helpful. Agents with >15% response rates include gemcitabine, topotecan, liposomal doxorubicin, and bevacizumab. Five-year survival correlates with the stage of disease: stage I, 90–95%; stage II, 70–80%; stage III, 25–40%; stage IV, 10–15% (Table 89-1). Prognosis is also influenced by histologic grade: 5-year survival is 88% for well-differentiated tumors, 58% for moderately differentiated tumors, and 27% for poorly differentiated tumors. Histologic type has less influence on outcome.

■ UNCOMMON OVARIAN TUMORS

Low Malignant Potential Tumors (Borderline Tumors) These type I tumors are found in younger women (age 30–50 years) and indolent in behavior, and few of these patients will succumb to

their tumors (10-year survival may approach 98%), although recurrence is not uncommon. Certain features, such as micropapillary histology and microinvasion, are linked to more aggressive behavior. Tumors of low malignant potential should be carefully distinguished from grade 1 serous carcinomas. Borderline tumor patients are managed primarily by surgery; chemotherapy and radiation therapy do not substantially alter survival.

Stromal Tumors Approximately 7% of ovarian neoplasms are stromal tumors, with ~1800 cases expected each year in the United States. Ovarian stromal tumors or sex cord tumors are most common in women in their fifties or sixties, but tumors can present at any age. These tumors arise from the mesenchymal components of the ovary, including both steroid-producing cells and fibroblasts. Most of these tumors are indolent tumors with limited metastatic potential and present as unilateral solid masses. These tumors primarily are discovered by the detection of an abdominal mass, sometimes with abdominal pain due to ovarian torsion, intratumoral hemorrhage, or rupture. Rarely, stromal tumors can produce estrogen and present with breast tenderness as well as precocious puberty in children, menstrual disturbances in reproductively active women, or postmenopausal bleeding. In some women, estrogen-associated secondary malignancies, such as endometrial or breast cancer, may present as synchronous malignancies. Sertoli-Leydig tumors often present with hirsutism and virilization due to increased production of androgens. Hormonally inert tumors include fibromas, which present as solitary masses often in association with ascites and occasionally hydrothorax, also known as Meig's syndrome. A subset of these tumors present in individuals with a variety of inherited disorders that predispose them to mesenchymal neoplasia including Ollier's disease (juvenile granulosa cell tumors) and Peutz-Jeghers syndrome (ovarian sex cord tumors). The treatment of these tumors is almost exclusively by surgical resection, without adjuvant chemotherapy. Chemotherapy with carboplatin and

paclitaxel is generally reserved for either unresectable or multiply recurrent tumors.

Germ Cell Tumors of the Ovary Germ cell tumors, like their counterparts in the testis, are cancers of germ cells. These totipotent cells contain the programming for differentiation to essentially all tissue types, and hence, the germ cell tumors include a histologic menagerie of bizarre tumors, including benign teratomas (dermoid cysts) and a variety of malignant tumors, such as dysgerminoma, immature teratomas, yolk sac malignancies, and choriocarcinomas. Benign teratoma (or dermoid cyst) is the most common germ cell neoplasm of the ovary and often presents in young women. These tumors include a complex mixture of differentiated tissue including tissues from all three germ layers. In older women, these differentiated tumors can develop malignant transformation, most commonly squamous cell carcinomas. Malignant germ cell tumors include dysgerminomas, yolk sac tumors, immature teratomas, and embryonal and choriocarcinomas. Germ cell tumors can present at all ages, but the peak age of presentation tends to be in adolescents. Typically, these tumors will become large ovarian masses, which eventually present as palpable low abdominal or pelvic masses. Like sex cord tumors, torsion or hemorrhage may present urgently or emergently as acute abdominal pain. Some germ cell tumors produce elevated levels of human chorionic gonadotropin (hCG) or α -fetoprotein (AFP). Unlike epithelial ovarian cancer, these tumors have a higher proclivity for nodal or hematogenous metastases. Germ cell tumors typically present in women who are of childbearing age, and because bilateral tumors are uncommon (except in dysgerminoma, 10–15%), the typical treatment is unilateral oophorectomy or salpingo-oophorectomy with lymph node sampling. Most commonly, women with advanced malignant germ cell tumors typically receive bleomycin, etoposide, and cisplatin (BEP) chemotherapy, in an analogous fashion to the treatment of testicular cancers. In the majority of these women, even those with advanced-stage disease, cure is expected. Dysgerminoma is the ovarian counterpart of testicular seminoma and is highly curable. Although the tumor is highly radiation-sensitive, radiation produces infertility in many patients. BEP chemotherapy is as effective or more so without causing infertility.

FALLOPIAN TUBE CANCER

Transport of the egg to the uterus occurs through the fallopian tube, with the distal ends of these tubes composed of fimbriae that drape about the ovarian surface and capture the egg as it erupts from the ovarian cortex. As described above, the majority of type 2 ovarian cancers are now thought to arise from the tubal epithelium. As might be expected, fallopian tube malignancies are typically of serous histology and share the same biology and recommended treatment as serous ovarian cancer. These tumors often present as clinically isolated adnexal masses, but like ovarian cancer, these tumors spread relatively early throughout the peritoneal cavity. Fallopian tubal cancers have a natural history and treatment that are essentially identical to ovarian cancer (Table 89-1).

CERVICAL CANCER

ETIOLOGY AND GENETICS

Cervical cancer is the second most common and the most lethal malignancy in women worldwide. Infection with high-risk strains of human papillomavirus (HPV) is the primary neoplastic-initiating event in the vast majority of women with invasive cervical cancer. This double-stranded DNA virus infects epithelium near the transformation zone of the cervix where underlying columnar epithelium becomes squamous epithelium. More than 60 types of HPV are known, with ~20 types having the ability to generate high-grade dysplasia and malignancy. HPV16 and 18 are the types most frequently associated with high-grade dysplasia, but types 31, 33, 35, 52, and 58 are also considered to be high-risk variants. The large majority of sexually active adults are exposed to HPV, and most women clear the infection without specific intervention. The 8-kb HPV genome encodes seven

early genes, most notably *E6* and *E7*, which can bind to *RB* and *p53*, respectively. High-risk types of HPV encode *E6* and *E7* molecules that are particularly effective at inhibiting the normal cell cycle checkpoint functions of these regulatory proteins, leading to immortalization but not full transformation of cervical epithelium. A minority of women will fail to clear the infection, with subsequent HPV integration into the host genome. Over as little as a few months to several years, some of these persistently infected women develop worsening dysplasia, a premalignant condition that, untreated, can progress to cervical carcinoma. Complete transformation to cancer occurs over a period of years and almost certainly requires the acquisition of other poorly defined genetic mutations within the infected and immortalized epithelium.

In 2018, ~570,000 new cases of cervical cancer occurred worldwide, with an estimated 311,000 deaths. Cancer incidence is particularly high in women residing in Central and South America, the Caribbean, and southern and eastern Africa. The mortality rate is disproportionately high in Africa. In the United States, an estimated 14,480 women will be diagnosed with cervical cancer in 2021 and ~4290 women will die of the disease.

In the integrated genomic characterization of cervical cancer by The Cancer Genome Atlas (TCGA), integration of HPV sequences was found in all of the HPV18-linked cancers and over three-quarters of the HPV16 cancers. The cervical tumors also showed a characteristic APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; a family of cytidine deaminases that edit DNA and are endogenous mutagenic enzymes) pattern of mutagenesis, with *ERBB3*, *CASP8*, and *TGFRB2* identified as significantly mutated genes presumably linked to progression from dysplasia to carcinoma. In the much smaller number of HPV-negative cancers, which are more common in older women, mutations in oncogenes *KRAS*, *ARID1A*, and *PTEN* were frequently seen. The clinical behavior of these cancers is likely to be different.

HPV INFECTION AND PREVENTION

The Pap smear is the primary detection method for asymptomatic preinvasive cervical dysplasia of squamous epithelial lining during a gynecologic exam. Because the delay between dysplasia and frank cervical cancer is years long, annual (or longer) screening and prevention strategies that detect precancerous dysplasia and carcinoma *in situ* can be implemented successfully. Annual or biannual cervical scraping for cytology (Pap smear) is highly effective in reducing the incidence of cervical cancer by early detection and subsequent surgical treatment of premalignant disease. The incorporation of HPV testing by polymerase chain reaction (PCR) or other molecular techniques increases the sensitivity of detecting cervical pathology but at the cost of lower sensitivity in that it identifies many women with transient infections who require no specific medical intervention. Unfortunately, both the collection of a Pap smear and its cytologic evaluation require infrastructure beyond the means of many middle- and low-income countries. High-throughput, low-technology prevention strategies and point-of-care testing are needed to identify and treat women bearing high-risk cervical dysplasia to prevent cancer development.

A primary prevention strategy relies on HPV vaccines. Currently approved vaccines include the recombinant proteins to the late proteins, L1 and L2 of HPV16 and 18, as well as other, less common cancer-causing isotypes 11, 31, 33, 45, 52, and 58. Vaccination of girls aged 11–13 years with two injections (1 year apart) before the initiation of sexual activity dramatically reduces the rate of high-risk HPV infection and subsequent dysplasia. Vaccination of both boys and girls is increasingly considered to reduce the risk of HPV-induced cancers of the pharynx. Partial protection is also provided against other HPV types, although vaccinated women are still at risk for HPV infection and still benefit from standard Pap smear screening.

CLINICAL PRESENTATIONS

Risk Factors Clinical risk factors include many HPV infection-linked features: a high number of sexual partners, early age of first intercourse, and history of venereal disease. Smoking is a cofactor;

heavy smokers have a higher risk of dysplasia with HPV infection. HIV infection, especially when associated with low CD4+ T-cell counts, is associated with a higher rate of high-grade dysplasia and likely a shorter latency period between infection and invasive disease. Histologically, the majority of cervical malignancies are squamous cell carcinomas associated with HPV, but adenocarcinomas are also HPV related, and both arise in the transitional zone of the endocervical canal; the lesions in the canal or cervical glands may not be seen by visual inspection of the cervix and can be missed by Pap smear screening. Less common malignancies, such as vulvar cancer, anal cancer, and pharyngeal cancer, are also linked to HPV infection.

Diagnosis of Cervical Cancer Early cancer of the cervix is asymptomatic, and this biology underlies the recommendations for routine gynecologic care. Larger, invasive carcinomas often have symptoms or signs including postcoital spotting or intermenstrual cycle bleeding or menometrorrhagia. Foul-smelling or persistent yellow discharge may also be present. Symptoms such as pelvic or sacral pain suggest lateral extension into the pelvic nerve plexus by either the primary tumor or a pelvic node metastasis and indicate advanced-stage disease. Likewise, flank pain from hydronephrosis from ureteral compression or deep-venous thrombosis from iliac vessel compression suggests either extensive nodal disease or direct extension of the primary tumor to the pelvic sidewall. The most common finding upon physical exam is a visible tumor on the cervix, but deeper tumors in the cervical os and glands should be considered. Larger tumors may be identified by inspection and biopsied directly. Staging of cervical cancer is performed by clinical exam. Stage I cervical tumors are confined to the cervix, whereas stage II tumors extend into the upper vagina or paracervical soft tissue (Fig. 89-2). Stage III tumors extend to the lower vagina or the pelvic sidewalls, whereas stage IV tumors invade the bladder or rectum or have spread to distant sites. While radiographic studies are not part of the formal clinical staging of cervical cancer, treatment planning requires them for appropriate therapy. CT can detect hydronephrosis indicative of pelvic sidewall disease but is not accurate at evaluating other pelvic structures. MRI is more accurate at estimating uterine extension and paracervical extension of disease into soft tissues typically bordered by broad and cardinal ligaments that support the uterus in the central pelvis. Very small stage I cervical tumors can be treated with a variety of surgical procedures, but minimally invasive surgery has inferior outcome compared to standard open hysterectomy. In young women desiring to maintain fertility,

radical trachelectomy removes the cervix with subsequent anastomosis of the upper vagina to the uterine corpus; however, subsequent pregnancies may be more problematic. Patients with large stage I cervical tumors (4 cm) confined to the cervix and all stage II to IV patients are treated with radiation therapy in combination with cisplatin-based chemotherapy. This multimodality treatment can offer the patient with advanced-stage disease a 40–80% chance of cure depending on the clinical circumstances. Platinum agents (cisplatin or carboplatin) combined with paclitaxel and bevacizumab are generally considered as the best palliative choice for metastatic cervical cancer patients. Secondary chemotherapy confers minimal improvement in most patients. Immunotherapy with immune checkpoint inhibitors or adoptive T-cell therapies are promising avenues for improved outcomes in recurrent, unresectable cancers of the cervix.

UTERINE CANCER

■ EPIDEMIOLOGY

Several different tumor types arise in the uterine corpus. Most tumors arise in the glandular lining and are endometrial adenocarcinomas. Benign (leiomyomas) and malignant tumors (leiomyosarcomas) can also arise in the uterine smooth muscle and have very different clinical features. The endometrioid histologic subtype is the most common gynecologic malignancy in the United States. In 2021, the American Cancer Society predicted that 66,570 new cancers of the uterine corpus in 2021 with 12,940 resulting deaths. Development of these tumors is a multistep process, with estrogen playing an important early role in driving endometrial gland proliferation. Relative overexposure to this class of hormones is the principal risk factor for the subsequent development of endometrioid tumors. In contrast, progestins drive glandular maturation and are protective. Hence, women with high endogenous or pharmacologic exposure to estrogens, especially if unopposed by progesterone, are at higher risk for endometrial cancer. Obese women, women treated with postmenopausal estrogens, or women with estrogen-producing tumors are at higher risk for endometrial cancer. In addition, long-term treatment with tamoxifen, which has antiestrogenic effects in breast tissue but can show weak estrogenic effects in uterine epithelium, is associated with an increased risk of endometrial cancer.

Genetics Women with a germline mutation in one of a series of DNA mismatch repair genes associated with the Lynch syndrome,

also known as hereditary nonpolyposis colon cancer (HNPCC) syndrome, are at increased risk for endometrioid endometrial carcinoma. These individuals have germline mutations in *MSH2*, *MLH1*, and, in rare cases, *PMS1* and *PMS2*. Individuals who carry these mutations typically have a family history of cancer and are at markedly increased risk for colon cancer and modestly increased risk for ovarian cancer and a variety of other tumors. Middle-aged women with HNPCC carry a 4% annual risk of endometrial cancer and a relative overall risk of ~200-fold as compared to age-matched women without HNPCC. In sporadic cancers, secondary events such as mutation of the *PI3K* gene or the loss of the *PTEN* tumor-suppressor gene likely serve as secondary genetic “hits” in the carcinogenesis related to estrogenic excess. The molecular events that underlie less common endometrial cancers such as clear cell and papillary serous tumors of the uterine corpus are not well understood.

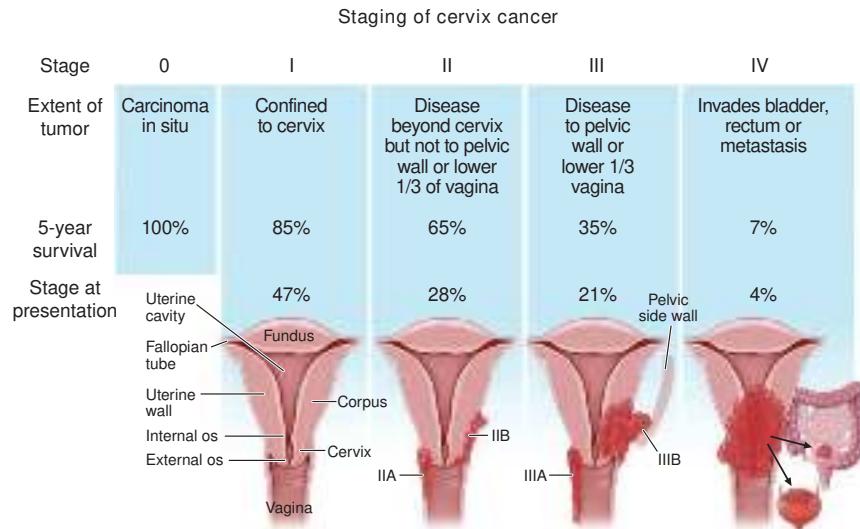


FIGURE 89-2 Anatomic display of the stages of cervix cancer defined by location, extent of tumor, frequency of presentation, and 5-year survival.

Approximately 75–80% of endometrial cancers are adenocarcinomas and have been characterized as type 1 (estrogen-linked) endometrial cancers and type 2 cancers that have less clear associations with estrogens (clear cell cancers, serous cancers, and mucinous cancers). Endometrial serous cancers show *TP53* loss of function and behave clinically more like ovarian cancers with high risk for systemic recurrence. Prognosis for endometrial cancer depends on stage, histologic grade, and depth of myometrial invasion.

■ CLINICAL PRESENTATION

The majority of women with tumors of the uterine corpus present with postmenopausal vaginal bleeding due to shedding of the malignant endometrial lining. Premenopausal women often will present with atypical bleeding between typical menstrual cycles. These signs typically bring a woman to the attention of a health care professional, and the majority of women present with early-stage disease in which the tumor is confined to the uterine corpus and, consequently, have a high cure rate. Diagnosis is typically established by endometrial biopsy. Type 1 tumors may spread to pelvic or para-aortic lymph nodes and are generally subjected to sentinel lymph node biopsy at the time of primary surgery. Serous tumors tend to have patterns of spread much more reminiscent of ovarian cancer, and patients may present with omental/peritoneal disease and sometimes ascites. Some women with endometrial cancer have a history of endometriosis. Some women presenting with uterine sarcomas will present with pelvic pain. Uterine sarcomas (carcinosarcomas and leiomyosarcomas) commonly are found by detection of symptomatic large pelvic masses that may or may not be associated with dysfunctional bleeding.

TREATMENT

Uterine Cancer

Most women with endometrial cancer have disease that is localized to the uterus (75% are stage I, Table 89-1), and definitive treatment typically involves a hysterectomy with removal of the ovaries and fallopian tubes. The resection of lymph nodes does not improve outcome, but sentinel node resection provides important staging and prognostic information. Node involvement defines stage IIIC disease. Tumor grade and depth of invasion are the two key prognostic variables in early-stage tumors, and women with low-grade and/or minimally invasive tumors (<50% myometrial penetration) are typically observed after definitive surgical therapy. Patients with high-grade tumors or tumors that are deeply invasive (stage IB) are at higher risk for pelvic recurrence or recurrence at the vaginal cuff, which is typically prevented by intravaginal brachytherapy.

The loss of one or more mismatch repair proteins results in microsatellite instability (MSI) with a larger number of mutations in the tumor. MSI testing should be routinely performed in endometrial cancers at the time of diagnosis to help with current and future treatment plans. MSI cancers, when recurrent or present at an advanced stage, are likely to respond to immune checkpoint therapy. Women with regional metastases or metastatic disease (3% of patients) with low-grade tumors can be treated with progesterone or tamoxifen. Poorly differentiated tumors lack hormone receptors and are typically resistant to hormonal manipulation. The role of adjuvant chemotherapy in stage I-II disease is generally restricted to serous endometrial cancers. For more advanced-stage cancers (stage III-IV), chemotherapy and/or immune checkpoint blockade are administered because of the higher rates of recurrent systemic disease. Carboplatin and paclitaxel combinations are the current standard of care. Chemotherapy for metastatic disease is delivered with palliative intent. Patients with advanced cancer and known mismatch repair deficits may respond particularly well to immunotherapy with antagonists of the PD-1/PD-L1 axis. Lenvatinib and pembrolizumab (even for microsatellite-stable tumors)

have become the most common second-line treatments, although survival data are not yet available. Other potentially active treatments include bevacizumab, mTOR inhibitors (e.g., temsirolimus), and anthracyclines. Carcinosarcomas of the uterus (also called Müllerian tumors) contain both mesenchymal and epithelial components but will often respond to paclitaxel and platinum complex therapy. Other uterine sarcomas require an entirely different approach and need histology-specific consideration. The most common are the leiomyosarcomas of the uterus, which are treated with docetaxel/gemcitabine at recurrence but do not appear to benefit from adjuvant therapy. Ifosfamide/doxorubicin and trabectedin can have some benefit in refractory disease.

GESTATIONAL TROPHOBlastic TUMORS

Gestational trophoblastic diseases represent a spectrum of neoplasia from benign hydatidiform mole to choriocarcinoma due to persistent trophoblastic disease associated most commonly with molar pregnancy but occasionally seen after normal gestation. The most common presentations of trophoblastic tumors are partial and complete molar pregnancies. These represent approximately 1 in 1500 conceptions in developed Western countries. The incidence widely varies globally, with areas in Southeast Asia having a much higher incidence of molar pregnancy. Regions with high molar pregnancy rates are often associated with diets low in carotene and animal fats.

■ RISK FACTORS

Trophoblastic tumors result from the outgrowth or persistence of placental tissue. They arise most commonly in the uterus but can also arise in other sites such as the fallopian tubes due to ectopic pregnancy. Risk factors include poorly defined dietary and environmental factors as well as conceptions at the extremes of reproductive age, with the incidence particularly high in females conceiving at younger than age 16 or older than age 50. In older women, the incidence of molar pregnancy might be as high as one in three, likely due to increased risk of abnormal fertilization of the aged ova. Most trophoblastic neoplasms are associated with complete moles, diploid tumors with all genetic material from the paternal donor (known as uniparental disomy). This is thought to occur when a single sperm fertilizes an enucleate egg that subsequently duplicates the paternal DNA. Trophoblastic proliferation occurs with exuberant villous stroma. If pseudopregnancy extends past the 12th week, fluid progressively accumulates within the stroma, leading to “hydropic changes.” Fetal development does not occur in complete moles.

Partial moles arise from the fertilization of an egg with two sperm cells; hence, two-thirds of genetic material is paternal in these triploid tumors. Hydropic changes are less dramatic, and fetal development can often occur through late first trimester or early second trimester, at which point spontaneous abortion is common. Laboratory findings will include excessively high human chorionic gonadotropin (hCG) and high AFP. The risk of persistent gestational trophoblastic disease after partial mole is ~5%. Complete and partial moles can be noninvasive or invasive. Myometrial invasion occurs in no more than one in six complete moles and a lower portion of partial moles.

■ PRESENTATION OF INVASIVE TROPHOBlastic DISEASE

The clinical presentation of molar pregnancy is changing in developed countries due to the early detection of pregnancy with home pregnancy kits and the very early use of Doppler and ultrasound to evaluate the early fetus and uterine cavity for evidence of a viable fetus. Thus, in these countries, the majority of women presenting with trophoblastic disease have their moles detected early and have typical symptoms of early pregnancy including nausea, amenorrhea, and breast tenderness. With uterine evacuation of early complete and partial moles, most women experience spontaneous remission of their disease as monitored by serial serum β -hCG levels. These women require no

chemotherapy. Patients with persistent elevation of β -hCG or rising β -hCG after uterine evacuation have persistent or actively growing gestational trophoblastic disease and require therapy. Most series suggest that between 15 and 25% of women will have evidence of persistent gestational trophoblastic disease after molar evacuation.

In women who lack access to prenatal care, presenting symptoms can be life-threatening, including the development of preeclampsia or even eclampsia. Hyperthyroidism can also be seen with very high β -hCG values. Evacuation of large moles can be associated with life-threatening complications including uterine perforation, volume loss, high-output cardiac failure, and adult respiratory distress syndrome (ARDS).

For women with evidence of rising β -hCG or radiologic confirmation of metastatic or persistent regional disease, prognosis can be estimated through a variety of scoring algorithms that identify women at low, intermediate, and high risk for requiring multiagent chemotherapy. In general, women with widely metastatic nonpulmonary disease, very elevated β -hCG, and prior normal antecedent term pregnancy are considered at high risk and typically require multiagent chemotherapy at an expert center for cure. Even very advanced gestational trophoblastic disease is almost uniformly curable when managed by an expert in this rare malignancy.

TREATMENT

Invasive Trophoblastic Disease

Management of invasive trophoblastic disease should be 100% curative, and complex patients should be managed by clinicians experienced in this disease. The management for a persistent and rising β -hCG after evacuation of a molar conception is typically chemotherapy, although surgery can play an important role for chemotherapy-resistant disease that is isolated in the uterus (especially if childbearing is complete) or to control hemorrhage. For women wishing to maintain fertility or with metastatic disease, the preferred treatment is chemotherapy. Trophoblastic disease is exquisitely sensitive to chemotherapy, and guided by serial serum β -hCG testing, successful, curative treatment is the rule. Single-agent treatment with dactinomycin or methotrexate cures 90% of women with low-risk disease. Patients with high-risk disease (very high β -hCG levels, presentation ≥ 4 months after pregnancy, brain or liver metastases, failure of methotrexate therapy) are typically treated with multiagent chemotherapy (etoposide, methotrexate, and dactinomycin, alternating with cyclophosphamide and vincristine [EMA-CO]), which is typically curative even in women with extensive metastatic disease. A regimen of cisplatin and etoposide alternating with etoposide/methotrexate/dactinomycin is used for the highest-risk patients. In the highest-risk patients with liver, lung, and brain metastases, hemorrhage from the rich tumor vasculature is a major risk during chemotherapy initiation. Cured women may become pregnant again without evidence of increased fetal or maternal complications.

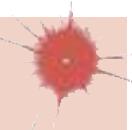
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90

Primary and Metastatic Tumors of the Nervous System

Lisa M. DeAngelis, Patrick Y. Wen



An estimated 87,000 people will be diagnosed with a primary brain tumor annually in the United States. At least 25,000 of these tumors are malignant, and most of these are gliomas. Meningiomas account for 35%, vestibular schwannomas 10%, and central nervous system (CNS) lymphomas ~2%. Brain metastases are three times more common than all primary brain tumors combined and are diagnosed in ~150,000 people each year. Metastases to the leptomeninges and epidural space of the spinal cord each occur in ~3–5% of patients with systemic cancer and are also a major cause of neurologic disability.

APPROACH TO THE PATIENT

Primary and Metastatic Tumors of the Nervous System

CLINICAL FEATURES

Brain tumors of any type can present with a variety of symptoms and signs that fall into two categories: general and focal; patients often have a combination of the two (Table 90-1). General symptoms include headache, with or without nausea or vomiting, cognitive difficulties, personality change, and gait disorder. These symptoms arise when the enlarging tumor and its surrounding edema cause an increase in intracranial pressure or compression of cerebrospinal fluid (CSF) circulation leading to hydrocephalus. The classic brain tumor headache predominates in the morning and improves during the day, but this pattern is seen in a minority of patients. Headaches are often holocephalic but can be ipsilateral to the side of a tumor. Occasionally, headaches have features of a typical migraine with unilateral throbbing pain associated with visual scotoma. Personality changes may include apathy and withdrawal from social situations, mimicking depression. Focal or lateralizing findings include hemiparesis, aphasia, or visual field defect. Lateralizing symptoms are typically subacute and progressive; language difficulties may be mistaken for confusion. Seizures are common, occurring in ~25% of patients with brain metastases or malignant gliomas, and are the presenting symptom in up to 90% of patients with a low-grade glioma. All seizures arising from a brain tumor will have a focal onset whether or not it is apparent clinically.

NEUROIMAGING

Cranial magnetic resonance imaging (MRI) is the preferred diagnostic test for any patient suspected of having a brain tumor and should be performed with gadolinium contrast administration. Computed tomography (CT) scan should be reserved for those patients unable to undergo MRI. Malignant brain tumors—whether primary or metastatic—typically enhance with gadolinium, have central areas of necrosis, and are surrounded by edema of the neighboring white matter. Low-grade gliomas usually do not enhance with gadolinium and are best appreciated on fluid-attenuated inversion recovery (FLAIR) MRI sequences. Meningiomas have a typical appearance on MRI because they are dural-based enhancing tumors with a dural tail and compress but do not invade the brain. Dural metastases or a dural lymphoma can have a similar appearance. Imaging is characteristic for many primary and metastatic tumors and sometimes will suffice to establish a diagnosis when the location precludes surgical intervention (e.g., brainstem glioma).

TABLE 90-1 Symptoms and Signs at Presentation of Brain Tumors

	HIGH-GRADE GLIOMA (%)	LOW-GRADE GLIOMA (%)	MENINGIOMA (%)	METASTASES (%)
Generalized				
Impaired cognitive function	50	10	30	60
Hemiparesis	40	10	36	60
Headache	50	40	37	50
Lateralizing				
Seizures	20	70+	17	18
Aphasia	20	<5	—	18
Visual field deficit	—	—	—	7

Functional MRI is useful in presurgical planning to define eloquent sensory, motor, or language cortex. Positron emission tomography (PET) is useful in determining the metabolic activity of the lesions seen on MRI; MR perfusion and spectroscopy can provide information on blood flow or tissue composition. These techniques may help distinguish tumor progression from tissue necrosis due to treatment with radiation and chemotherapy. Neuroimaging is the only test necessary to diagnose a brain tumor. Laboratory tests are rarely useful, although patients with metastatic disease may have elevation of a serum tumor marker (e.g., β human chorionic gonadotropin [β -hCG] from testicular cancer). Additional testing such as cerebral angiogram, electroencephalogram (EEG), or lumbar puncture is rarely indicated or helpful.

TREATMENT

Brain Tumors

Therapy of any intracranial malignancy requires both symptomatic and definitive treatments. Definitive treatment is based on the specific tumor type and includes surgery, radiotherapy, and chemotherapy. However, symptomatic treatments apply to brain tumors of any type. Most high-grade malignancies are accompanied by substantial surrounding edema, which contributes to neurologic disability and raised intracranial pressure. Glucocorticoids are highly effective at reducing perilesional edema and improving neurologic function, often within hours of administration. Dexamethasone has been the glucocorticoid of choice because of its relatively low mineralocorticoid activity; initial doses are 8–12 mg/d. Glucocorticoids rapidly ameliorate symptoms and signs, but their long-term use causes substantial toxicity including insomnia, weight gain, diabetes mellitus, steroid myopathy, and personality changes. Consequently, a taper is indicated as definitive treatment is administered and the patient improves.

Patients with brain tumors who present with seizures require antiepileptic drug therapy. Prophylactic antiepileptic drugs are used in the perioperative setting, but there is no role for extended use in patients who have not had a seizure. The agents of choice are those drugs that do not induce the hepatic microsomal enzyme system. These include levetiracetam, topiramate, lamotrigine, valproic acid, and lacosamide (Chap. 425). Other drugs, such as phenytoin and carbamazepine, are used less frequently because they are potent enzyme inducers that can interfere with both glucocorticoid and chemotherapy metabolism. Venous thromboembolic disease occurs in 20–30% of patients with high-grade gliomas or brain metastases. Prophylactic anticoagulants should be used during hospitalization and in nonambulatory patients. Those who have had either a deep vein thrombosis or a pulmonary embolus can receive therapeutic doses of anticoagulation safely and without increasing the risk for hemorrhage into the tumor. Inferior vena cava filters are reserved for patients with absolute contraindications to anticoagulation such as recent craniotomy.

PRIMARY BRAIN TUMORS

EPIDEMIOLOGY

No etiology has been identified for most primary brain tumors. The only established risk factors are exposure to ionizing radiation (meningiomas, gliomas, and schwannomas) and immunosuppression (primary CNS lymphoma). There is no proven evidence for any association with exposure to electromagnetic fields including cellular telephones, head injury, foods containing *N*-nitroso compounds, or occupational risk factors. A small minority of patients have a family history of brain tumors. Some of these familial cases are associated with genetic syndromes (Table 90-2).

MOLECULAR PATHOGENESIS

As with other neoplasms, brain tumors arise as a result of a multistep process driven by the sequential acquisition of genetic alterations. These include loss of tumor-suppressor genes (e.g., *p53*, cyclin-dependent kinase inhibitor 2A and 2B [*CDKN2A/B*], and phosphatase and tensin homolog on chromosome 10 [*PTEN*]) and amplification and overexpression of protooncogenes such as the epidermal growth factor receptor (*EGFR*) and platelet-derived growth factor receptors (*PDGFR*). The accumulation of these genetic abnormalities results in uncontrolled cell growth and tumor formation. Many brain tumors, including glioblastomas, are characterized by significant molecular heterogeneity, which contributes to the difficulty in developing effective therapies.

Important progress has been made in understanding the molecular pathogenesis of several types of brain tumors, including glioblastoma and medulloblastoma, allowing them to be separated into different subtypes with different prognoses. This has led the World Health Organization (WHO) to issue an update on the classification of CNS tumors in 2016 that for the first time incorporates molecular parameters in addition to traditional histology into the diagnosis of brain tumors.

INTRINSIC “MALIGNANT” TUMORS

DIFFUSE GLIOMAS

Gliomas are the most common type of malignant primary brain tumor and are derived, based on their presumed lineage, into astrocytomas and oligodendrogliomas. These tumors are classified based on two highly recurrent molecular alterations, isocitrate dehydrogenase (*IDH*) mutations and 1p/19q codeletion, in addition to more conventional histopathologic parameters. Most lower-grade astrocytomas have *IDH* mutations but intact 1p/19q and often mutations in *ATRX* and *p53*. Oligodendrogliomas usually have *IDH* mutations and codeletion of 1p/19q. A minority of astrocytomas and oligodendrogliomas that lack *IDH* mutations (20–30%) have a worse prognosis.

Diffuse gliomas can present rarely as widespread infiltration of the brain tissue without a focal mass. Such tumors usually present with cognitive problems, and the MRI demonstrates confluent, typically nonenhancing areas of increased signal on FLAIR sequences without significant mass effect. Formerly known as gliomatosis cerebri, these lesions are now categorized by the pathology identified on biopsy, but they can be diagnostically challenging when the nature of the imaging

TABLE 90-2 Genetic Syndromes Associated with Primary Brain Tumors

SYNDROME	INHERITANCE	GENE/PROTEIN	ASSOCIATED TUMORS
Cowden's syndrome	AD	Mutations of <i>PTEN</i> (ch10p23)	Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease), meningioma, astrocytoma Breast, endometrial, thyroid cancer, trichilemmomas
Familial schwannomatosis	Sporadic Hereditary	Mutations in <i>INI1/SNF5</i> (ch22q11)	Schwannomas, gliomas
Gardner's syndrome	AD	Mutations in <i>APC</i> (ch5q21)	Medulloblastoma, glioblastoma, craniopharyngioma Familial polyposis, multiple osteomas, skin and soft tissue tumors
Gorlin syndrome (basal cell nevus syndrome)	AD	Mutations in <i>Patched 1</i> gene (ch9q22.3)	Medulloblastomas Basal cell carcinoma
Li-Fraumeni syndrome	AD	Mutations in <i>p53</i> (ch17p13.1)	Gliomas, medulloblastomas Sarcomas, breast cancer, leukemias, others
Lynch syndrome	AD	Mutations in <i>MSH2</i> , <i>MSH1</i> , <i>MSH6</i> , <i>PMS2</i>	Glioblastoma and other gliomas Gastrointestinal, endometrial, and other cancers
Multiple endocrine neoplasia 1 (Wermer's syndrome)	AD	Mutations in <i>Menin</i> (ch11q13)	Pituitary adenoma, malignant schwannomas Parathyroid and pancreatic islet cell tumors
NF1	AD	Mutations in <i>NF1/neurofibromin</i> (ch17q12-22)	Schwannomas, astrocytomas, optic nerve gliomas, meningiomas Neurofibromas, neurofibrosarcomas, others
NF2	AD	Mutations in <i>NF2/merlin</i> (ch22q12)	Bilateral vestibular schwannomas, astrocytomas, multiple meningiomas, ependymomas
TSC (Bourneville disease)	AD	Mutations in <i>TSC1/TSC2</i> (ch9q34/16)	Subependymal giant cell astrocytoma, ependymomas, glioma, gangliouroma, hamartoma
Turcot syndrome	AD AR	Mutations in <i>APC</i> (ch5) <i>hMLH1</i> (ch3p21)	Gliomas, medulloblastomas Adenomatous colon polyps, adenocarcinoma
VHL	AD	Mutations in <i>VHL</i> gene (ch3p25)	Hemangioblastomas Retinal angiomas, renal cell carcinoma, pheochromocytoma, pancreatic tumors and cysts, endolymphatic sac tumors of the middle ear

^aVarious DNA mismatch repair gene mutations may cause a similar clinical phenotype, also referred to as Turcot syndrome, in which there is a predisposition to nonpolyposis colon cancer and brain tumors.

Abbreviations: AD, autosomal dominant; APC, adenomatous polyposis coli; AR, autosomal recessive; ch, chromosome; NF, neurofibromatosis; PTEN, phosphatase and tensin homologue; TSC, tuberous sclerosis complex; VHL, von Hippel-Lindau.

abnormalities is unclear. Often diagnosis is delayed until the patient develops worsening deficits or there is clear progression on imaging. Treatment is then determined by the pathology.

■ ASTROCYTOMAS

These are infiltrative tumors with a presumptive glial cell of origin. The WHO classifies astrocytomas into four prognostic grades based on histologic features: grade I (pilocytic astrocytoma, subependymal giant cell astrocytoma); grade II (astrocytoma); grade III (anaplastic astrocytoma); and grade IV (glioblastoma). Grades I and II are considered low-grade, and grades III and IV high-grade, astrocytomas.

Low-Grade Astrocytoma • GRADE I ASTROCYTOMAS Pilocytic astrocytomas (WHO grade I) are the most common tumor of childhood. They occur typically in the cerebellum but may also be found elsewhere in the neuraxis, including the optic nerves and brainstem. Frequently they appear as cystic lesions with an enhancing mural nodule. Often they have *BRAF* fusions or mutations. These are well-demarcated lesions that are potentially curable if they can be resected completely. Giant cell subependymal astrocytomas are usually found in the ventricular wall of patients with tuberous sclerosis. They often do not require intervention but can be treated surgically or with inhibitors of the mammalian target of rapamycin (mTOR).

GRADE II ASTROCYTOMAS These are infiltrative tumors that usually present with seizures in young adults. They appear as nonenhancing tumors with increased T2/FLAIR signal (Fig. 90-1). If feasible, patients should undergo maximal surgical resection, although complete resection is rarely possible because of the invasive nature of the tumor. In patients at higher risk for recurrence (subtotal resection or above the age of 40 years), there is evidence that radiation therapy (RT) followed by PCV (procarbazine, cyclohexylchloroethylnitrosourea [CCNU], and vincristine) chemotherapy may possibly be of benefit. The tumor

transforms to a malignant astrocytoma in most patients, leading to variable survival with a median of ~5–10 years.

High-Grade Astrocytoma • GRADE III ANAPLASTIC ASTROCYTOMA These account for ~15–20% of high-grade

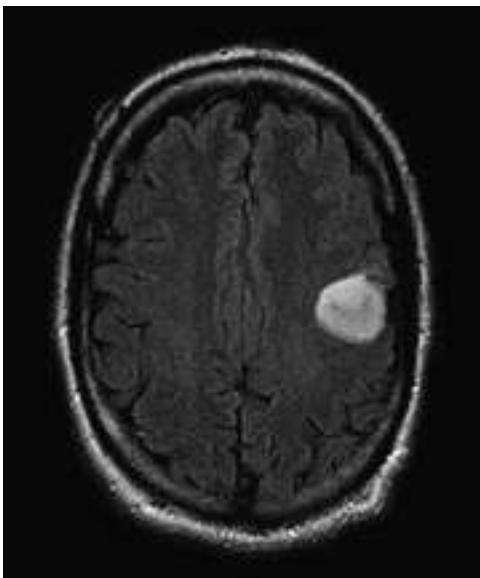


FIGURE 90-1 Fluid-attenuated inversion recovery (FLAIR) MRI of a left frontal low-grade astrocytoma. This lesion did not enhance.

astrocytomas. They generally present in the fourth and fifth decades of life as variably enhancing tumors. Treatment is the same as for glioblastoma, consisting of maximal safe surgical resection followed by RT and adjuvant temozolomide alone or RT with concurrent and adjuvant temozolomide.

GRADE IV ASTROCYTOMA (GLIOBLASTOMA) Glioblastoma accounts for the majority of high-grade astrocytomas. Approximately 10% of glioblastomas have *IDH* mutations. These tend to arise from lower-grade tumors (secondary glioblastomas) and have a better prognosis. In the next update of the WHO classification, the term glioblastoma will be restricted to tumors without *IDH* mutations. Glioblastomas with *IDH* mutations have a different biology and better prognosis and will be termed astrocytomas, *IDH*-mutant, grade 4. In addition, astrocytomas without the classic histologic features of glioblastoma (necrosis and endothelial proliferation) but that have the molecular features of glioblastoma (epidermal growth factor amplification, combined with whole chromosome 7 gain and 10 loss, or telomerase reverse transcriptase [*TERT*] promoter mutations) will also be considered glioblastomas.

Glioblastomas are the most common malignant primary brain tumor, with >12,000 cases diagnosed each year in the United States. Patients usually present in the sixth and seventh decades of life with headache, seizures, or focal neurologic deficits. The tumors appear as ring-enhancing masses with central necrosis and surrounding edema (Fig. 90-2). These are highly infiltrative tumors, and the areas of increased T2/FLAIR signal surrounding the main tumor mass contain invading tumor cells. Treatment involves maximal surgical resection followed by partial-field external-beam RT (6000 cGy in thirty 200-cGy fractions) with concomitant temozolomide, followed by 6 months of adjuvant temozolomide. With this regimen, median survival is increased to 14.6–18 months compared to only 12 months with RT alone, and 5-year survival is ~10%. Efforts to increase the dose of RT locally using brachytherapy or stereotactic radiosurgery (SRS) have failed to improve the outcome and these treatments are not used. Patients whose tumor contains the DNA repair enzyme O⁶-methylguanine-DNA methyltransferase (MGMT) are relatively resistant to temozolomide and have a worse prognosis compared to those whose tumors contain low levels of MGMT as a result of silencing of the *MGMT* gene by promoter hypermethylation. Implantation of biodegradable polymers containing carmustine chemotherapy into the tumor bed after resection of the tumor or addition of tumor treating fields (scalp electrodes delivering low-intensity electric currents) produces a modest improvement in survival.

For elderly patients aged >65–70 years, a hypofractionated RT regimen of 40 Gy over 3 weeks with temozolomide is well tolerated and likely leads to similar outcomes as the 6-week standard RT regimen.

Despite optimal therapy, glioblastomas invariably recur. Treatment options for recurrent disease may include reoperation, re-irradiation with bevacizumab, and alternate chemotherapeutic regimens. Bevacizumab, a humanized vascular endothelial growth factor (VEGF) monoclonal antibody, has activity in recurrent glioblastoma, increasing progression-free survival but not overall survival and reducing peritumoral edema and glucocorticoid use (Fig. 90-3). Immune checkpoint inhibitors have been successful in a variety of solid tumors but have failed to demonstrate substantial activity in glioblastoma. A recent phase III trial comparing bevacizumab with nivolumab in recurrent glioblastoma demonstrated an identical median overall survival of 9.8–10 months in the two arms, with similar toxicities. Treatment decisions for patients with recurrent glioblastoma must be made on an individual basis, taking into consideration such factors as previous therapy, time to relapse, performance status, and quality of life. Whenever feasible, patients should be enrolled in clinical trials. Novel therapies

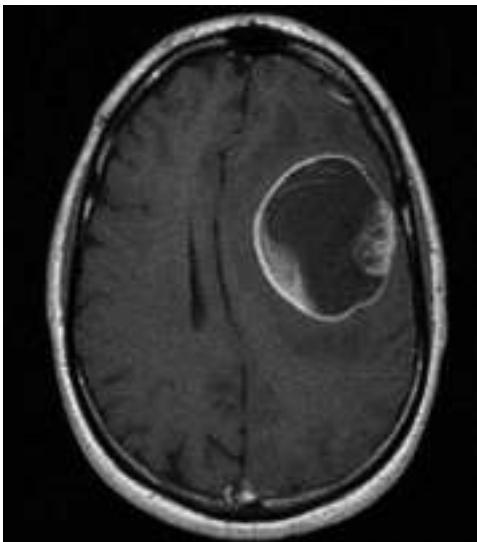
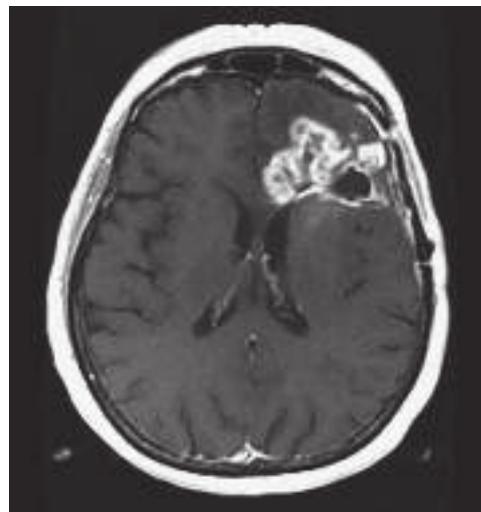
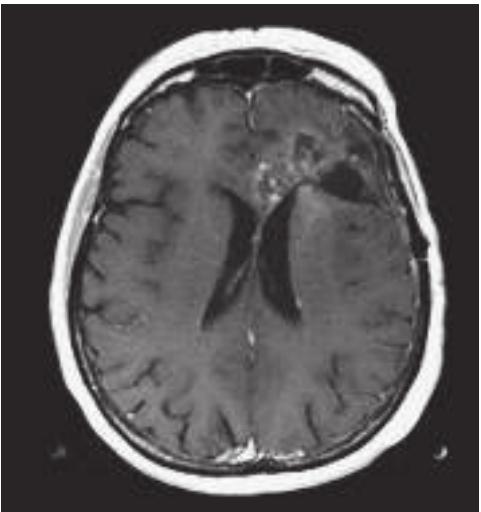


FIGURE 90-2 Postgadolinium T1 MRI of a large cystic left frontal glioblastoma.



A



B

FIGURE 90-3 Postgadolinium T1 MRI of a recurrent glioblastoma before (A) and after (B) administration of bevacizumab. Note the decreased enhancement and mass effect.

undergoing evaluation in patients with glioblastoma include targeted molecular agents directed at receptor tyrosine kinases and signal transduction pathways; immunotherapy using vaccines, novel checkpoint inhibitors, or chimeric antigen receptor (CAR) T cells; oncolytic viruses; antiangiogenic agents; chemotherapeutic agents that cross the blood-brain barrier more effectively than currently available drugs; and infusion of radiolabeled drugs and targeted toxins into the tumor and surrounding brain by means of convection-enhanced delivery.

The most important adverse prognostic factors in patients with glioblastomas are older age, absence of *IDH* mutations, unmethylated MGMT promoter, poor Karnofsky performance status, and unresectable tumor.

Gliosarcomas are a variant of glioblastoma containing both an astrocytic and a sarcomatous component and are treated in the same way as glioblastomas.

■ OLIGODENDROGLIOMA

Oligodendrogiomas account for ~15–20% of gliomas. They are characterized by codeletion of 1p/19q and have *IDH* mutations. Oligodendroglomas are classified by the WHO into oligodendroglomas (grade II) or anaplastic oligodendroglomas (AOs) (grade III). Oligodendroglomas have distinctive pathologic features such as perinuclear clearing—giving rise to a “fried-egg” appearance—and a reticular pattern of blood vessel growth. Some tumors have both an oligodendroglial as well as an astrocytic component. With molecular testing, it is now clear that almost all of these mixed tumors (oligoastrocytomas) are genetically either astrocytomas or oligodendroglomas. As a result, the diagnosis of oligoastrocytoma is now rarely made unless molecular testing is not available.

Grade II oligodendroglomas are generally more responsive to therapy and have a better prognosis than pure astrocytic tumors. These tumors present similarly to grade II astrocytomas in young adults. The tumors are nonenhancing and often partially calcified. They should be treated with surgery and, in patients with residual disease or aged >40 years, RT and chemotherapy. Patients with oligodendroglomas have a median survival in excess of 10 years.

AOs present in the fourth and fifth decades as variably enhancing tumors. They are more responsive to therapy than grade III astrocytomas. Treatment involves maximal safe resection followed by RT and PCV or temozolomide chemotherapy. Median survival of patients with AO is in excess of 10 years.

■ EPENDYMOGRAMS

Ependymomas are tumors derived from ependymal cells that line the ventricular surface. They account for ~5% of childhood tumors, frequently arise from the wall of the fourth ventricle in the posterior fossa, are associated with *RELA* fusions, and are classified as type A and B ependymoma subtypes. Although adults can have intracranial ependymomas, they occur more commonly in the spine, especially in the filum terminale of the spinal cord where they have a myxopapillary histology. Ependymomas that can be completely resected are potentially curable. Partially resected ependymomas will recur and require irradiation. The less common anaplastic ependymoma is more aggressive and is treated with resection and RT; chemotherapy has limited efficacy. Subependymomas are slow-growing benign lesions arising in the wall of ventricles that often do not require treatment.

■ OTHER LESS COMMON GLIOMAS

Gangliogliomas and pleomorphic xanthoastrocytomas occur in young adults. They behave as more indolent forms of grade I gliomas and are usually treated with surgery. Frequently they will have *BRAF V600E* mutations. Brainstem gliomas usually occur in children or young adults and often have *H3K27M* mutations. Despite treatment with RT and chemotherapy, the prognosis is poor, with a median survival of only 1 year.

■ PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

Primary central nervous system lymphoma (PCNSL) is a rare non-Hodgkin's lymphoma accounting for <3% of primary brain tumors. For

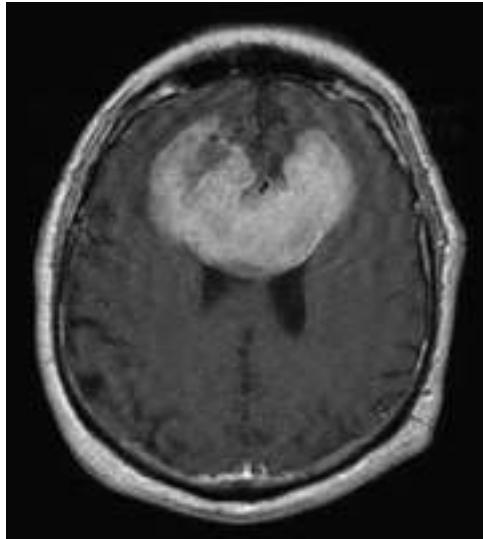


FIGURE 90-4 Postgadolinium T1 MRI demonstrating a large bifrontal primary central nervous system lymphoma (PCNSL). The periventricular location and diffuse enhancement pattern are characteristic of lymphoma.

unclear reasons, its incidence is increasing, particularly in immunocompetent, older individuals.

PCNSL in immunocompetent patients is usually a diffuse large B-cell lymphoma. Immunocompromised patients, especially those infected with the human immunodeficiency virus (HIV) or organ transplant recipients, are at risk for PCNSL that is typically large cell with immunoblastic and more aggressive features. Epstein-Barr virus (EBV) plays an important role in the pathogenesis of PCNSL in this population. These patients are usually severely immunocompromised, with CD4 counts of <50/mL.

Immunocompetent patients with PCNSL are older (median 60 years) than those with HIV-related PCNSL (median 31 years). PCNSL usually presents as a mass lesion, with neuropsychiatric symptoms, lateralizing signs, or seizures. Ocular and leptomeningeal involvement each occur in 15–20% of patients, and involvement of these compartments may be asymptomatic. Rarely, it may present as isolated ocular lymphoma or as primary leptomeningeal lymphoma. When restricted to the leptomeninges, it may present as a subacute or chronic meningitis that causes progressive cranial and spinal nerve dysfunction. CSF cytologic examination or flow cytometry is required to establish the diagnosis.

On contrast-enhanced MRI, PCNSL usually appears as a densely enhancing tumor (Fig. 90-4). Immunocompetent patients have solitary lesions more often than immunosuppressed patients. Frequently there is involvement of the basal ganglia, corpus callosum, or periventricular region. Stereotactic biopsy is necessary to obtain a histologic diagnosis. Whenever possible, glucocorticoids should be withheld until after the biopsy has been obtained because they have a cytolytic effect on lymphoma cells and may lead to nondiagnostic tissue. In addition, patients should be tested for HIV, and the extent of disease should be assessed by performing PET or CT of the body, MRI of the spine, CSF analysis, and slit-lamp examination of the eye. Bone marrow biopsy and testicular ultrasound are occasionally performed.

TREATMENT

Primary Central Nervous System Lymphoma

PCNSL is more sensitive to glucocorticoids, chemotherapy, and RT than other primary brain tumors. Durable complete responses and long-term survival are possible with these treatments. High-dose methotrexate, a folate antagonist that interrupts DNA synthesis, produces response rates ranging from 35 to 80% and median survival

of up to 50 months. The combination of methotrexate with other chemotherapeutic agents such as cytarabine increases the response rate to 70–100%. The addition of whole-brain RT (WBRT) to methotrexate-based chemotherapy prolongs progression-free survival but not overall survival, but it is associated with delayed neurotoxicity, especially in patients aged >60 years. As a result, full-dose RT is frequently omitted, but there may be a role for reduced-dose RT. The anti-CD20 monoclonal antibody rituximab is often incorporated into the chemotherapy regimen, although there are studies questioning its benefit. For some patients, high-dose chemotherapy with autologous stem cell rescue may offer the best chance of preventing relapse. At least 50% of patients will eventually develop recurrent disease. Treatment options include RT for patients who have not had prior irradiation, retreatment with methotrexate, as well as other chemotherapeutic agents such as temozolamide and pemetrexed. High-dose chemotherapy with autologous stem cell rescue may be appropriate in selected patients with relapsed disease. Bruton's tyrosine kinase (BTK) inhibitors such as ibrutinib, immunomodulatory drugs such as pomalidomide and lenalidomide, and immune checkpoint inhibitors have shown promising preliminary activity and are being evaluated in clinical trials, as are CAR T cells.

PCNSL IN IMMUNOCOMPROMISED PATIENTS

PCNSL in immunocompromised patients often produces multiple ring-enhancing lesions that can be difficult to differentiate from metastases or infections such as toxoplasmosis. The diagnosis is usually established by examination of the CSF for cytology and EBV DNA; toxoplasmosis serologic testing; brain PET imaging for hypermetabolism of the lesions, which, although nonspecific, can be consistent with tumor; and, if necessary, brain biopsy. Since the advent of highly active antiretroviral drugs, the incidence of HIV-related PCNSL has declined. These patients are preferably treated with high-dose methotrexate-based regimens and initiation of highly active antiretroviral therapy; WBRT is reserved for those who cannot tolerate systemic chemotherapy. In organ transplant recipients, reduction of immunosuppression may improve outcome.

MEDULLOBLASTOMAS

Medulloblastomas are the most common malignant brain tumor of childhood, accounting for ~20% of all primary CNS tumors among children. They arise from granule cell progenitors or from multipotent progenitors from the ventricular zone. Approximately 5% of children with medulloblastoma have an inherited syndrome, such as Gorlin, Turcot, or Li-Fraumeni, which predisposes to the development of medulloblastoma. Histologically, medulloblastomas are highly cellular tumors with abundant dark staining, round nuclei, and rosette formation (Homer-Wright rosettes). In the 2016 WHO pathologic classification, they have been divided into four molecular subgroups: (1) WNT-activated (primarily affects children and has the best outcome); (2) SHH-activated (affects adults, infants, and children, with the younger patients having the better outcome and adults doing poorly); (3) non-WNT/non-SHH, group 3 (frequently has disseminated CNS disease at diagnosis and has the worst outcome); and (4) non-WNT/non-SHH, group 4 (30% have metastases at diagnosis, but 5-year progression-free survival is 95%). Regardless of subtype, patients present with headache, ataxia, and signs of brainstem involvement. On MRI, they appear as densely enhancing tumors in the posterior fossa, sometimes associated with hydrocephalus. Treatment involves maximal surgical resection, craniospinal irradiation, and chemotherapy with agents such as cisplatin, lomustine, cyclophosphamide, and vincristine. Approximately 70% of patients overall have long-term survival but usually at the cost of significant neurocognitive impairment. A major goal of current research is to improve survival while minimizing long-term complications, and clinical trials are now being designed for specific molecular subgroups.

PINEAL REGION TUMORS

A large variety of tumors can arise in the region of the pineal gland. These typically present with headache, visual symptoms, and hydrocephalus.

Patients may have Parinaud's syndrome characterized by impaired upgaze and accommodation. Some pineal tumors such as pineocytomas and benign teratomas can be treated by surgical resection. Germinomas respond to irradiation, whereas pineoblastomas and nongerminomatous germ cell tumors require craniospinal radiation and chemotherapy.

EXTRINSIC “BENIGN” TUMORS

■ MENINGIOMAS

Meningiomas are diagnosed with increasing frequency as more people undergo neuroimaging for various indications. They are now the most common primary brain tumor, accounting for ~35% of the total. Their incidence increases with age. They tend to be more common in women and in patients with neurofibromatosis type 2 (NF2). They also occur more commonly in patients with a history of cranial irradiation.

Meningiomas arise from the dura mater and are composed of neoplastic meningothelial (arachnoidal cap) cells. They are most commonly located over the cerebral convexities, especially adjacent to the sagittal sinus, but they can also occur in the skull base and along the dorsum of the spinal cord. Meningiomas are classified by the WHO into three histologic grades of increasing aggressiveness: grade I (benign), grade II (atypical), and grade III (malignant).

Many meningiomas are found incidentally following neuroimaging for unrelated reasons. They can also present with headaches, seizures, or focal neurologic deficits. On imaging studies, they have a characteristic appearance usually of a densely enhancing extra-axial tumor arising from the dura (Fig. 90-5). Typically they have a dural tail, consisting of thickened, enhanced dura extending like a tail from the mass. The main differential diagnosis of meningioma is a dural metastasis.

If the meningioma is small and asymptomatic, no intervention is necessary and the lesion can be observed with serial MRI studies. Larger, symptomatic lesions should be resected. If complete resection is achieved, the patient is cured. Incompletely resected tumors tend to recur, although the rate of recurrence can be very slow with grade I tumors. Tumors that cannot be resected, or can only be partially removed, may benefit from external-beam RT or SRS. These treatments may also be helpful in patients whose tumor has recurred after surgery. Hormonal therapy and chemotherapy are currently unproven.

Rarer tumors that resemble meningiomas include hemangiopericytomas and solitary fibrous tumors. Since they share similar molecular alterations (*NAB2-STAT6* fusion), the 2016 WHO classification introduced the combined term *solitary fibrous tumor/hemangiopericytoma* for this entity. These tumors are treated with surgery and RT but have a higher propensity to recur locally or metastasize systemically.

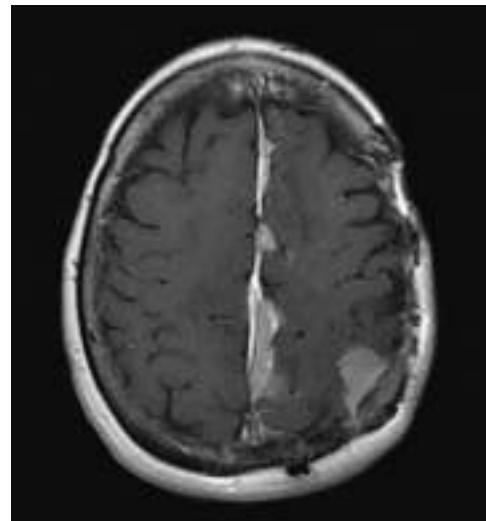


FIGURE 90-5 Postgadolinium T1 MRI demonstrating multiple meningiomas along the falx and left parietal cortex.

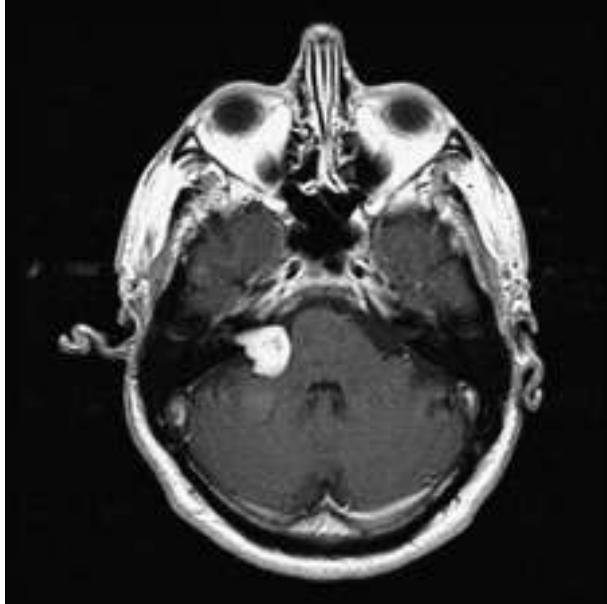


FIGURE 90-6 Postgadolinium MRI of a right vestibular schwannoma. The tumor can be seen to involve the internal auditory canal.

SCHWANNOMAS

These are generally benign tumors arising from the Schwann cells of cranial and spinal nerve roots. The most common schwannomas, termed *vestibular schwannomas* or *acoustic neuromas*, arise from the vestibular portion of the eighth cranial nerve and account for ~9% of primary brain tumors. Patients with NF2 have a high incidence of vestibular schwannomas that are frequently bilateral. Schwannomas arising from other cranial nerves, such as the trigeminal nerve (cranial nerve V), occur with much lower frequency. Neurofibromatosis type 1 (NF1) is associated with an increased incidence of schwannomas of the spinal nerve roots.

Vestibular schwannomas may be found incidentally on neuroimaging or present with progressive unilateral hearing loss, dizziness, tinnitus, or, less commonly, symptoms resulting from compression of the brainstem and cerebellum. On MRI, they appear as densely enhancing lesions, enlarging the internal auditory canal and often extending into the cerebellopontine angle (Fig. 90-6). The differential diagnosis includes meningioma. Very small, asymptomatic lesions can be observed with serial MRIs. Larger lesions should be treated with surgery or SRS. The optimal treatment will depend on the size of the tumor, symptoms, and the patient's preference. In patients with small vestibular schwannomas and relatively intact hearing, early surgical intervention increases the chance of preserving hearing.

PITUITARY TUMORS

These are discussed in detail in [Chap. 380](#).

CRANIOPHARYNGIOMAS

Craniopharyngiomas are rare, usually suprasellar, partially calcified, solid, or mixed solid-cystic benign tumors that arise from remnants of Rathke's pouch. They have a bimodal distribution, occurring predominantly in children but also between the ages of 55 and 65 years. They present with headaches, visual impairment, and impaired growth in children and hypopituitarism in adults. Treatment involves surgery, RT, or a combination of the two. The papillary subtype of craniopharyngiomas often has *BRAF* V600E mutations and can be treated with RAF/MEK inhibitors.

OTHER BENIGN TUMORS

Dysembryoplastic Neuroepithelial Tumors (DNTs) These are benign, supratentorial tumors, usually in the temporal lobe. They

typically occur in children and young adults with a long-standing history of seizures. Surgical resection is curative.

Epidermoid Cysts These consist of squamous epithelium surrounding a keratin-filled cyst. They are usually found in the cerebellopontine angle and the intrasellar and suprasellar regions. They may present with headaches, cranial nerve abnormalities, seizures, or hydrocephalus. MRI demonstrates an extra-axial lesion with characteristics that are similar to CSF but have restricted diffusion. Treatment involves surgical resection.

Dermoid Cysts Like epidermoid cysts, dermoid cysts arise from epithelial cells that are retained during closure of the neural tube. They contain both epidermal and dermal structures such as hair follicles, sweat glands, and sebaceous glands. Unlike epidermoid cysts, these tumors usually have a midline location. They occur most frequently in the posterior fossa, especially the vermis, fourth ventricle, and suprasellar cistern. On MRI, dermoid cysts resemble lipomas, demonstrating T1 hyperintensity and variable signal on T2. Symptomatic dermoid cysts can be treated with surgery.

Colloid Cysts These usually arise in the anterior third ventricle and may present with headaches, hydrocephalus, and, very rarely, sudden death. Surgical resection is curative, or a third ventriculostomy may relieve the obstructive hydrocephalus and be sufficient therapy.

NEUROCUTANEOUS SYNDROMES PHAKOMATOSES

A number of genetic disorders are characterized by cutaneous lesions and an increased risk of brain tumors. Most of these disorders have an autosomal dominant inheritance with variable penetrance.

■ NEUROFIBROMATOSIS TYPE 1 von RECKLINGHAUSEN'S DISEASE

NF1 is an autosomal dominant disorder with variable penetrance and an incidence of ~1 in 2600–3000. Approximately one-half of cases are familial; the remainder are caused by new mutations arising in patients with unaffected parents. The *NFI* gene is located on chromosome 17q11.2 and encodes neurofibromin, a guanosine triphosphatase (GTPase) activating protein (GAP) that is a negative regulator of the RAS–mitogen-activated protein (MAP) kinase signaling pathway, which includes the downstream kinase MEK. It is a classic tumor suppressor, and biallelic loss can result in a variety of nervous system tumors including neurofibromas, plexiform neurofibromas, optic nerve gliomas, astrocytomas, and meningiomas. In addition to neurofibromas, which appear as multiple, soft, rubbery cutaneous tumors, other cutaneous manifestations of NF1 include café-au-lait spots and axillary freckling. NF1 is also associated with hamartomas of the iris termed Lisch nodules, pheochromocytomas, pseudoarthrosis of the tibia, scoliosis, epilepsy, and mental retardation. The MEK inhibitor selumetinib has activity against inoperable plexiform neurofibromas and is the only treatment that targets the dysregulated signaling pathway.

■ NEUROFIBROMATOSIS TYPE 2

NF2 is less common than NF1, with an incidence of 1 in 25,000–40,000. It is an autosomal dominant disorder with full penetrance. As with NF1, approximately one-half of cases arise from new mutations. The *NF2* gene on 22q encodes a cytoskeletal protein, merlin (moesin, ezrin, radixin-like protein), that functions as a tumor suppressor. NF2 is characterized by bilateral vestibular schwannomas in >90% of patients, multiple meningiomas, and spinal ependymomas and astrocytomas. Treatment of bilateral vestibular schwannomas can be challenging because the goal is to preserve hearing for as long as possible. These patients may also have diffuse schwannomatosis that may affect the cranial, spinal, or peripheral nerves; posterior subcapsular lens opacities; and retinal hamartomas.

■ TUBEROUS SCLEROSIS BOURNEVILLE DISEASE

This is an autosomal dominant disorder with an incidence of ~1 in 5000–10,000 live births. It is caused by mutations in either the *TSC1*

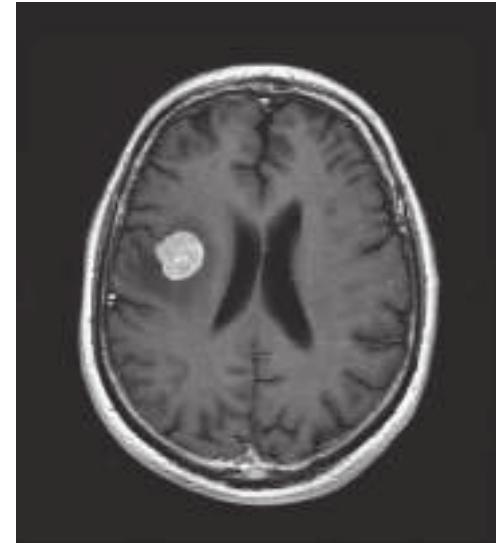
gene, which maps to chromosome 9q34 and encodes a protein termed hamartin, or the *TSC2* gene, which maps to chromosome 16p13.3 and encodes the protein tuberin. Hamartin forms a complex with tuberin, which inhibits cellular signaling through mTOR, and acts as a negative regulator of the cell cycle. Patients with tuberous sclerosis may have seizures, mental retardation, adenoma sebaceum (facial angiofibromas), shagreen patch, hypomelanotic macules, periungual fibromas, renal angiomyolipomas, and cardiac rhabdomyomas. These patients have an increased incidence of subependymal nodules, cortical tubers, and subependymal giant cell astrocytomas (SEGAs). Patients frequently require anticonvulsants for seizures. SEGAs do not always require therapeutic intervention, but the most effective therapy is with the mTOR inhibitors sirolimus or everolimus, which often decrease seizures as well as SEGA size.

TUMORS METASTATIC TO THE BRAIN

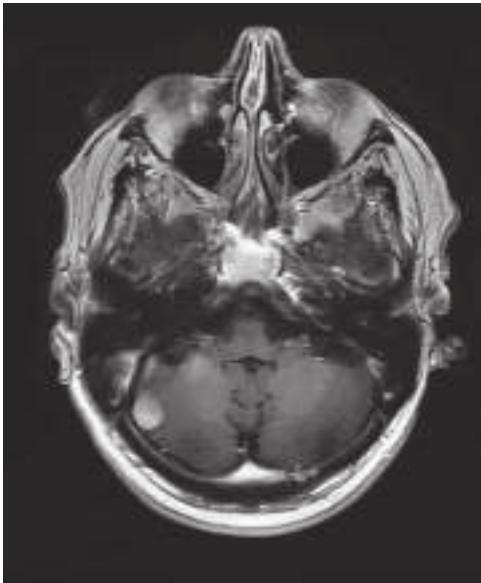
Brain metastases arise from hematogenous spread and frequently originate from a lung primary or are associated with pulmonary metastases. Most metastases develop at the gray matter–white matter junction in the watershed distribution of the brain where intravascular tumor cells lodge in terminal arterioles. The distribution of metastases in the brain approximates the proportion of blood flow such that ~85% of all metastases are supratentorial and 15% occur in the posterior fossa. The most common sources of brain metastases are lung and breast carcinomas; melanoma has the greatest propensity to metastasize to the brain, being found in 80% of patients at autopsy (Table 90-3). Other tumor types such as ovarian and esophageal carcinoma rarely metastasize to the brain. Prostate and breast cancers also have a propensity to metastasize to the dura and can mimic meningioma. Leptomeningeal metastases are common from hematologic malignancies and also breast and lung cancers. Spinal cord compression primarily arises in patients with prostate and breast cancer, tumors with a strong propensity to metastasize to the axial skeleton.

■ DIAGNOSIS OF METASTASES

Brain metastases are best visualized on MRI, where they usually appear as well-circumscribed lesions (Fig. 90-7). The amount of perilesional edema can be highly variable, with large lesions causing minimal edema and sometimes very small lesions causing extensive edema. Enhancement may be in a ring pattern or diffuse. Occasionally, intracranial metastases will hemorrhage; melanoma, thyroid, and kidney cancer have the greatest propensity to hemorrhage, but the most common cause of a hemorrhagic metastasis is lung cancer because it accounts for the majority of brain metastases. The radiographic appearance of brain metastasis is nonspecific, and similar-appearing lesions can occur with infection including brain abscesses, demyelinating lesions, sarcoidosis, radiation necrosis in a previously treated patient, or a primary brain tumor that may be a second malignancy in a patient with systemic cancer. Biopsy is rarely necessary for diagnosis because imaging alone in the appropriate clinical situation usually suffices. However, in ~10%



A



B

FIGURE 90-7 Postgadolinium T1 MRI of multiple brain metastases from non-small-cell lung cancer involving the right frontal (A) and right cerebellar (B) hemispheres. Note the diffuse enhancement pattern and absence of central necrosis.

TABLE 90-3 Frequency of Nervous System Metastases by Common Primary Tumors

	BRAIN (%)	LM (%)	ESCC (%)
Lung	41	17	15
Breast	19	57	22
Melanoma	10	12	4
Prostate	1	1	10
GIT	7	—	5
Renal	3	2	7
Lymphoma	<1	10	10
Sarcoma	7	1	9
Other	11	—	18

Abbreviations: ESCC, epidural spinal cord compression; GIT, gastrointestinal tract; LM, leptomeningeal metastases.

of patients, a systemic cancer may present with a brain metastasis, and if there is not an easily accessible systemic site to biopsy, a brain lesion must be removed for diagnostic purposes.

TREATMENT

Tumors Metastatic to the Brain

DEFINITIVE TREATMENT

The number and location of brain metastases often determine the therapeutic options. The patient's overall condition and current or potential control of systemic disease are also major determinants. Brain metastases are single in approximately one-half of patients and multiple in the other half.

RADIATION THERAPY

The standard treatment for brain metastases has previously been WBRT usually administered to a total dose of 3000 cGy in 10 fractions. This affords rapid palliation, and ~80% of patients improve with glucocorticoids and RT. However, it is not curative, is associated with neurocognitive toxicity, and produces median survival of only 4–6 months. Recent data demonstrate that hippocampal avoidance during WBRT preserves cognitive function without increasing the risk of an intracranial relapse. If feasible, SRS has become the primary radiation oncology approach to brain metastases. It can be delivered through a variety of equally effective techniques including the gamma knife, linear accelerator, proton beam, or CyberKnife, all of which can deliver highly focused doses of RT, usually in a single fraction. SRS can effectively sterilize the visible lesions and afford local disease control in 80–90% of patients. Some patients have been cured of their brain metastases using SRS, whereas this is distinctly rare with WBRT. Traditionally SRS was used only for patients with 1–3 metastases, but recent data suggest that SRS can effectively treat up to 10 lesions. It is, however, confined to lesions of ≤ 3 cm and is most effective in metastases of ≤ 1 cm. The addition of WBRT to SRS improves disease control in the nervous system but does not prolong survival and thus is rarely employed.

SURGERY

Randomized controlled trials have demonstrated that surgical extirpation of a single brain metastasis followed by WBRT is superior to WBRT alone. Removal of two lesions or a single symptomatic mass, particularly if compressing the ventricular system, can also be useful. This is particularly important in patients who have highly radioresistant lesions such as renal carcinoma. Surgical resection can produce rapid amelioration of symptoms, improve control of edema, and result in prolonged survival. WBRT administered after complete resection of a brain metastasis improves disease control but does not prolong survival. Some centers administer focal RT or even SRS to a resected cavity, especially if there is concern that tumor has been left behind, but most avoid postoperative WBRT because of its cognitive effects.

CHEMOTHERAPY

Chemotherapy is becoming increasingly useful for brain metastases. Metastases from tumor types that are highly chemosensitive, such as germ cell tumors or small-cell lung cancer, may respond to chemotherapeutic regimens chosen according to the underlying malignancy. Increasingly, data demonstrate responsiveness of brain metastases to chemotherapy including targeted therapeutics, such as for patients with lung cancer harboring *EGFR* mutations that sensitize them to EGFR inhibitors. Immunotherapy is also effective against those primary tumors that are sensitive to this approach, such as melanoma. Antiangiogenic agents such as bevacizumab are effective in the treatment of CNS metastases in those primary tumors for which it is approved.

LEPTOMENINGEAL METASTASES

Leptomeningeal metastases are also described as carcinomatous meningitis, meningeal carcinomatosis, or, in the case of specific tumors, leukemic or lymphomatous meningitis. Among the hematologic malignancies, acute leukemias most commonly metastasize to the subarachnoid space, followed in frequency by aggressive diffuse lymphomas. Among solid tumors, breast and lung carcinomas and melanoma most frequently spread in this fashion. Tumor cells reach the subarachnoid space via the arterial circulation or occasionally through retrograde flow in venous systems that drain metastases along the bony spine or cranium. In addition, leptomeningeal metastases may develop as a direct consequence of prior brain metastases and occur in almost 40% of patients who have a metastasis resected from the cerebellum.

CLINICAL FEATURES

Leptomeningeal metastases are characterized by multilevel symptoms and signs along the neuraxis. Combinations of lumbar and cervical

radiculopathies, cranial neuropathies, seizures, confusion, and encephalopathy from hydrocephalus or raised intracranial pressure may be present. Focal deficits such as hemiparesis or aphasia are rarely due to leptomeningeal metastases unless there is direct brain infiltration. New-onset limb pain in patients with breast cancer, lung cancer, or melanoma should prompt consideration of leptomeningeal spread.

LABORATORY AND IMAGING DIAGNOSIS

Leptomeningeal metastases are particularly challenging to diagnose because identification of tumor cells in the subarachnoid compartment may be elusive. MRI can be definitive when there are clear tumor nodules adherent to the cauda equina or spinal cord, enhancing cranial nerves, or subarachnoid enhancement on brain imaging (Fig. 90-8).



FIGURE 90-8 Postgadolinium MRI images of extensive leptomeningeal metastases from breast cancer. Nodules along the dorsal surface of the spinal cord (A) and cauda equina (B) are seen.

Imaging is diagnostic in ~75% of patients and is more often positive in patients with solid tumors. Demonstration of tumor cells in the CSF is definitive and often considered the gold standard. However, CSF cytologic examination is positive in only 50% of patients on the first lumbar puncture and still misses 10% after three CSF samples. New technologies, such as rare cell capture, enhance identification of tumor cells in the CSF; molecular profiling of the CSF can also identify tumor-specific mutations, indicating malignancy in the leptomeninges. CSF cytologic examination is most useful in hematologic malignancies, especially when combined with flow cytometry to identify a clonal population. Accompanying CSF abnormalities include an elevated protein concentration and an elevated white blood cell count; hypoglycorrachia is noted in <25% of patients but is useful when present. Identification of tumor markers may be helpful in some solid tumors.

TREATMENT

Leptomeningeal Metastases

The treatment of leptomeningeal metastasis is palliative because there is no curative therapy. RT to the symptomatically involved areas, such as skull base for cranial neuropathy, can relieve pain and sometimes improve function. Craniospinal irradiation (CSI) is avoided because it has significant toxicity with myelosuppression and gastrointestinal irritation as well as limited effectiveness. However, recent data on proton beam CSI suggest better disease control with fewer systemic toxicities. Systemic chemotherapy, targeted therapeutics, and immunotherapy have all demonstrated efficacy in the appropriate setting. Alternatively, intrathecal chemotherapy can be effective, particularly in hematologic malignancies. This is optimally delivered through an intraventricular cannula (Ommaya reservoir) rather than by lumbar puncture. Few drugs can be delivered safely into the subarachnoid space, and they have a limited spectrum of antitumor activity, perhaps accounting for the relatively poor response to this approach, particularly in solid tumors. In addition, impaired CSF flow dynamics can compromise intrathecal drug delivery. Surgery has a limited role in leptomeningeal metastasis. A ventriculoperitoneal shunt can relieve raised intracranial pressure; once placed, intrathecal drug cannot be used.

EPIDURAL METASTASIS

Epidural metastasis occurs in 3–5% of patients with a systemic malignancy and causes neurologic compromise by compressing the spinal cord or cauda equina. The most common cancers that metastasize to the epidural space are those malignancies that spread to bone, such as breast and prostate. Lymphoma can cause bone involvement and compression, but it can also invade an intervertebral foramen and cause spinal cord compression without bone destruction. The thoracic spine is affected most commonly, followed by the lumbar and then cervical spine.

CLINICAL FEATURES

Back pain is the presenting symptom of epidural metastasis in virtually all patients; the pain may precede neurologic findings by weeks or months. The pain is usually exacerbated by lying down; by contrast, arthritic pain is often relieved by recumbency. Leg weakness is seen in ~50% of patients, as is sensory dysfunction. Sphincter problems are present in ~25% of patients at diagnosis.

DIAGNOSIS

Diagnosis is established by imaging, preferably with an MRI of the entire spine (Fig. 90-9). Contrast is not required to identify bony or epidural lesions. Any patient with cancer who has severe back pain should undergo an MRI. Plain films, bone scans, or even CT scans may show bone metastases, but only MRI can reliably delineate epidural tumor. For patients unable to have an MRI, CT myelography should be performed to outline the epidural space. The differential diagnosis of epidural tumor includes epidural abscess, acute or chronic hematomas, epidural lipomatosis, and, rarely, extramedullary hematopoiesis.



FIGURE 90-9 Postgadolinium T1 MRI showing circumferential epidural tumor around the thoracic spinal cord from esophageal cancer.

TREATMENT

Epidural Metastasis

Epidural metastasis requires immediate treatment. A randomized controlled trial demonstrated the superiority of surgical resection followed by RT compared to RT alone. However, patients must be able to tolerate surgery, and the surgical procedure of choice is a complete removal of the mass, which is typically anterior to the spinal canal, necessitating an extensive approach and resection. Otherwise, RT is the mainstay of treatment and can be used for patients with radiosensitive tumors, such as lymphoma, or for those unable to undergo surgery. SRS is increasingly being used, especially for radioresistant tumor types or for re-irradiation. Chemotherapy is rarely used for epidural metastasis unless the patient has minimal to no neurologic deficit and a highly chemosensitive tumor such as lymphoma or germinoma. Patients generally fare well if treated before there is a severe neurologic deficit. Recovery from paraparesis is better after surgery than with RT alone, but survival is often short due to widespread metastatic tumor.

NEUROLOGIC TOXICITY OF THERAPY

TOXICITY FROM RADIOTHERAPY

RT can cause a variety of toxicities in the CNS. These are usually described based on their relationship in time to the administration of RT: acute (occurring within days of RT), early delayed (months), or late delayed (years). In general, the acute and early delayed syndromes resolve and do not result in persistent deficits, whereas the late delayed toxicities are usually permanent and sometimes progressive.

Acute Toxicity Acute cerebral toxicity may occur during RT to the brain. RT can cause a transient disruption of the blood-brain barrier, resulting in edema and elevated intracranial pressure. This is usually manifest as headache, lethargy, nausea, and vomiting and can be both prevented and treated with the administration of glucocorticoids. There is no acute RT toxicity that affects the spinal cord.

Early Delayed Toxicity Early delayed toxicity is usually apparent weeks to months after completion of cranial irradiation and is likely due to focal demyelination. Clinically it may be asymptomatic or take the form of worsening or reappearance of a preexisting neurologic deficit. At times, a contrast-enhancing lesion can be seen on MRI/CT

that can mimic the tumor for which the patient received the RT. For patients with a malignant glioma, this has been described as “pseudoprogression” because it mimics tumor recurrence on MRI, but it represents inflammation and necrotic debris engendered by effective therapy. This is seen with increased frequency when chemotherapy, particularly temozolomide, is given concurrently with RT. Pseudoprogression can resolve on its own or, if very symptomatic, may require glucocorticoids, resection, or bevacizumab.

In the spinal cord, early delayed RT toxicity is manifest as a Lhermitte symptom with paresthesias of the limbs or along the spine when the patient flexes the neck. Although frightening, it is benign, resolves on its own, and does not portend more serious problems.

Late Delayed Toxicity Late delayed toxicities are the most serious because they are often irreversible and cause severe neurologic deficits. In the brain, late toxicities can take several forms, the most common of which include radiation necrosis and leukoencephalopathy. Radiation necrosis is a focal mass of necrotic tissue that is contrast enhancing on CT/MRI and may be associated with significant edema. This may appear identical to pseudoprogression but is seen months to years after RT and is always symptomatic. Clinical symptoms and signs include seizures and findings referable to the location of the necrotic mass. The necrosis is caused by the effect of RT on cerebral vasculature with fibrinoid necrosis and occlusion of blood vessels. It can mimic tumor radiographically, but unlike tumor, it is typically hypometabolic on a PET scan and has reduced perfusion on perfusion MR sequences. It may require resection for diagnosis and treatment unless it can be managed with glucocorticoids. There are reports of improvement with hyperbaric oxygen or bevacizumab, but symptomatic benefit does not always accompany radiographic improvement.

Leukoencephalopathy is seen most commonly after WBRT as opposed to focal RT. On T2 or FLAIR MR sequences, there is diffusely increased signal seen throughout the hemispheric white matter, often bilaterally and symmetrically. There tends to be a periventricular predominance that may be associated with atrophy and ventricular enlargement. Clinically, patients develop cognitive impairment, a gait disorder, and later urinary incontinence, all of which can progress over time. These symptoms mimic those of normal pressure hydrocephalus, and placement of a ventriculoperitoneal shunt can improve function in some patients but does not reverse the deficits completely. Increased age is a risk factor for leukoencephalopathy but not for radiation necrosis. Necrosis appears to depend on an unidentified predisposition.

Other late neurologic toxicities include endocrine dysfunction if the pituitary or hypothalamus was included in the RT port. An RT-induced neoplasm can occur many years after therapeutic RT for either a prior CNS or a head and neck tumor; accurate diagnosis requires surgical resection or biopsy. In addition, RT causes accelerated atherosclerosis, which can cause stroke either from intracranial vascular disease or carotid plaque from neck irradiation.

The peripheral nervous system is relatively resistant to RT toxicities. Peripheral nerves are rarely affected by RT, but the plexus is more vulnerable. Plexopathy develops more commonly in the brachial than in the lumbosacral distribution. It must be differentiated from tumor progression in the plexus, which is usually visualized by CT/MRI or PET scan demonstrating tumor infiltrating the region. Clinically, tumor progression is usually painful, whereas RT-induced plexopathy is painless. Radiation plexopathy is also more commonly associated with lymphedema and myokymia of the affected limb. Sensory loss and weakness are seen in both.

■ TOXICITY FROM CHEMOTHERAPY

Neurotoxicity is second to myelosuppression as the dose-limiting toxicity of chemotherapeutic agents (Table 90-4). Chemotherapy causes peripheral neuropathy from many commonly used agents, and the type of neuropathy can vary depending on the drug. Vincristine causes paresthesias but little sensory loss and is associated with motor dysfunction, autonomic impairment (frequently ileus), and, rarely, cranial nerve compromise. Cisplatin causes large-fiber sensory loss resulting in sensory ataxia but little cutaneous sensory loss and no weakness.

TABLE 90-4 Neurologic Signs Caused by Agents Commonly Used in Patients with Cancer

Acute encephalopathy (delirium)	Seizures
Methotrexate (high-dose IV, IT)	Methotrexate
Cisplatin	Etoposide (high-dose)
Vincristine	Cisplatin
Asparaginase	Vincristine
Procarbazine	Asparaginase
5-Fluorouracil (\pm levamisole)	Nitrogen mustard
Cytarabine (high-dose)	Carmustine
Nitrosoureas (high-dose or arterial)	Dacarbazine (intraarterial or high-dose)
Ifosfamide	Busulfan (high-dose)
Etoposide (high-dose)	Myelopathy (IT drugs)
Bevacizumab (PRES)	Methotrexate
Chronic encephalopathy (dementia)	Cytarabine
Methotrexate	Thiotepa
Carmustine	Peripheral neuropathy
Cytarabine	Vinca alkaloids
Fludarabine	Cisplatin
Visual loss	Procarbazine
Tamoxifen	Etoposide
Gallium nitrate	Teniposide
Cisplatin	Cytarabine
Fludarabine	Taxanes
Cerebellar dysfunction/ataxia	Suramin
5-Fluorouracil (\pm levamisole)	Bortezomib
Cytarabine	
Procarbazine	

Abbreviations: IT, intrathecal; IV, intravenous; PRES, posterior reversible encephalopathy syndrome.

The taxanes also cause a predominantly sensory neuropathy. Agents such as bortezomib and thalidomide also cause neuropathy. Sometimes a severe neuropathy emerges after multiple neurotoxic agents have been used together or in sequence.

Encephalopathy and seizures are common toxicities from chemotherapeutic drugs. Ifosfamide can cause a severe encephalopathy, which is reversible with discontinuation of the drug and the use of methylene blue for severely affected patients. Fludarabine also causes a severe global encephalopathy that may be permanent. Bevacizumab and other anti-VEGF agents can cause posterior reversible encephalopathy syndrome. Cisplatin can cause hearing loss and less frequently vestibular dysfunction. Immunotherapy with monoclonal antibodies such as ipilimumab or nivolumab can cause an autoimmune hypophysitis, Guillain-Barré syndrome, or an autoimmune encephalitis.

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100,000 population, but the incidence varies with age. Soft tissue sarcomas constitute 0.7% of all cancers in the general population and 6.5% of all cancers in children.

■ EPIDEMIOLOGY

Malignant transformation of a benign soft tissue tumor is extremely rare, with the exception that malignant peripheral nerve sheath tumors (neurofibrosarcoma, malignant schwannoma) can arise from neurofibromas in patients with neurofibromatosis. Several etiologic factors have been implicated in soft tissue sarcomas.

Environmental Factors Trauma or previous injury is rarely involved, but sarcomas can arise in scar tissue resulting from a prior operation, burn, fracture, or foreign body implantation. Chemical carcinogens such as polycyclic hydrocarbons, asbestos, and dioxin may be involved in the pathogenesis.

Iatrogenic Factors Sarcomas in bone or soft tissues occur in patients who are treated with radiation therapy. The tumor nearly always arises in the irradiated field. The risk increases with time.

Viruses Kaposi's sarcoma (KS) in patients with HIV type 1, classic KS, and KS in HIV-negative homosexual men is caused by human herpesvirus (HHV) 8 (Chap. 195). No other sarcomas are associated with viruses.

Immunologic Factors Congenital or acquired immunodeficiency, including therapeutic immunosuppression, increases the risk of sarcoma.

■ GENETIC CONSIDERATIONS

 Li-Fraumeni syndrome is a familial cancer syndrome in which affected individuals have germline abnormalities of the tumor-suppressor gene *p53* and an increased incidence of soft tissue sarcomas and other malignancies, including breast cancer, osteosarcoma, brain tumor, leukemia, and adrenal carcinoma (Chap. 71). Neurofibromatosis 1 (NF-1, peripheral form, von Recklinghausen's disease) is characterized by multiple neurofibromas and café-au-lait spots. Neurofibromas occasionally undergo malignant degeneration to become malignant peripheral nerve sheath tumors. The gene for *NFI* is located in the pericentromeric region of chromosome 17 and encodes neurofibromin, a tumor-suppressor protein with guanosine 5'-triphosphate (GTP)ase-activating activity that inhibits ras function (Chap. 90). Germline mutation of the *RBL* locus (chromosome 13q14) in patients with inherited retinoblastoma is associated with the development of osteosarcoma in those who survive the retinoblastoma and of soft tissue sarcomas unrelated to radiation therapy. Other soft tissue tumors, including desmoid tumors, lipomas, leiomyomas, neuroblastomas, and paragangliomas, occasionally show a familial predisposition.

Ninety percent of synovial sarcomas contain a characteristic chromosomal translocation t(X;18)(p11;q11) involving a nuclear transcription factor on chromosome 18 called *SYT* and two breakpoints on X. Patients with translocations to the second X breakpoint (*SSX2*) may have longer survival than those with translocations involving *SSX1*.

Insulin-like growth factor (IGF) type II is produced by some sarcomas and may act as an autocrine growth factor and as a motility factor that promotes metastatic spread. IGF-II stimulates growth through IGF-I receptors, but its effects on motility are through different receptors. If secreted in large amounts, IGF-II may produce hypoglycemia (Chaps. 93 and 406). A large international sarcoma kindred study including 1162 patients and 6545 Caucasian controls revealed that about half the patients with sarcoma have putatively pathogenic mono- and polygenic variation in previously reported and new cancer genes, some of them representing therapeutically actionable targets. These patients were diagnosed with sarcoma at an earlier age compared to controls.

■ CLASSIFICATION

Approximately 20 different groups of sarcomas are recognized on the basis of the pattern of differentiation toward normal tissue. For example, rhabdomyosarcoma shows evidence of skeletal muscle fibers

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Soft Tissue and Bone Sarcomas and Bone Metastases

Shreyaskumar R. Patel



Sarcomas are rare (<1% of all malignancies) mesenchymal neoplasms that arise in bone and soft tissues. These tumors are usually of mesodermal origin, although a few are derived from neuroectoderm, and they are biologically distinct from the more common epithelial malignancies. Sarcomas affect all age groups; 15% are found in children <15 years of age, and 40% occur after age 55 years. Sarcomas are one of the most common solid tumors of childhood and are the fifth most common cause of cancer deaths in children. Sarcomas may be divided into two groups, those derived from bone and those derived from soft tissues.

SOFT TISSUE SARCOMAS

Soft tissues include muscles, tendons, fat, fibrous tissue, synovial tissue, vessels, and nerves. Approximately 60% of soft tissue sarcomas arise in the extremities, with the lower extremities involved three times as often as the upper extremities. Thirty percent arise in the trunk, with the retroperitoneum accounting for 40% of all trunk lesions. The remaining 10% arise in the head and neck.

■ INCIDENCE

Approximately 13,130 new cases of soft tissue sarcomas occurred in the United States in 2020. The annual age-adjusted incidence is 3 per

with cross-striations; leiomyosarcomas contain interlacing fascicles of spindle cells resembling smooth muscle; and liposarcomas contain adipocytes. When precise characterization of the group is not possible, the tumors are called *unclassified sarcomas*. All of the primary bone sarcomas can also arise from soft tissues (e.g., extraskeletal osteosarcoma). The entity *malignant fibrous histiocytoma* (MFH) includes many tumors previously classified as fibrosarcomas or as pleomorphic variants of other sarcomas and is characterized by a mixture of spindle (fibrous) cells and round (histiocytic) cells arranged in a storiform pattern with frequent giant cells and areas of pleomorphism. As immunohistochemical suggestion of differentiation, particularly myogenic differentiation, may be found in a significant fraction of these patients, many are now characterized as poorly differentiated leiomyosarcomas, and the terms *undifferentiated pleomorphic sarcoma* (UPS) and *myxofibrosarcoma* are replacing MFH and myxoid MFH.

For purposes of treatment, most soft tissue sarcomas can be considered together. However, some specific tumors have distinct features. For example, *liposarcoma* can have a spectrum of behaviors. Pleomorphic liposarcomas and dedifferentiated liposarcomas behave like other high-grade sarcomas; in contrast, well-differentiated liposarcomas (better termed *atypical lipomatous tumors*) lack metastatic potential, and myxoid liposarcomas metastasize infrequently, but, when they do, they have a predilection for unusual metastatic sites containing fat, such as the retroperitoneum, mediastinum, and subcutaneous tissue. Rhabdomyosarcomas, Ewing's sarcoma, and other small-cell sarcomas tend to be more aggressive and are more responsive to chemotherapy than other soft tissue sarcomas.

Gastrointestinal stromal tumors (GISTs), previously classified as gastrointestinal leiomyosarcomas, are now recognized as a distinct entity within soft tissue sarcomas. Its cell of origin resembles the interstitial cell of Cajal, which controls peristalsis. The majority of malignant GISTs have activating mutations of the *c-kit* gene that result in ligand-independent phosphorylation and activation of the KIT receptor tyrosine kinase, leading to tumorigenesis. Approximately 5–10% of tumors will have a mutation in the platelet-derived growth factor receptor α (*PDGFRA*). GISTs that are wild type for both *KIT* and *PDGFRA* mutations may show mutations in *SDH B, C, or D* and may be driven by the IGF-I pathway.

■ DIAGNOSIS

The most common presentation is an asymptomatic mass. Mechanical symptoms referable to pressure, traction, or entrapment of nerves or muscles may be present. All new and persistent or growing masses should be biopsied, either by a small incision or by a cutting needle (core-needle biopsy) placed so that it can be encompassed in the subsequent excision without compromising a definitive resection. Lymph node metastases occur in 5%, except in synovial and epithelioid sarcomas, clear-cell sarcoma (melanoma of the soft parts), angiosarcoma, and rhabdomyosarcoma, where nodal spread may be seen in 17%. The pulmonary parenchyma is the most common site of metastases. Exceptions are GISTs, which metastasize to the liver; myxoid liposarcomas, which seek fatty tissue; and clear cell sarcomas, which may metastasize to bones. Central nervous system metastases are rare, except in alveolar soft part sarcoma.

Radiographic Evaluation Imaging of the primary tumor is best with plain radiographs and magnetic resonance imaging (MRI) for tumors of the extremities or head and neck and by computed tomography (CT) for tumors of the chest, abdomen, or retroperitoneal cavity. A radiograph and CT scan of the chest are important for the detection of lung metastases. Other imaging studies may be indicated, depending on the symptoms, signs, or histology.

■ STAGING AND PROGNOSIS

The histologic grade and size of the primary tumor are the most important prognostic factors. The current American Joint Committee on Cancer (AJCC) staging system is shown in Table 91-1. Prognosis is related to the stage. Cure is common in the absence of metastatic disease, but a small number of patients with metastases can also be cured. Historically, most patients with stage IV disease used to die within 12 months, but

TABLE 91-1 American Joint Commission on Cancer Staging System for Sarcomas, Eighth Edition

T1	Tumor ≤ 5 cm in greatest dimension
T2	Tumor > 5 cm and ≤ 10 cm in greatest dimension
T3	Tumor > 10 cm and ≤ 15 cm in greatest dimension
T4	Tumor > 15 cm in greatest dimension
N0	No regional lymph node metastasis or unknown lymph node status
N1	Regional lymph node metastasis
M0	No distant metastasis
M1	Distant metastasis
Stage Groups	
Stage IA	T1; N0; M0; G1
Stage IB	T2, T3, T4; N0; M0; G1
Stage II	T1; N0; M0; G2/3
Stage IIIA	T1A, T2; N0; M0; G2/3
Stage IIIB	T3, T4; N0; M0; G2/3
Stage IV	Any T; N1; M0; any G Any T; any N; M1; any G

with availability of multiple lines of treatments, median survival in second-line and beyond ranges from 13 to 14 months, and some patients may live with stable or slowly progressive disease for many years.

TREATMENT

Soft Tissue Sarcomas

AJCC stage I patients are adequately treated with surgery alone. Stage II patients are considered for adjuvant radiation therapy. Stage III patients may benefit from neoadjuvant or adjuvant chemotherapy. Stage IV patients are managed primarily with systemic therapy, with or without other modalities.

SURGERY

Soft tissue sarcomas tend to grow along fascial planes, with the surrounding soft tissues compressed to form a pseudocapsule that gives the sarcoma the appearance of a well-encapsulated lesion. This is invariably deceptive because “shelling out,” or marginal excision, of such lesions results in a 50–90% probability of local recurrence. Wide excision with a negative margin, incorporating the biopsy site, is the standard surgical procedure for local disease. The adjuvant use of radiation therapy and/or chemotherapy improves the local control rate and permits the use of limb-sparing surgery with a local control rate (85–90%) comparable to that achieved by radical excisions and amputations. Limb-sparing approaches are indicated except when negative margins are not obtainable, when the risks of radiation are prohibitive, or when neurovascular structures are involved so that resection will result in serious functional consequences to the limb.

RADIATION THERAPY

External-beam radiation therapy is an adjuvant to limb-sparing surgery for improved local control. Preoperative radiation therapy allows the use of smaller fields and smaller doses but results in a higher rate of wound complications. Postoperative radiation therapy must be given to larger fields, because the entire surgical bed must be encompassed, and in higher doses to compensate for hypoxia in the operated field. This results in a higher rate of late complications. Brachytherapy or interstitial therapy, in which the radiation source is inserted into the tumor bed, is comparable in efficacy (except in low-grade lesions), less time consuming, and less expensive.

With the advent of stereotactic body radiotherapy (SBRT), the role of radiation therapy in oligometastatic disease in various visceral sites is being investigated and evolving.

ADJUVANT CHEMOTHERAPY

Chemotherapy is the mainstay of treatment for Ewing's sarcomas/primitive neuroectodermal tumors (PNETs) and rhabdomyosarcomas. Meta-analysis of 14 randomized trials in non-small-cell sarcomas revealed a significant improvement in local control and disease-free survival in favor of doxorubicin-based chemotherapy. Overall survival improvement was 4% for all sites and 7% for the extremity site. An updated meta-analysis including four additional trials with doxorubicin and ifosfamide combination reported a statistically significant 6% survival advantage in favor of chemotherapy. A chemotherapy regimen including an anthracycline and ifosfamide with growth factor support improved overall survival by 19% for high-risk (high-grade, ≥ 5 cm primary, or locally recurrent) extremity soft tissue sarcomas. Long-term follow-up of a trial evaluating neoadjuvant use of the same combination confirms survival advantage and reports a 10-year survival of 61%. A more contemporary randomized trial compared the standard anthracycline and ifosfamide combination to specific histology-tailored chemotherapy as an active control and confirmed superiority of the standard regimen.

ADVANCED DISEASE

Metastatic soft tissue sarcomas are largely incurable, but up to 20% of patients who achieve a complete response become long-term survivors. The therapeutic intent, therefore, is to produce a complete remission with chemotherapy (<10%) and/or surgery (30–40%). Surgical resection of metastases, whenever possible, is an integral part of the management. Some patients benefit from repeated surgical excision of metastases. The two most active chemotherapeutic agents are doxorubicin and ifosfamide. These drugs show a steep dose-response relationship in sarcomas. Gemcitabine with or without docetaxel has become an established second-line regimen and is particularly active in patients with UPS and leiomyosarcomas. Dacarbazine also has some modest activity. Taxanes have selective activity in angiosarcomas, and vincristine, etoposide, and irinotecan are effective in rhabdomyosarcomas and Ewing's sarcomas. Pazopanib, an inhibitor of the vascular endothelial growth factor, platelet-derived growth factor (PDGF), and c-kit, is now approved for patients with advanced soft tissue sarcomas excluding liposarcomas after failure of chemotherapy. Two additional chemotherapy drugs have gained approval from the U.S. Food and Drug Administration (FDA). Trabectedin was compared to dacarbazine in a large phase 3 randomized study in advanced leiomyosarcomas and liposarcomas after failure of an anthracycline and resulted in significant improvement in progression-free survival. Eribulin was also tested in a similar trial and showed improvement in survival, predominantly in the liposarcoma subgroup, and is therefore now approved for that subset. Tazemetostat, an EZH2 inhibitor, is now approved for use in metastatic epithelioid sarcomas characterized by loss of tumor-suppressor gene *INIL*, resulting in activation of the EZH2 pathway. Imatinib targets KIT and PDGF tyrosine kinase activity and is standard therapy for advanced/metastatic GISTs and dermatofibrosarcoma protuberans. Imatinib is also indicated as adjuvant therapy for completely resected primary GISTs. Three years of adjuvant imatinib appear to be superior to 1 year of therapy for high-risk GISTs, although the optimal treatment duration remains unknown. Sunitinib and regorafenib are approved for second- and third-line use, respectively, in metastatic GIST after failure of or intolerance to imatinib. Ripekinib, an inhibitor of c-kit and PDGFRA, was approved for fourth-line use in metastatic GIST based on a placebo-controlled randomized trial reporting an improved median progression-free and overall survival. Avapritinib also received approval for use in the specific molecular subset of *PDGFRA* D842V-mutant metastatic GIST.

BONE SARCOMAS

INCIDENCE AND EPIDEMIOLOGY

Bone sarcomas are rarer than soft tissue sarcomas; they accounted for only 0.2% of all new malignancies and 3600 new cases in the United States in 2020. Several benign bone lesions have the potential for

malignant transformation. Enchondromas and osteochondromas can transform into chondrosarcoma; fibrous dysplasia, bone infarcts, and Paget's disease of bone can transform into either UPS or osteosarcoma.

CLASSIFICATION

Benign Tumors The common benign bone tumors include enchondroma, osteochondroma, chondroblastoma, and chondromyxoid fibroma, of cartilage origin; osteoid osteoma and osteoblastoma, of bone origin; fibroma and desmoplastic fibroma, of fibrous tissue origin; hemangioma, of vascular origin; and giant cell tumor, of unknown origin.

Malignant Tumors The most common malignant tumors of bone are plasma cell tumors ([Chap. 111](#)). The four most common malignant nonhematopoietic bone tumors are osteosarcoma, chondrosarcoma, Ewing's sarcoma, and UPS. Rare malignant tumors include chordoma (of notochordal origin), malignant giant cell tumor, adamantinoma (of unknown origin), and hemangioendothelioma (of vascular origin).

Musculoskeletal Tumor Society Staging System Sarcomas of bone are staged according to the Musculoskeletal Tumor Society staging system based on grade and compartmental localization. A Roman numeral reflects the tumor grade: stage I is low grade, stage II is high grade, and stage III includes tumors of any grade that have lymph node or distant metastases. In addition, the tumor is given a letter reflecting its compartmental localization. Tumors designated A are intracompartimental (i.e., confined to the same soft tissue compartment as the initial tumor), and tumors designated B are extracompartimental (i.e., extending into the adjacent soft tissue compartment or into bone). The tumor-node-metastasis (TNM) staging system is shown in [Table 91-2](#).

TABLE 91-2 Staging System for Bone Sarcomas

Primary tumor (T)	TX	Primary tumor cannot be assessed
	T0	No evidence of primary tumor
	T1	Tumor ≤ 8 cm in greatest dimension
	T2	Tumor >8 cm in greatest dimension
	T3	Discontinuous tumors in the primary bone site
Regional lymph nodes (N)	NX	Regional lymph nodes cannot be assessed
	N0	No regional lymph node metastasis
	N1	Regional lymph node metastasis
Distant metastasis (M)	MX	Distant metastasis cannot be assessed
	M0	No distant metastasis
	M1	Distant metastasis
	M1a	Lung
	M1b	Other distant sites
Histologic grade (G)	GX	Grade cannot be assessed
	G1	Well differentiated—low grade
	G2	Moderately differentiated—low grade
	G3	Poorly differentiated—high grade
	G4	Undifferentiated—high grade (Ewing's is always classed G4)
Stage Grouping		
Stage IA	T1	N0
Stage IB	T2	N0
Stage IIA	T1	N0
Stage IIB	T2	N0
Stage III	T3	N0
Stage IVA	Any T	N0
Stage IVB	Any T	N1
	Any T	Any N
		Any M
		M1b
		Any G

■ OSTEOSARCOMA

Osteosarcoma, accounting for almost 45% of all bone sarcomas, is a spindle cell neoplasm that produces osteoid (unmineralized bone) or bone. Approximately 60% of all osteosarcomas occur in children and adolescents in the second decade of life, and 10% occur in the third decade of life. Osteosarcomas in the fifth and sixth decades of life are frequently secondary to either radiation therapy or transformation in a preexisting benign condition, such as Paget's disease. Males are affected 1.5–2 times as often as females. Osteosarcoma has a predilection for metaphyses of long bones; the most common sites of involvement are the distal femur, proximal tibia, and proximal humerus. The classification of osteosarcoma is complex, but 75% of osteosarcomas fall into the "classic" category, which includes osteoblastic, chondroblastic, and fibroblastic osteosarcomas. The remaining 25% are classified as "variants" on the basis of (1) clinical characteristics, as in the case of osteosarcoma of the jaw, postradiation osteosarcoma, or Paget's osteosarcoma; (2) morphologic characteristics, as in the case of telangiectatic osteosarcoma, small-cell osteosarcoma, or epithelioid osteosarcoma; or (3) location, as in parosteal or periosteal osteosarcoma. Diagnosis usually requires a synthesis of clinical, radiologic, and pathologic features. Patients typically present with pain and swelling of the affected area. A plain radiograph reveals a destructive lesion with a moth-eaten appearance, a spiculated periosteal reaction (sunburst appearance), and a cuff of periosteal new bone formation at the margin of the soft tissue mass (Codman's triangle). A CT scan of the primary tumor is best for defining bone destruction and the pattern of calcification, whereas MRI is better for defining intramedullary and soft tissue extension. A chest radiograph and CT scan are used to detect lung metastases. Metastases to the bony skeleton should be imaged by a bone scan or by fluorodeoxyglucose positron emission tomography (FDG-PET). Almost all osteosarcomas are hypervascular and PET-avid. Pathologic diagnosis is established either with a core-needle biopsy, where feasible, or with an open biopsy with an appropriately placed incision that does not compromise future limb-sparing resection. Most osteosarcomas are high grade. The most important predictive factor for long-term survival is response to chemotherapy. Preoperative chemotherapy followed by limb-sparing surgery (which can be accomplished in >80% of patients) followed by postoperative chemotherapy is standard management. The effective drugs are doxorubicin, ifosfamide, cisplatin, and high-dose methotrexate with leucovorin rescue. The various combinations of these agents that have been used have all been about equally successful. Long-term survival rates in extremity osteosarcoma range from 60 to 80%. Osteosarcoma is radioresistant; radiation therapy has no role in the routine management. UPS is considered a part of the spectrum of osteosarcoma and is managed similarly. A randomized trial has shown improved progression-free survival with regorafenib compared to placebo.

■ CHONDROSARCOMA

Chondrosarcoma, which constitutes ~20–25% of all bone sarcomas, is a tumor of adulthood and old age with a peak incidence in the fourth to sixth decades of life. It has a predilection for the flat bones, especially the shoulder and pelvic girdles, but can also affect the diaphyseal portions of long bones. Chondrosarcomas can arise de novo or as a malignant transformation of an enchondroma or, rarely, of the cartilaginous cap of an osteochondroma. Chondrosarcomas have an indolent natural history and typically present as pain and swelling. Radiographically, the lesion may have a lobular appearance with mottled or punctate or annular calcification of the cartilaginous matrix. It is difficult to distinguish low-grade chondrosarcoma from benign lesions by x-ray or histologic examination. The diagnosis is therefore influenced by clinical history and physical examination. A new onset of pain, signs of inflammation, and progressive increase in the size of the mass suggest malignancy. The histologic classification is complex, but most tumors fall within the classic category. Like other bone sarcomas, high-grade chondrosarcomas spread to the lungs. Most chondrosarcomas are resistant to chemotherapy, and surgical resection of primary or recurrent tumors, including pulmonary metastases, is the mainstay of therapy with the exception of two histologic variants. Dedifferentiated chondrosarcoma has a high-grade osteosarcoma or a malignant fibrous histiocytoma component

that responds to chemotherapy. Mesenchymal chondrosarcoma, a rare variant composed of a small-cell element, also is responsive to systemic chemotherapy and is treated like Ewing's sarcoma.

■ EWING'S SARCOMA

Ewing's sarcoma, which constitutes ~10–15% of all bone sarcomas, is common in adolescence and has a peak incidence in the second decade of life. It typically involves the diaphyseal region of long bones and also has an affinity for flat bones. The plain radiograph may show a characteristic "onion peel" periosteal reaction with a generous soft tissue mass, which is better demonstrated by CT or MRI. This mass is composed of sheets of monotonous, small, round, blue cells and can be confused with lymphoma, embryonal rhabdomyosarcoma, and small-cell carcinoma. The presence of p30/32, the product of the *mic-2* gene (which maps to the pseudoautosomal region of the X and Y chromosomes), is a cell-surface marker for Ewing's sarcoma (and other members of the Ewing family of tumors, previously also called PNETs). Most PNETs arise in soft tissues; they include peripheral neuroepithelioma, Askin's tumor (chest wall), and esthesioneuroblastoma. Glycogen-filled cytoplasm detected by staining with periodic acid-Schiff is also characteristic of Ewing's sarcoma cells. The classic cytogenetic abnormality associated with this disease is a reciprocal translocation of the long arms of chromosomes 11 and 22, t(11;22), which creates a chimeric gene product of unknown function with components from the *fli-1* gene on chromosome 11 and *ews* on chromosome 22. This disease is very aggressive, and it is therefore considered a systemic disease. Common sites of metastases are lung, bones, and bone marrow. Systemic chemotherapy is the mainstay of therapy, often being used before surgery. Doxorubicin, cyclophosphamide or ifosfamide, etoposide, vincristine, and dactinomycin are active drugs. Topotecan or irinotecan in combination with an alkylating agent is often used in relapsed patients. Local treatment for the primary tumor includes surgical resection, usually with limb salvage or radiation therapy. Patients with lesions below the elbow and below the mid-calf have a 5-year survival rate of 80% with effective treatment. Ewing's sarcoma at first presentation is a curable tumor, even in the presence of obvious metastatic disease, especially in children <11 years old.

TUMORS METASTATIC TO BONE

Bone is a common site of metastasis for carcinomas of the prostate, breast, lung, kidney, bladder, and thyroid and for lymphomas and sarcomas. Prostate, breast, and lung primaries account for 80% of all bone metastases. Metastatic tumors of bone are more common than primary bone tumors. Tumors usually spread to bone hematogenously, but local invasion from soft tissue masses also occurs. In descending order of frequency, the sites most often involved are the vertebrae, proximal femur, pelvis, ribs, sternum, proximal humerus, and skull. Bone metastases may be asymptomatic or may produce pain, swelling, nerve root or spinal cord compression, pathologic fracture, or myelophthisis (replacement of the marrow). Symptoms of hypercalcemia may be noted in cases of bony destruction.

Pain is the most frequent symptom. It usually develops gradually over weeks, is usually localized, and often is more severe at night. When patients with back pain develop neurologic signs or symptoms, emergency evaluation for spinal cord compression is indicated (Chap. 75). Bone metastases exert a major adverse effect on quality of life in cancer patients.

Cancer in the bone may produce osteolysis, osteogenesis, or both. Osteolytic lesions result when the tumor produces substances that can directly elicit bone resorption (vitamin D-like steroids, prostaglandins, or parathyroid hormone-related peptide) or cytokines that can induce the formation of osteoclasts (interleukin 1 and tumor necrosis factor). Osteoblastic lesions result when the tumor produces cytokines that activate osteoblasts. In general, purely osteolytic lesions are best detected by plain radiography, but they may not be apparent until they are >1 cm. These lesions are more commonly associated with hypercalcemia and with the excretion of hydroxyproline-containing peptides indicative of matrix destruction. When osteoblastic activity is prominent, the lesions may be readily detected using radionuclide

716 bone scanning (which is sensitive to new bone formation), and the radiographic appearance may show increased bone density or sclerosis. Osteoblastic lesions are associated with higher serum levels of alkaline phosphatase and, if extensive, may produce hypocalcemia. Although some tumors may produce mainly osteolytic lesions (e.g., kidney cancer) and others mainly osteoblastic lesions (e.g., prostate cancer), most metastatic lesions produce both types of lesion and may go through stages where one or the other predominates.

In older patients, particularly women, it may be necessary to distinguish metastatic disease of the spine from osteoporosis. In osteoporosis, the cortical bone may be preserved, whereas cortical bone destruction is usually noted with metastatic cancer.

TREATMENT

Metastatic Bone Disease

Treatment of metastatic bone disease depends on the underlying malignancy and the symptoms. Some metastatic bone tumors are curable (lymphoma, Hodgkin's disease), and others are treated with palliative intent. Pain may be relieved by local radiation therapy. Hormonally responsive tumors are responsive to hormone inhibition (antiandrogens for prostate cancer, antiestrogens for breast cancer). Strontium-89, samarium-153, and radium-223 are bone-seeking radionuclides that can exert antitumor effects and relieve symptoms. Denosumab, a monoclonal antibody that binds to RANK ligand, inhibits osteoclastic activity and increases bone mineral density. Bisphosphonates such as pamidronate may relieve pain and inhibit bone resorption, thereby maintaining bone mineral density and reducing risk of fractures in patients with osteolytic metastases from breast cancer and multiple myeloma. Careful monitoring of serum electrolytes and creatinine is recommended. Monthly administration prevents bone-related clinical events and may reduce the incidence of bone metastases in women with breast cancer. When the integrity of a weight-bearing bone is threatened by an expanding metastatic lesion that is refractory to radiation therapy, prophylactic internal fixation is indicated. Overall survival is related to the prognosis of the underlying tumor. Bone pain at the end of life is particularly common; an adequate pain relief regimen including sufficient amounts of narcotic analgesics is required. The management of hypercalcemia is discussed in [Chap. 410](#).

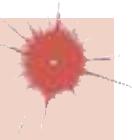
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Carcinoma of Unknown Primary

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Carcinoma (or cancer) of unknown primary (CUP) is a biopsy-proven malignancy for which the anatomic site of origin remains unidentified after a standardized detailed diagnostic evaluation. CUP is one of the 10 most frequently diagnosed cancers globally, accounting for 3–5% of all malignancies. Most investigators limit CUP to epithelial or undifferentiated cancers and do not include lymphomas, metastatic melanomas, and metastatic sarcomas because these cancers have specific histology and stage-based management guidelines, even in the absence of a primary site. CUP can occur in patients of all age groups including adolescents and young adults.

The emergence of sophisticated imaging, robust immunohistochemistry (IHC), and genomic and proteomic tools has challenged the “unknown” designation. Additionally, effective targeted therapies in several cancers and tissue agnostic biomarker-driven therapies have endorsed a change in paradigm from empiricism to a personalized approach to CUP management. The reasons cancers present as CUP remain unclear. One hypothesis is that the primary tumor either regresses after seeding the metastasis or remains so small that it is not detected. It is possible that CUP falls on the continuum of cancer presentation where the primary has been contained or eliminated by the natural body defenses, including the immune system. Alternatively, CUP may represent a specific malignant event that results in an increase in metastatic spread or survival relative to the primary. Whether the CUP metastases truly define a clone that is genetically and phenotypically unique to this diagnosis remains to be determined.

Since liver is a common site of CUP presentation, intrahepatic cholangiocarcinoma (ICC) can be often misdiagnosed as CUP. Of note, the incidence of ICC is increasing, whereas at the same time, that of CUP is declining. Improvements in diagnostic technologies including next-generation sequencing and other molecular techniques and awareness among clinicians to differentiate the two are possibly contributing to an increased recognition and incidence of ICC.

CUP BIOLOGY

Studies looking for unique signature abnormalities in CUP tumors have not been positive. Abnormalities in chromosomes 1 and 12 and other complex cytogenetic abnormalities have been reported. Aneuploidy has been described in 70% of CUP patients with metastatic adenocarcinoma or undifferentiated carcinoma. The overexpression of various genes, including *RAS*, *BCL2* (40%), *HER2* (11%), and *P53* (26–53%), has been identified in CUP samples, but they are found in many other malignancies and have no effect on response to therapy or survival. The extent of angiogenesis in CUP relative to that in metastases from known primaries has also been evaluated, but no consistent findings have emerged. Although current comprehensive genomic profiling efforts may help identify targeted therapeutic approaches to improve outcomes for this disease as discussed below, they have failed thus far to reveal a distinct molecular signature. More comprehensive and integrated multiomic efforts are needed to provide insights into CUP biology through recognition of molecular aberrations that especially drive metastatic growth.

APPROACH TO THE PATIENT

Carcinoma (or Cancer) of Unknown Primary

Initial CUP evaluation has two goals: search for the primary tumor based on pathologic evaluation of the metastases and determine the extent of disease. Focused evaluation directed by clinicopathologic

cues allows for judicious and efficient use of diagnostic tests. Obtaining a thorough medical history from CUP patients is essential, including paying particular attention to previous surgeries, removed lesions, and family medical history to assess potential hereditary cancers. Adequate physical examination, including a digital rectal examination in men and breast and pelvic examinations in women, should be performed based on clinical presentation. Finally, all patients with CUP, in the absence of contraindication, must undergo a computed tomographic (CT) scan of chest, abdomen and pelvis as a part of their standard work-up.

■ ROLE OF SERUM TUMOR MARKERS AND CYTOGENETICS

Most tumor markers, including carcinoembryonic antigen (CEA), CA-125, CA 19-9, and CA 15-3, when elevated, are nonspecific and not helpful in determining the primary site. Men who present with adenocarcinoma and predominant osteoblastic metastasis should undergo a prostate-specific antigen (PSA) test. In patients with undifferentiated or poorly differentiated carcinoma (especially with a midline tumor), elevated β -human chorionic gonadotropin (β -hCG) and α fetoprotein (AFP) levels suggest the possibility of an extragonadal germ cell (testicular) tumor. With the availability of advanced immunohistochemistry (IHC), cytogenetic studies are rarely needed.

■ ROLE OF IMAGING STUDIES

In the absence of contraindications, a baseline IV contrast computed tomography (CT) scan of the chest, abdomen, and pelvis is the standard of care. This helps to search for the primary tumor, evaluate the extent of disease, and select the most accessible biopsy site. With precise imaging and reporting, latent primary cancers, defined as appearance of a new primary cancer after a latent period of several months to years, is uncommon and seen in $\leq 5\%$ of CUP patients, usually in patients with very indolent presentations and/or highly responsive metastatic cancers that allows a latent primary to emerge (grow) over time.

Mammography should be performed in all women who present with metastatic adenocarcinoma, specifically in those with isolated axillary lymphadenopathy. Magnetic resonance imaging (MRI) of the breast can be considered in patients with axillary adenopathy and suspected occult primary breast carcinoma following a negative mammography and ultrasound. The results of these imaging modalities can influence surgical management; a negative MRI of the breast predicts a low tumor yield at mastectomy.

A conventional workup for a squamous cell carcinoma and cervical CUP (neck lymphadenopathy with no known primary tumor) includes a CT scan or MRI and invasive studies, including indirect and direct laryngoscopy, bronchoscopy, and upper endoscopy. Ipsilateral (or bilateral) staging tonsillectomy has been recommended for these patients. 18-Fluorodeoxyglucose positron emission tomography (18-FDG-PET) scans are useful in this patient population and may help guide the biopsy; determine the extent of disease; facilitate the appropriate treatment, including planning radiation fields; and help with disease surveillance. A smaller radiation field encompassing the metastatic adenopathy decreases the risk of chronic xerostomia. Several studies have evaluated the utility of PET in patients with squamous cervical CUP, and head and neck primary tumors were identified in $\sim 21\text{--}30\%$.

The diagnostic contribution of PET to the evaluation of other CUP presentations (outside of the neck adenopathy indication) remains controversial and is not routinely recommended. PET-CT can be helpful for patients with bone metastases and those deemed candidates for aggressive multimodality therapy (surgical intervention/radiation) such as patients with solitary metastatic disease because the identification of disease in addition to the solitary metastatic site may affect treatment planning.

Invasive studies, including upper endoscopy, colonoscopy, and bronchoscopy, should be limited to symptomatic patients or those with laboratory, imaging, or pathologic abnormalities that suggest that these techniques will result in a high yield in finding a primary cancer.

■ ROLE OF PATHOLOGIC STUDIES

A detailed pathologic examination of the most accessible biopsied tissue specimen is mandatory in CUP patients. Pathologic evaluation typically consists of hematoxylin and eosin stains and IHC tests. The importance of adequate tissue acquisition cannot be overemphasized in CUP. In addition to pathologic evaluation, tissue is also needed for tests of biomarkers of targeted agents, immunotherapy, and clinical trials.

Light Microscopy Evaluation Adequate tissue obtained preferably by excisional biopsy or core needle biopsy (instead of only a fine-needle aspiration) is stained with hematoxylin and eosin and subjected to light microscopic examination. On light microscopy, 60–65% of CUP is adenocarcinoma, and 5% is squamous cell carcinoma. The remaining 30–35% is poorly differentiated adenocarcinoma, poorly differentiated carcinoma, or poorly differentiated neoplasm. A small percentage of lesions are diagnosed as neuroendocrine cancers (2%), mixed tumors (adenosquamous or sarcomatoid carcinomas), or undifferentiated neoplasms (Table 92-1).

Role of IHC Analysis IHC stains are peroxidase-labeled antibodies against specific tumor antigens that are used to define tumor lineage. The number of available IHC stains is ever-increasing. However, a tiered and uniform approach to tissue evaluation in the CUP setting is lacking. For CUP cases, more is not necessarily better, and IHC stains should be used in conjunction with the patient's clinical presentation and imaging studies to select the best therapy. Communication between the clinician and pathologist is essential. No stain is 100% sensitive or specific, and under-/overinterpretation should be avoided. Poor differentiation, even in known primary tumors, decreases sensitivity of hallmark IHC markers. PSA and thyroglobulin tissue markers, which are positive in prostate and thyroid cancer, respectively, are the most specific of the current marker panel. However, these cancers rarely present as CUP, so the yield of these tests may be low. Figure 92-1 delineates a simple algorithm for immunohistochemical staining in CUP cases. Table 92-2 lists additional tests that may be useful to further define the tumor lineage. A more comprehensive algorithm may improve the diagnostic accuracy but can make the process complex and increase cost. With the use of IHC markers, electron microscopic analysis, which is time-consuming and expensive, is rarely needed.

There are >20 subtypes of cytokeratin (CK) intermediate filaments with different molecular weights and differential expression in various cell types and cancers. Monoclonal antibodies to specific CK subtypes have been used to help classify tumors according to their site of origin; commonly used CK stains in adenocarcinoma CUP are CK7 and CK20. CK7 is found in tumors of the lung, ovary, endometrium, breast, and upper gastrointestinal tract including pancreaticobiliary cancers, whereas CK20 is normally expressed in the gastrointestinal epithelium, urothelium, and Merkel cells. The nuclear CDX-2 transcription factor, which is the product of a homeobox gene necessary for intestinal organogenesis, is often used to aid in the diagnosis of gastrointestinal adenocarcinomas. However, CDX-2 positivity can be seen with enteric or mucinous differentiation in tumors from diverse primary sites (e.g., mucinous ovarian cancers).

Thyroid transcription factor 1 (TTF-1) nuclear staining is frequently positive in lung and thyroid cancers. Approximately 68% of adenocarcinomas and 25% of squamous cell lung cancers stain positive for TTF-1, which helps differentiate a lung primary tumor from metastatic

TABLE 92-1 Major Histologies in Carcinoma of Unknown Primary

HISTOLOGY	PROPORTION, %
Well to moderately differentiated adenocarcinoma	60
Squamous cell cancer	5
Poorly differentiated adenocarcinoma, poorly differentiated carcinoma	30
Neuroendocrine	2
Undifferentiated malignancy	3

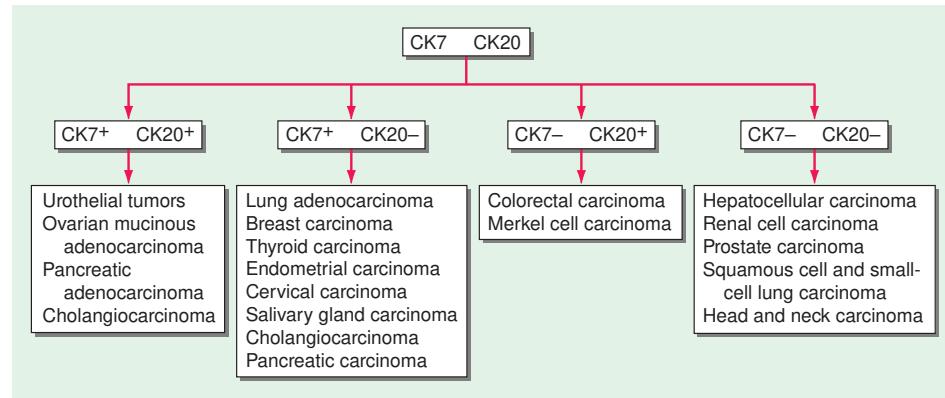


FIGURE 92-1 Approach to cytokeratin (CK7 and CK20) markers used in adenocarcinoma of unknown primary.

adenocarcinoma in a pleural effusion, the mediastinum, or the lung parenchyma.

Gross cystic disease fibrous protein-15 (GCDFP-15), a 15-kDa monomer protein, is a marker of apocrine differentiation that is detected in 62–72% of breast carcinomas. GATA3 is being increasingly used in the CUP setting when there is concern for a breast primary and can be particularly useful as a marker for metastatic breast carcinoma, especially triple-negative and metaplastic carcinomas, which lack specific endocrine markers of mammary origin. UROIII, high-molecular-weight cytokeratin, thrombomodulin, and CK20 are the markers used to diagnose lesions of urothelial origin.

TABLE 92-2 Select Immunohistochemical Stains Useful in the Diagnosis of CUP

LIKELY PRIMARY PROFILE	COMMONLY CONSIDERED IHC TO ASSIST IN DIFFERENTIAL DIAGNOSIS OF CUP ^a
Breast	ER, GCDFP-15, gammaglobin, HER2/neu, GATA3
Ovarian/mullerian	ER, WT1, CK7, PAX8, PAX2
Lung adenocarcinoma	TTF-1; nuclear staining, napsin A, SP-A1
Germ cell	β -hCG, AFP, OCT3/4, CKIT, CD30 (embryonal), SALL4
Prostate	PSA, α -methylacyl CoA racemase/P504S (AMACR/P504S), P501S (prostein), PSMA, NKX3-1
Intestinal	CK7, CK20, CDX-2, CEA
Neuroendocrine	Chromogranin, synaptophysin, CD56
Sarcoma	Desmin (desmoid tumors), factor VIII (angiosarcomas), CD31, smooth muscle actin (leiomyosarcoma), MyoD1 (rhabdomyosarcoma)
Renal	RCC, CD10, PAX8, CD10
Hepatocellular carcinoma	Hep Par-1, Arg-1, glyican-3
Melanoma	S100, SOX-10, vimentin, HMB-45, tyrosinase, melan-A
Urothelial	CK7, CK20, thrombomodulin, uroplakin III
Mesothelioma	Calretinin, WT1, D2-40, mesothelin
Lymphoma	LCA, CD3, CD4, CD5, CD20, CD45
SOC	p63, p40 (lung SCC), CK5/6

^aPatterns emerging from coexpression of stains are better than individual stains to suggest putative primary site. Even with optimization, no IHC panel is 100% sensitive or specific (e.g., ovarian mucinous carcinoma can exhibit positivity with intestinal markers).

Abbreviations: AFP, α fetoprotein; Arg-1, arginase-1; β -hCG, β -human chorionic gonadotropin; CEA, carcinoembryonic antigen; CUP, carcinoma of unknown primary; ER, estrogen receptor; GCDFP-15, gross cystic disease fibrous protein-15; IHC, immunohistochemistry; LCA, leukocyte common antigen; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; SOC, squamous cell carcinoma; SP-A1, surfactant protein A precursor; TTF, thyroid transcription factor; WT, Wilms' tumor.

IHC performs the best when used in groups that give rise to patterns that are strongly indicative of certain profiles. For example, the TTF-1/CK7+ and CK20+/CDX-2+/CK7- phenotypes have been reported as very suggestive of lung and lower gastrointestinal cancer profiles, respectively. Despite their practical utility, these patterns have not been validated prospectively in CUP patients. IHC is not without its limitations; several factors affect tissue antigenicity (antigen retrieval, specimen processing, and fixation), interpretation of stains in tumor (nuclear, cytoplasmic, membrane) versus normal tissue, inter- and intraobserver variability, variable performance of different antibodies said to recognize the same antigen, and tissue heterogeneity and inadequacy (given small biopsy sizes). Communication with the pathologist is critical to determine if additional tissue will be beneficial in the pathologic evaluation. Pathologic features should not always supersede clinical or radiologic findings when considering testing for biomarkers of therapeutic response (e.g., epidermal growth factor receptor [EGFR], ALK mutations, human epidermal growth factor receptor 2 [HER-2]).

Role of Cancer Classifier Molecular Profiling In the absence of a known primary, developing therapeutic strategies for CUP is challenging. The current diagnostic yield with imaging and immunochemistry is ~20–30% for CUP patients. To reduce diagnostic uncertainty, sophisticated molecular analytics have been applied to CUP samples. These include gene expression profiling, messenger RNA (mRNA), microRNA, and epigenetic profiling to classify the CUP cancer.

Gene expression profiles are most commonly generated using quantitative reverse transcriptase polymerase chain reaction (RT-PCR) or DNA microarray. Neural network programs are then used to develop predictive algorithms from the gene expression profiles. Typically, a training set of gene profiles from known cancers (preferably from metastatic sites) is used to train the software. Comprehensive gene expression databases that have become available for common malignancies are then applied to CUP samples, and the program can then be used to predict the putative origin of a CUP sample.

mRNA- or microRNA-based tissue of origin cancer classifier assays have also been studied in prospective and retrospective CUP trials. More recently, a classifier based on microarray DNA methylation signatures has been studied and validated in known cancers. The DNA methylation profiling predicted a primary cancer in 87% of the 216 CUP patients.

Despite the sophistication of the cancer classifier molecular assays, most of the CUP studies have evaluated assay *performance*, although the challenge with validating the accuracy of an assay for CUP is that, by definition, the primary cancer diagnosis cannot be verified. Thus, current estimates of tissue of origin test accuracy have relied on indirect metrics, including comparison with pathology/IHC, clinical presentation, appearance of latent primaries, and autopsies. Using

these measures, the assays suggest a plausible primary in ~70–80% of patients studied. Three outcomes-based studies have been performed. First, a single-arm study reported a median survival of 12.5 months for patients who received assay-directed site-specific therapy. Second, a phase 2 trial of site-specific therapy, including molecularly targeted therapy, based on predicted tumor site from an algorithm using gene expression and alteration profile showed a 1-year survival of 53.1%. However, a randomized clinical trial evaluating site-specific therapy directed by gene expression profiling versus empirical chemotherapy with paclitaxel and carboplatin failed to show a significant improvement in 1-year survival (44% vs 55%, $p = .264$) with this approach. Firm conclusions of therapeutic impact cannot be drawn from these studies given the sample size, design, statistical biases, confounding variables including use of subsequent lines of (empiric) therapy, and heterogeneity of the CUP cancers. Additional studies are needed to better understand the clinical impact of tissue of origin profiling tools and how these assays complement IHC and help guide therapy.

Role of Next-Generation Sequencing A significant push is being made toward personalized medicine across all cancer types with the goal of identifying driver mutation(s) in a patient who can be treated with targeted agents independent of the site of origin. A retrospective study of 200 CUP tumor specimens reported on genomic alterations using the hybrid capture–based FoundationOne assay. The authors reported that a large number of CUP samples (85%) harbored at least one clinically relevant genomic alteration with the potential to influence and personalize therapy. The mean number of genomic alterations was 4.2 per tumor, and the most common genetic alterations included *TP53* (55%), *KRAS* (20%), *CDKN2A* (19%), and *ARID1A* (11%). The adenocarcinoma CUP tumors were more frequently driven by genetic alterations in the receptor tyrosine kinase (RTK)/Ras/mitogen-activated protein kinase (MAPK) signaling pathway than nonadenocarcinoma CUP tumors. Although, druggable genetic lesions seen in CUP are comparable to those in defined large entities, whether molecularly stratified approaches for CUP will successfully improve outcomes remains to be seen and clinical trials are needed. In a single-arm phase 2 study of 97 patients with molecularly

targeted therapy, five patients were found to have targetable *EGFR* mutations. Of these, four patients were treated with afatinib, an anti-*EGFR* drug, and two patients achieved a progression-free survival of >6 months. The emerging role of assays looking for circulating tumor cells, so-called liquid biopsies, within known tumor types has stirred interest in their potential utility in CUP.

Ongoing histology and cellular-context agnostic prospective clinical trials are studying the presence of actionable mutations and matching patients to the right targeted drug. Should this approach eventually be appropriately validated, CUP would be a natural fit for genomic alteration (GA)-based targeted therapy independent of tumor site. Immune checkpoint inhibitors (pembrolizumab) for microsatellite instability high (MSI-H) or deficient mismatch repair (dMMR) tumors and NTRK inhibitors for *NTRK* fusion–positive tumors can help a small minority of CUP patients.

TREATMENT

Carcinoma (or Cancer) of Unknown Primary

GENERAL CONSIDERATIONS

The treatment of CUP continues to evolve, albeit slowly. The median survival of most patients with disseminated CUP is ~6–10 months. Systemic chemotherapy is the primary treatment modality in most patients with disseminated disease, but the careful integration of surgery, radiation therapy, and even periods of observation is important in the overall management of this condition (Figs. 92-2 and 92-3). Prognostic factors include performance status, site and number of metastases, response to chemotherapy, and serum lactate dehydrogenase (LDH) levels. Culine and colleagues developed a prognostic model using performance status and serum LDH levels, which allowed the assignment of patients into two subgroups with divergent outcomes. Raghav and colleagues developed a prognostic nomogram to provide individualized survival estimates for patients with CUP based on baseline gender, ECOG performance status, histology, number of metastatic

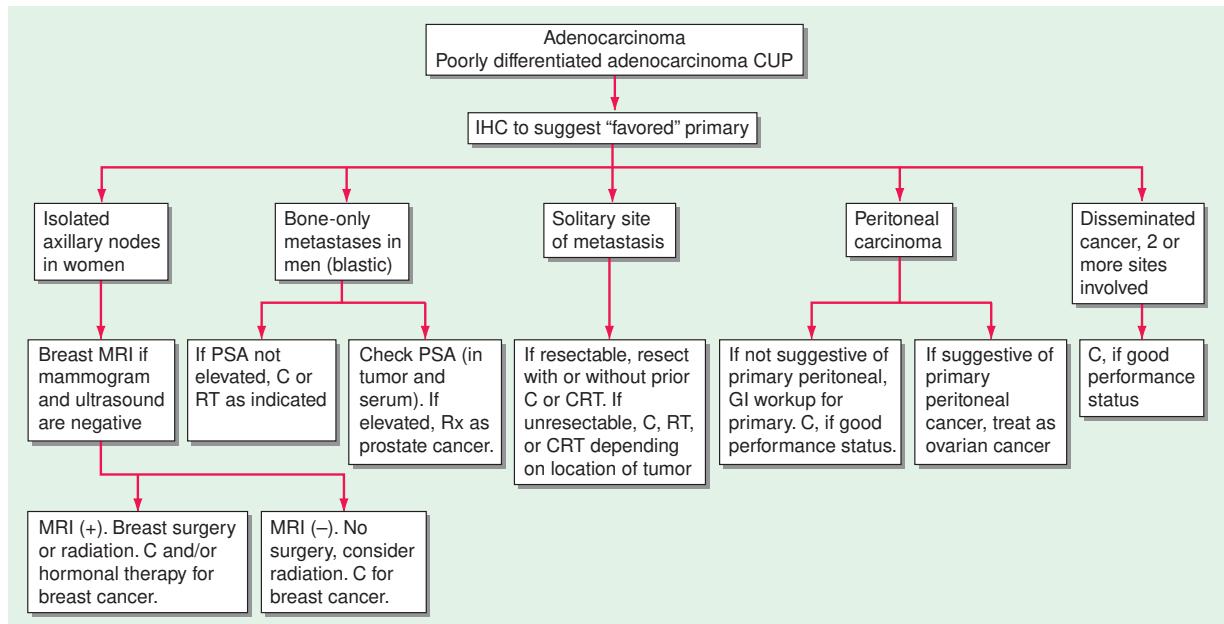


FIGURE 92-2 Treatment algorithm for adenocarcinoma and poorly differentiated adenocarcinoma of unknown primary (CUP). C, chemotherapy; CRT, chemoradiation; GI, gastrointestinal; IHC, immunohistochemistry; MRI, magnetic resonance imaging; PSA, prostate-specific antigen; RT, radiation.

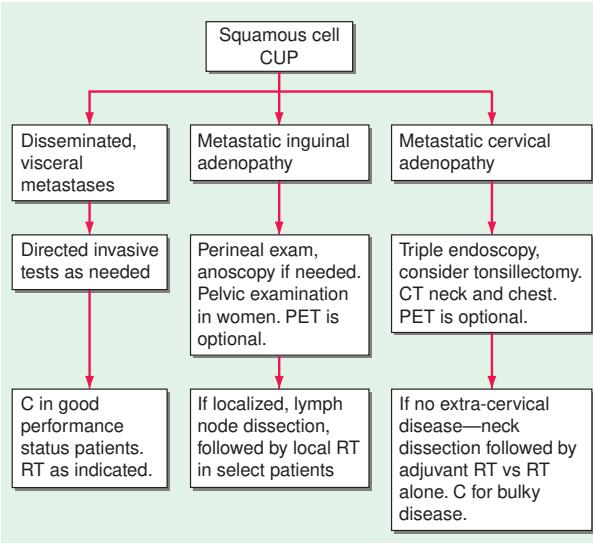


FIGURE 92-3 Treatment algorithm for squamous cell carcinoma of unknown primary (CUP). C, chemotherapy; CT, computed tomography; PET, positron emission tomography; RT, radiation.

sites and neutrophil-lymphocyte ratio. Future prospective trials using this prognostic model are warranted. Clinically, some CUP diagnoses fall into a favorable prognostic subset. Others, including those with disseminated CUP, have a more unfavorable prognosis.

TREATMENT OF FAVORABLE CUP SUBSETS

Women with Isolated Axillary Adenopathy Women with isolated axillary adenopathy with adenocarcinoma or carcinoma are usually treated for stage II or III breast cancer based on pathologic findings. These patients should undergo a breast MRI if mammogram and ultrasound are negative. Radiation therapy to the ipsilateral breast is indicated if the MRI of the breast is positive. Chemotherapy and/or hormonal therapy are indicated based on patient's age (premenopausal or postmenopausal), nodal disease bulk, and hormone receptor and HER2 status (Chap. 79). It is important to verify that the pathology suggests a breast cancer profile (morphology, IHC breast markers including estrogen receptor, mammaglobin, GCDFP-15, GATA3, HER-2 gene expression) before embarking on a breast cancer therapeutic program.

Women with Peritoneal Carcinomatosis The term *primary peritoneal papillary serous carcinoma* (PPSC) has been used to describe CUP with carcinomatosis with the pathologic and laboratory (elevated CA-125 antigen) characteristics of ovarian cancer but no ovarian primary tumor identified on transvaginal sonography or laparotomy. Studies suggest that ovarian cancer and PPSC, which are both of müllerian origin, have similar gene expression profiles. Similar to patients with ovarian cancer, patients with PPSC are candidates for cytoreductive surgery, followed by adjuvant taxane- and platinum-based chemotherapy. In one retrospective study of 258 women with peritoneal carcinomatosis who had undergone cytoreductive surgery and chemotherapy, 22% of patients had a complete response to chemotherapy; the median survival duration was 18 months (range 11–24 months). However, not all peritoneal carcinomatosis in women is PPSC. Careful pathologic evaluation can help diagnose a colon cancer profile (CDX-2+, CK20+, CK7-) or a pancreaticobiliary cancer or even a mislabeled peritoneal mesothelioma (calretinin, D2-40 positive; BerEP4, MOC-31 negative).

Poorly Differentiated Carcinoma with Midline Adenopathy (Chap. 88) Men with poorly differentiated or undifferentiated carcinoma who present with midline adenopathy should be evaluated for extragonadal germ cell malignancy. If diagnosed and treated as such, they often experience a good response to treatment with platinum-based combination chemotherapy. Response rates of >50% have been noted, and long-term survival rates of 10–15% have been reported. Older patients, especially smokers, who present with mediastinal adenopathy are more likely to have a lung or head and neck cancer profile.

Neuroendocrine Cancer (Chap. 84) Low-grade neuroendocrine tumor (NET) often has an indolent course, and treatment decisions are based on symptoms and tumor bulk. Urine 5-HIAA and serum chromogranin may be elevated and can be followed as markers. Often the patient is treated with somatostatin analogues alone for hormone-related symptoms (diarrhea, flushing, nausea). Specific local therapies or systemic therapy would only be indicated if the patient is symptomatic with local pain secondary to significant growth of the metastasis or the hormone-related symptoms are not controlled with endocrine therapy. Novel therapy options have demonstrated benefit in patients with low-grade NET, including sunitinib (which targets the vascular endothelial growth factor pathway), everolimus (which inhibits the mammalian target of rapamycin), and lutetium-177 dotatate (a somatostatin peptide receptor radioligand). Patients with high-grade NET are treated with platinum-based doublet therapy; 20–25% show a complete response, and up to 10% patients with limited/oligo presentations survive for >5 years. Some degree of neuroendocrine differentiation can be seen in diverse poorly differentiated carcinomas.

Squamous Cell Carcinoma Presenting as Neck Adenopathy Patients with early-stage squamous cell carcinoma involving the cervical lymph nodes are candidates for node dissection and radiation therapy, which can result in long-term survival. The role of chemotherapy in these patients is undefined, although chemoradiation therapy or induction chemotherapy is often used and is beneficial in bulky N2/N3 lymph node disease.

Solitary Metastatic Sites Patients with solitary metastases can also experience good treatment outcomes. Some patients who present with locoregional disease are candidates for aggressive trimodality (chemotherapy, radiation, and surgery) management—both prolonged disease-free survival and, occasionally, cure are possible.

Men with Blastic Skeletal Metastases and Elevated PSA (Chap. 87) Blastic bone-only metastasis is a rare presentation, and elevated serum PSA or tumor staining with PSA may provide confirmatory evidence of prostate cancer in these patients. Those with elevated levels are candidates for hormonal or other therapy for prostate cancer, although it is important to rule out other primary tumors (lung most common).

MANAGEMENT OF DISSEMINATED CUP

Patients who present with liver, brain, and adrenal metastatic disease usually have a poor prognosis. Patients with peritoneal carcinomatosis secondary to metastatic adenocarcinoma have a broad differential diagnosis, which includes mainly gastrointestinal cancers including gastric, appendiceal, colon, and pancreaticobiliary cancers.

Traditionally, platinum-based combination chemotherapy regimens have been used to treat CUP. Several broadly used regimens have been studied in the past two decades; these include paclitaxel-carboplatin, gemcitabine-cisplatin, gemcitabine-oxaliplatin, and irinotecan and fluoropyrimidine-based therapies. These chemotherapeutic agents used as empiric regimens have shown response rates of 25–40%, and their use obtains median survival times of 6–13 months.

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Paraneoplastic Syndromes: Endocrinologic/Hematologic



J. Larry Jameson, Dan L. Longo

Outside of favorable subsets, there is a small group of patients with a “definitive” IHC profile. These patients usually have a single diagnosis based on their clinicopathologic presentation and are often treated for the putative primary tumor. This does not guarantee a response, although it increases the probability of response when select drugs are chosen from a class of drugs known to be effective in that cancer type. Efforts should be made to search for biomarkers of response to tumor-agnostic effective therapies such as immunotherapy for MSI-H/dMMR tumors. Patients who do not fall into those categories are candidates for broad-spectrum platinum-based regimens, clinical trials, and additional trial-based genomic and proteomic tests. Today, we do not have many effective drugs for several CUP cancer profiles, and treatments overlap for some cancers. Immunotherapy has been an area of active interest due to robust and durable responses in cancers with known primaries and has shown some activity in CUP. However, biomarkers of response and immune-sensitive subsets need to be defined within CUP.

SUMMARY

Patients with CUP should undergo a directed diagnostic search for the primary tumor on the basis of clinical and pathologic data. Subsets of patients have prognostically favorable disease, as defined by clinical or histologic criteria, and may substantially benefit from aggressive treatment; in these patients, prolonged survival can be expected. However, for most patients who present with advanced CUP, the prognosis remains poor with early resistance to available cytotoxic therapy. The current focus has shifted away from empirical chemotherapeutic trials to understanding the metastatic phenotype, tissue of origin profiling in select patients, and next-generation sequencing to identify actionable mutations in CUP patients. As novel therapies evolve in cancers with known primaries, investigations to assess their value in CUP will likely have an impact on management of CUP patients.

FURTHER READING

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Neoplastic cells can produce a variety of substances that can alter the physiology of hormonal, hematologic, dermatologic, rheumatologic, renal, and neurologic systems. *Paraneoplastic syndromes* refer to the disorders that accompany benign or malignant tumors but are not directly related to mass effects or invasion. Tumors of neuroendocrine origin, such as small-cell lung carcinoma (SCLC) and carcinoids are common causes of paraneoplastic syndromes, but they have been associated with many types of tumors that produce peptide hormones, cytokines, and growth factors and induce the production of antibodies. Studies of the prevalence of paraneoplastic syndromes indicate that they are more common than is generally appreciated. The signs, symptoms, and metabolic alterations associated with paraneoplastic disorders are easily overlooked in the context of a malignancy and its treatment. Consequently, atypical clinical manifestations in a patient with cancer should prompt consideration of a paraneoplastic syndrome. The most common hormonal and hematologic syndromes associated with underlying neoplasia will be discussed here.

ENDOCRINE PARANEOPLASTIC SYNDROMES

Etiology Hormones can be produced from eutopic or ectopic sources. *Eutopic* refers to the expression of a hormone from its normal tissue of origin, whereas *ectopic* refers to hormone production from an atypical tissue source. For example, adrenocorticotrophic hormone (ACTH) is expressed eutopically by the corticotrope cells of the anterior pituitary, but it can be expressed ectopically in SCLC. Many hormones are produced at low levels from tissues other than the classic endocrine source. Thus, ectopic expression is often a quantitative change rather than an absolute change in tissue expression. Nevertheless, the term *ectopic expression* is firmly entrenched and conveys the abnormal physiology associated with hormone production by neoplastic cells. In addition to high levels of hormones, ectopic expression is often characterized by abnormal regulation of hormone production (e.g., defective feedback control in ectopic ACTH) and peptide processing (resulting in large, unprocessed precursor peptide such as proopiomelanocortin [POMC]).

Many different molecular mechanisms can cause ectopic hormone production. In rare instances, genetic rearrangements account for aberrant hormone expression. For example, translocation of the parathyroid hormone (*PTH*) gene can result in high levels of *PTH* expression in tissues other than the parathyroid gland because the genetic rearrangement brings the *PTH* gene under the control of atypical regulatory elements. A related phenomenon is well documented in many forms of leukemia and lymphoma, in which somatic genetic rearrangements confer a growth advantage and alter cellular differentiation and function. Although genetic rearrangements cause selected cases of ectopic hormone production, this mechanism is rare, as many tumors are associated with excessive production of numerous peptides. Cellular dedifferentiation probably underlies most cases of ectopic hormone production. Many cancers are poorly differentiated, and certain tumor products, such as human chorionic gonadotropin (hCG), PTH-related protein (PTHRP), and α fetoprotein, are characteristic of gene expression at earlier developmental stages. In contrast, the propensity of certain cancers to produce particular hormones (e.g., squamous cell carcinomas produce PTHRP) suggests that dedifferentiation is partial or that selective pathways are derepressed. These expression profiles probably reflect epigenetic modifications that alter transcriptional

repression, microRNA expression, and other pathways that govern cell differentiation.

In SCLC, the pathway of differentiation has been relatively well defined. The neuroendocrine phenotype is dictated in part by the basic-helix-loop-helix (bHLH) transcription factor human achaete-scute homologue 1 (hASH1), which is expressed at abnormally high levels in SCLC associated with ectopic ACTH. The abnormal expression of hASH1 and other developmental transcription factors appears to provide a link between cell proliferation and differentiation.

Ectopic hormone production might be considered merely epiphenomenon associated with cancer if it did not cause clinical manifestations. Excessive and unregulated production of hormones such as ACTH, PTHrP, and vasopressin can lead to substantial morbidity and complicate the cancer treatment plan. Moreover, the paraneoplastic endocrinopathies may be a presenting clinical feature of underlying malignancy and prompt the search for an unrecognized tumor.

A large number of paraneoplastic endocrine syndromes have been described, linking overproduction of particular hormones with specific types of tumors. However, certain recurring syndromes emerge from this group (Table 93-1). The most common paraneoplastic endocrine syndromes include hypercalcemia from overproduction of PTHrP and other factors, hyponatremia from excess vasopressin, and Cushing's syndrome from ectopic ACTH.

■ HYPERCALCEMIA CAUSED BY ECTOPIC PRODUCTION OF PTHrP

(See also Chap. 410)

Etiology Humoral hypercalcemia of malignancy (HHM) occurs in up to 20% of patients with cancer. HHM is most common in cancers of the lung, head and neck, skin, esophagus, breast, and genitourinary tract and in multiple myeloma and lymphomas, as well as metastases associated with these, and other cancers. There are several distinct humoral causes of HHM, but it is caused most commonly by overproduction of PTHrP. In addition to acting as a circulating humoral factor, bone metastases (e.g., breast, multiple myeloma) may produce PTHrP and other chemokines, leading to local osteolysis and hypercalcemia. PTHrP may also affect the initiation and progression of tumors by acting through pro-survival and chemokine pathways.

PTHrP is structurally related to PTH and binds to the PTH receptor, explaining the similar biochemical features of HHM and hyperparathyroidism. PTHrP plays a key physiologic role in skeletal development and regulates cellular proliferation and differentiation in other tissues, including skin, bone marrow, breast, and hair follicles. The mechanism of PTHrP induction in malignancy is incompletely understood; however, tumor-bearing tissues commonly associated with HHM normally produce PTHrP during development or cell renewal. PTHrP expression is stimulated by hedgehog pathways and Gli transcription factors that are active in many malignancies. Transforming growth factor β (TGF- β), which is produced by many tumors, also stimulates PTHrP. Mutations in certain oncogenes, such as Ras, also can activate PTHrP expression, as does loss of the tumor suppressor, p53. In addition to its role in HHM, the PTHrP pathway may also provide a potential target for therapeutic intervention to impede cancer growth.

TABLE 93-1 Paraneoplastic Syndromes Caused by Ectopic Hormone Production

PARANEOPLASTIC SYNDROME	ECTOPIC HORMONE	TYPICAL TUMOR TYPES ^a
Common		
Hypercalcemia of malignancy	Parathyroid hormone-related protein (PTHrP) 1,25-Dihydroxyvitamin D Parathyroid hormone (PTH) (rare) Prostaglandin E ₂ (PGE ₂) (rare)	Squamous cell (head and neck, lung, skin), breast, genitourinary, gastrointestinal; osteolytic metastases Lymphomas Lung, ovary Renal, lung
Syndrome of inappropriate antidiuretic hormone secretion (SIADH)	Vasopressin	Lung (squamous, small cell), gastrointestinal, genitourinary, ovary
Cushing's syndrome	Adrenocorticotrophic hormone (ACTH) Corticotropin-releasing hormone (CRH) (rare) Ectopic expression of gastric inhibitory peptide (GIP), luteinizing hormone (LH)/human chorionic gonadotropin (hCG), other G protein-coupled receptors (rare)	Lung (small cell, bronchial carcinoid, adenocarcinoma, squamous), thymus, pancreatic islet, medullary thyroid carcinoma, pheochromocytoma Pancreatic islet, carcinoid, lung, prostate Macronodular adrenal hyperplasia
Less Common		
Non-islet cell hypoglycemia	Insulin-like growth factor type II (IGF-II) Insulin (rare)	Mesenchymal tumors, sarcomas, adrenal, hepatic, gastrointestinal, kidney, prostate Cervix (small-cell carcinoma)
Male feminization	hOG ^b	Testis (embryonal, seminomas), germinomas, choriocarcinoma, lung, hepatic, pancreatic islet
Diarrhea or intestinal hypermotility	Calcitonin ^c Vasoactive intestinal peptide (VIP)	Lung, colon, breast, medullary thyroid carcinoma Pancreas, pheochromocytoma, esophagus
Rare		
Oncogenic osteomalacia	Fibroblast growth factor 23 (FGF23) or phosphatonin	Hemangiopericytomas, osteoblastomas, fibromas, sarcomas, giant cell tumors, prostate, lung
Acromegaly	Growth hormone-releasing hormone (GHRH) Growth hormone (GH)	Pancreatic islet, bronchial, and other carcinoids Lung, pancreatic islet
Hyperthyroidism	Thyroid-stimulating hormone (TSH)	Hydatidiform mole, embryonal tumors, struma ovarii
Hypertension	Renin	Juxtaglomerular tumors, kidney, lung, pancreas, ovary
Consumptive hypothyroidism	Type 3 deiodinase	Hepatic and other hemangiomas

^aOnly the most common tumor types are listed. For most ectopic hormone syndromes, an extensive list of tumors has been reported to produce one or more hormones.

^bhCG is produced ectopically by trophoblastic tumors. Certain tumors produce disproportionate amounts of the hCG α or hCG β subunit. High levels of hCG rarely cause hyperthyroidism because of weak binding to the TSH receptor. ^cCalcitonin is produced ectopically by medullary thyroid carcinoma and is used as a tumor marker.

Another relatively common cause of HHM is excess production of 1,25-dihydroxyvitamin D. Like granulomatous disorders associated with hypercalcemia, lymphomas can produce an enzyme that converts 25-hydroxyvitamin D to the more active 1,25-dihydroxyvitamin D, leading to enhanced gastrointestinal calcium absorption. Other causes of HHM include tumor-mediated production of osteolytic cytokines and inflammatory mediators.

Clinical Manifestations The typical presentation of HHM is a patient with a known malignancy who is found to be hypercalcemic on routine laboratory tests. Less often, hypercalcemia is the initial presenting feature of malignancy. Particularly when calcium levels are markedly increased ($>3.5 \text{ mmol/L}$ [$>14 \text{ mg/dL}$]), patients may experience fatigue, mental status changes, polyuria, dehydration, or symptoms of nephrolithiasis. Hypercalcemia can shorten ST segments and QT intervals, as well as bundle branch blocks and bradycardia.

Diagnosis Features that favor HHM, as opposed to primary hyperparathyroidism, include known malignancy, recent onset of hypercalcemia, and very high serum calcium levels. Like hyperparathyroidism, hypercalcemia caused by PTHrP is accompanied by hypercalciuria and hypophosphatemia. Patients with HHM typically have metabolic alkalosis rather than hyperchloremic acidosis, as is seen in hyperparathyroidism. Measurement of PTH is useful to exclude primary hyperparathyroidism; the PTH level should be suppressed in HHM. An elevated PTHrP level confirms the diagnosis, and it is increased in ~80% of hypercalcemic patients with cancer. 1,25-Dihydroxyvitamin D levels may be increased in patients with lymphoma.

TREATMENT

Humoral Hypercalcemia of Malignancy

The management of HHM begins with removal of excess calcium in the diet, medications, or IV solutions. Saline rehydration (typically 200–500 mL/h) is used to dilute serum calcium and promote calciuresis; exercise caution in patients with cardiac, hepatic, or renal insufficiency. Forced diuresis with furosemide (20–80 mg IV in escalating doses) or other loop diuretics can enhance calcium excretion but provides relatively little value except in life-threatening hypercalcemia. When used, loop diuretics should be administered only after complete rehydration and with careful monitoring of fluid balance. Oral phosphorus (e.g., 250 mg Neutra-Phos 3–4 times daily) should be given until serum phosphorus is $>1 \text{ mmol/L}$ ($>3 \text{ mg/dL}$). Bisphosphonates such as pamidronate (60–90 mg IV), zoledronate (4–8 mg IV), and etidronate (7.5 mg/kg per day PO for 3–7 consecutive days) can reduce serum calcium within 1–2 days and suppress calcium release for several weeks. Bisphosphonate infusions can be repeated, or oral bisphosphonates can be used for chronic treatment. Denosumab (120 mg SC weekly for 4 weeks and then monthly) can be used in patients who do not respond adequately to bisphosphonates. It acts as a decoy receptor for RANK ligand to mitigate stimulation of osteoclasts. Cinacalcet (30 mg PO bid to 90 mg PO qid) stimulates calcium-sensing receptors to suppress PTH secretion and is therefore applicable in parathyroid carcinoma and rare cases of ectopic PTH-producing tumors. Hypercalcemia associated with lymphomas, multiple myeloma, or leukemia may respond to glucocorticoid treatment (e.g., prednisone 40–100 mg PO in four divided doses). Dialysis should be considered in severe hypercalcemia when saline hydration and bisphosphonate treatments are not possible or are too slow in onset. Previously used agents such as calcitonin and mithramycin have little utility now that bisphosphonates and other agents are available.

■ ECTOPIC VASOPRESSIN: TUMOR ASSOCIATED SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE

(See also Chap. 53)

Etiology Vasopressin is an antidiuretic hormone normally produced by the posterior pituitary gland. Ectopic vasopressin production by tumors is a common cause of the syndrome of inappropriate antidiuretic hormone (SIADH), occurring in at least half of patients with SCLC. SIADH also can be caused by a number of nonneoplastic conditions, including central nervous system (CNS) trauma, infections, and medications (Chap. 381). Compensatory responses to SIADH, such as decreased thirst, may mitigate the development of hyponatremia. However, with prolonged production of excessive vasopressin, the osmostat controlling thirst and hypothalamic vasopressin secretion may become reset. In addition, intake of free water, orally or intravenously, can quickly worsen hyponatremia because of reduced renal diuresis.

Tumors with neuroendocrine features, such as SCLC and carcinoids, are the most common sources of ectopic vasopressin production, but it also occurs in other forms of lung cancer and with CNS lesions, head and neck cancer, and genitourinary, gastrointestinal, and ovarian cancers. The mechanism of activation of the vasopressin gene in these tumors is unknown, but the frequent concomitant expression of the adjacent oxytocin gene suggests derepression of this locus.

Clinical Manifestations Most patients with ectopic vasopressin secretion are asymptomatic and are identified because of the presence of hyponatremia on routine chemistry testing. Symptoms may include weakness, lethargy, nausea, confusion, depressed mental status, and seizures. The severity of symptoms reflects the rapidity of onset as well as the severity of hyponatremia. Hyponatremia usually develops slowly but may be exacerbated by the administration of IV fluids or the institution of new medications.

Diagnosis The diagnostic features of ectopic vasopressin production are the same as those of other causes of SIADH (Chaps. 53 and 381). Hyponatremia and reduced serum osmolality occur in the setting of an inappropriately normal or increased urine osmolality. Urine sodium excretion is normal or increased unless volume depletion is present. Other causes of hyponatremia should be excluded, including renal, adrenal, or thyroid insufficiency. Physiologic sources of vasopressin stimulation (CNS lesions, pulmonary disease, nausea), adaptive circulatory mechanisms (hypotension, heart failure, hepatic cirrhosis), and medications, including many chemotherapeutic agents, also should be considered as possible causes of hyponatremia. Vasopressin measurements are not usually necessary to make the diagnosis.

TREATMENT

Ectopic Vasopressin: Tumor-Associated SIADH

Most patients with ectopic vasopressin production develop hyponatremia over several weeks or months. The disorder should be corrected gradually unless mental status is altered or there is risk of seizures. Rapid correction can cause brain dehydration and central pontine myelinolysis. Treatment of the underlying malignancy may reduce ectopic vasopressin production, but this response is slow if it occurs at all. Fluid restriction to less than urine output, plus insensible losses, is often sufficient to correct hyponatremia partially. However, strict monitoring of the amount and types of liquids consumed or administered intravenously is required for fluid restriction to be effective. Salt tablets and saline are not helpful unless volume depletion is also present. Demeclocycline (150–300 mg orally 3–4 times daily) can be used to inhibit vasopressin action on the renal distal tubule, but its onset of action is relatively slow (1–2 weeks). The vaptan class of drugs acts by inhibiting vasopressin receptors (V_{1A} , V_{1B} , V_2) in the renal collecting ducts. Conivaptan, a nonpeptide V_2 -receptor antagonist, can be administered either PO (20–120 mg bid) or IV (10–40 mg) and is particularly effective when used in combination with fluid restriction in euvolemic hyponatremia. Tolvaptan (15 mg PO daily) is another vasopressin antagonist. The dose can be increased to 30–60 mg/d based on response. Severe hyponatremia ($\text{Na} < 115 \text{ meq/L}$) or mental status changes may require treatment with hypertonic (3%) or normal saline infusion together with furosemide to enhance free water clearance. The rate of sodium

correction should be slow (0.5–1 meq/L per hour) to prevent rapid fluid shifts and the possible development of central pontine myelinolysis.

CUSHING'S SYNDROME CAUSED BY ECTOPIC ACTH PRODUCTION

(See also [Chap. 386](#))

Etiology Ectopic ACTH production accounts for 10–20% of cases of Cushing's syndrome. The syndrome is particularly common in neuroendocrine tumors. SCLC is the most common cause of ectopic ACTH, followed by bronchial and thymic carcinoids, islet cell tumors, other carcinoids, and pheochromocytomas. Ectopic ACTH production is caused by increased expression of the proopiomelanocortin (*POMC*) gene, which encodes ACTH, along with melanocyte-stimulating hormone (MSH), β lipotropin, and several other peptides. In many tumors, there is abundant but aberrant expression of the *POMC* gene from an internal promoter, proximal to the third exon, which encodes ACTH. However, because this product lacks the signal sequence necessary for protein processing, it is not secreted. Increased production of ACTH arises instead from less abundant, but unregulated, *POMC* expression from the same promoter site used in the pituitary. Because tumors lack many of the enzymes needed to process the *POMC* polypeptide, it is typically released as multiple large, biologically inactive fragments along with relatively small amounts of fully processed, active ACTH.

Rarely, corticotropin-releasing hormone (CRH) is produced by pancreatic islet cell tumors, SCLC, medullary thyroid cancer, carcinoids, or prostate cancer. When levels are high enough, CRH can cause pituitary corticotrope hyperplasia and Cushing's syndrome. Tumors that produce CRH sometimes also produce ACTH, raising the possibility of a paracrine mechanism for ACTH production.

A distinct mechanism for ACTH-independent Cushing's syndrome involves ectopic expression of various G protein-coupled receptors in adrenal nodules. Ectopic expression of the gastric inhibitory peptide (GIP) receptor is the best-characterized example of this mechanism. In this case, meals induce GIP secretion, which inappropriately stimulates adrenal growth and glucocorticoid production.

Clinical Manifestations The clinical features of hypercortisolism are detected in only a fraction of patients with documented ectopic ACTH production. Patients with ectopic ACTH syndrome generally exhibit less marked weight gain and centripetal fat redistribution, probably because the exposure to excess glucocorticoids is relatively brief and because cachexia reduces the propensity for weight gain and fat deposition. The ectopic ACTH syndrome is associated with several clinical features that distinguish it from other causes of Cushing's syndrome (e.g., pituitary adenomas, adrenal adenomas, iatrogenic glucocorticoid excess). The metabolic manifestations of ectopic ACTH syndrome are dominated by fluid retention and hypertension, hypokalemia, metabolic alkalosis, glucose intolerance, and occasionally steroid psychosis. The very high ACTH levels often cause increased pigmentation, reflecting increased activity of MSH derived from the *POMC* precursor peptide. The extraordinarily high glucocorticoid levels in patients with ectopic sources of ACTH can lead to marked skin fragility and easy bruising. In addition, the high cortisol levels often overwhelm the renal 11 β -hydroxysteroid dehydrogenase type II enzyme, which normally inactivates cortisol and prevents it from binding to renal mineralocorticoid receptors. Consequently, in addition to the excess mineralocorticoids produced by ACTH stimulation of the adrenal gland, high levels of cortisol exert activity through the mineralocorticoid receptor, leading to severe hypokalemia.

Diagnosis The diagnosis of ectopic ACTH syndrome is usually not difficult in the setting of a known malignancy. Urine-free cortisol levels fluctuate but are typically greater than two to four times normal, and the plasma ACTH level is usually >22 pmol/L (>100 pg/mL). A suppressed ACTH level excludes this diagnosis and indicates an ACTH-independent cause of Cushing's syndrome (e.g., adrenal or exogenous glucocorticoid). In contrast to pituitary sources of ACTH,

most ectopic sources of ACTH do not respond to glucocorticoid suppression. Therefore, high-dose dexamethasone (8 mg PO) suppresses 8:00 . . . serum cortisol (50% decrease from baseline) in ~80% of pituitary ACTH-producing adenomas but fails to suppress ectopic ACTH in ~90% of cases. Bronchial and other carcinoids are well-documented exceptions to these general guidelines, as these ectopic sources of ACTH may exhibit feedback regulation indistinguishable from pituitary adenomas, including suppression by high-dose dexamethasone, and ACTH responsiveness to adrenal blockade with metyrapone. If necessary, petrosal sinus catheterization can be used to evaluate a patient with ACTH-dependent Cushing's syndrome when the source of ACTH is unclear. After CRH stimulation, a 3:1 petrosal sinus:peripheral ACTH ratio strongly suggests a pituitary ACTH source. Imaging studies (computed tomography or magnetic resonance imaging) are also useful in the evaluation of suspected carcinoid lesions, allowing biopsy and characterization of hormone production using special stains. If available, positron emission tomography or octreotide scanning may identify some sources of ACTH production.

TREATMENT

Cushing's Syndrome Caused by Ectopic ACTH Production

The morbidity associated with the ectopic ACTH syndrome can be substantial. Patients may experience depression or personality changes because of extreme cortisol excess. Metabolic derangements, including diabetes mellitus and hypokalemia, can worsen fatigue. Poor wound healing and predisposition to infections can complicate the surgical management of tumors, and opportunistic infections caused by organisms such as *Pneumocystis carinii* and mycoses are often the cause of death in patients with ectopic ACTH production. These patients have increased risk of venous thromboembolism reflecting the combination of malignancy and altered coagulation factor profiles. Depending on prognosis and treatment plans for the underlying malignancy, measures to reduce cortisol levels are often indicated. Treatment of the underlying malignancy may reduce ACTH levels but is rarely sufficient to reduce cortisol levels to normal. Adrenalectomy is not practical for most of these patients but should be considered during surgery for the malignancy or if the underlying tumor is not resectable and the prognosis is otherwise favorable (e.g., carcinoid). Medical therapy with ketoconazole (300–600 mg PO bid), metyrapone (250–500 mg PO every 6 h), mitotane (3–6 g PO in four divided doses, tapered to maintain low cortisol production), etomidate (0.1–0.3 mg/kg/h IV), or other agents that block steroid synthesis or action is often the most practical strategy for managing the hypercortisolism associated with ectopic ACTH production. Glucocorticoid replacement should be provided to prevent adrenal insufficiency ([Chap. 386](#)). Unfortunately, many patients eventually progress despite medical blockade. Mifepristone (200–1000 mg PO qd) inhibits both glucocorticoid and progesterone receptors, has rapid onset of action, and improves glucose intolerance and hypertension in a subset of patients. ACTH-neutralizing antibodies and ACTH receptor blockers are under investigation, as are selective inhibitors of the glucocorticoid receptor.

TUMOR INDUCED HYPOGLYCEMIA CAUSED BY EXCESS PRODUCTION OF INSULIN LIKE GROWTH FACTOR TYPE II

(See also [Chap. 406](#)) Mesenchymal tumors, hemangiopericytomas, hepatocellular tumors, adrenal carcinomas, and a variety of other large tumors have been reported to produce excessive amounts of insulin-like growth factor type II (IGF-II) precursor, which binds weakly to insulin receptors and more strongly to IGF-I receptors, leading to insulin-like actions. The gene encoding IGF-II resides on chromosome 11p15, a locus that is normally imprinted (that is, expression

is exclusively from a single parental allele). Biallelic expression of the IGF-II gene occurs in a subset of tumors, suggesting loss of methylation and loss of imprinting as a mechanism for gene induction. In addition to increased IGF-II production, IGF-II bioavailability is increased due to complex alterations in circulating binding proteins. Increased IGF-II suppresses growth hormone (GH) and insulin, resulting in reduced IGF binding protein 3 (IGFBP-3), IGF-I, and acid-labile subunit (ALS). The reduction in ALS and IGFBP-3, which normally sequester IGF-II, causes it to be displaced to a small circulating complex that has greater access to insulin target tissues. For this reason, circulating IGF-II levels may not be markedly increased despite causing hypoglycemia. In addition to IGF-II–mediated hypoglycemia, tumors may occupy enough of the liver to impair gluconeogenesis.

In most cases, a tumor causing hypoglycemia is clinically apparent (usually >10 cm in size), and hypoglycemia develops in association with fasting. As with other causes of hypoglycemia, patients may present with sweating, tremors, palpitations, confusion, seizures, or coma. The diagnosis is made by documenting low serum glucose and suppressed insulin levels in association with symptoms of hypoglycemia. Serum IGF-II levels may not be increased (IGF-II assays may not detect IGF-II precursors), but an elevated IGF-II/IGF-I ratio greater than 10:1 is suggestive. Increased IGF-II mRNA expression is found in most of these tumors. Any medications associated with hypoglycemia should be eliminated. Treatment of the underlying malignancy, if possible, may reduce the predisposition to hypoglycemia. Frequent meals and IV glucose, especially during sleep or fasting, are often necessary to prevent hypoglycemia. Glucagon and glucocorticoids have also been used to enhance glucose production. Antibodies that inhibit IGF-II action are under development.

■ HUMAN CHORIONIC GONADOTROPIN

hCG is composed of α and β subunits and can be produced as intact hormone, which is biologically active, or as uncombined biologically inert subunits. Ectopic production of intact hCG occurs most often in association with testicular embryonal tumors, germ cell tumors, extragonadal germinomas, lung cancer, hepatoma, and pancreatic islet tumors. Eutopic production of hCG occurs with trophoblastic malignancies. hCG α subunit production is particularly common in lung cancer and pancreatic islet cancer. In men, high hCG levels stimulate steroidogenesis and aromatase activity in testicular Leydig cells, resulting in increased estrogen production and the development of gynecomastia. Precocious puberty in boys or gynecomastia in men should prompt measurement of hCG and consideration of a testicular tumor or another source of ectopic hCG production. Most women are asymptomatic. hCG is easily measured. Treatment should be directed at the underlying malignancy.

■ ONCOGENIC OSTEOMALACIA

Hypophosphatemic oncogenic osteomalacia, also called tumor-induced osteomalacia (TIO), is caused by excessive production of fibroblast growth factor 23 (FGF23), previously referred to as phosphatonin. Oncogenic osteomalacia is characterized by markedly reduced serum phosphorus and renal phosphate wasting, leading to muscle weakness, bone pain, and osteomalacia. Serum calcium and PTH levels are normal. FGF23 inhibits the renal conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, resulting in low levels of 1,25-dihydroxyvitamin D. Oncogenic osteomalacia is usually caused by benign mesenchymal tumors, such as hemangiopericytomas, fibromas, and giant cell tumors, often of the skeletal extremities or head. It has also been described in sarcomas and in patients with prostate or lung cancer. Resection of the tumor reverses the disorder, confirming its humoral basis. FGF23 levels are increased in some, but not all, patients with osteogenic osteomalacia. FGF23 forms a ternary complex with the klotho protein and renal FGF receptors to reduce renal phosphate reabsorption. Treatment involves removal of the tumor, if possible, and supplementation with phosphate and vitamin D. Octreotide treatment reduces phosphate wasting in some patients with tumors that express somatostatin receptor subtype 2. Octreotide scans may also be useful in detecting these tumors. The calcium-sensing

receptor agonist, cinacalcet, has been effective in some patients, apparently by reducing PTH-mediated phosphaturia. FGF receptor inhibitors hold promise as future therapies targeted either to pathways that stimulate FGF23 production (e.g., FGFR1) or inhibit its action (e.g., FGF23 receptor).

■ CONSUMPTIVE HYPOTHYROIDISM

Newborns with hepatic hemangiomas can develop a rare form of hypothyroidism caused by overexpression of type 3 deiodinase (D3), an enzyme that degrades and inactivates thyroxine (T_4) and triiodothyronine (T_3). The very high expression of D3 and consumption of thyroid hormones apparently outstrip the thyroid gland's rate of hormone production. The disorder is characterized by low T_4 , low T_3 , high TSH, and markedly elevated reverse T_3 (rT_3), reflecting the degradation of T_4 to rT_3 . In addition to treating the underlying hemangioma (rarely other tumor types), patients are treated with -thyroxine replacement, titrated to normalize TSH. Steroids and propranolol may provide benefit, perhaps by inhibiting growth factor pathways thought to stimulate D3 production.

HEMATOLOGIC SYNDROMES

The elevation of granulocyte, platelet, and eosinophil counts in most patients with myeloproliferative disorders is caused by the proliferation of the myeloid elements due to the underlying disease rather than to a paraneoplastic syndrome. The paraneoplastic hematologic syndromes in patients with solid tumors are less well characterized than are the endocrine syndromes because the ectopic hormone(s) or cytokines responsible have not been identified in most of these tumors (Table 93-2). The extent of the paraneoplastic syndromes parallels the course of the cancer. With very rare exception, red cell, white cell or platelet numbers are self-limited and not associated with symptomatic abnormalities. In some circumstances, elevations in platelet counts can be a marker that influences prognosis. By far, the most consequential hematologic abnormality in cancer patients is hypercoagulability.

■ ERYTHROCYTOSIS

Ectopic production of erythropoietin by cancer cells causes most paraneoplastic erythrocytosis. The ectopically produced erythropoietin stimulates the production of red blood cells (RBCs) in the bone marrow and raises the hematocrit. Other lymphokines and hormones produced by cancer cells may stimulate erythropoietin release but have not been proved to cause erythrocytosis.

Most patients with erythrocytosis have an elevated hematocrit (>52% in men, >48% in women) that is detected on a routine blood

TABLE 93-2 Paraneoplastic Hematologic Syndromes

SYNDROME	PROTEINS	CANCERS TYPICALLY ASSOCIATED WITH SYNDROME
Erythrocytosis	Erythropoietin	Renal cancers, hepatocarcinoma, cerebellar hemangioblastomas
Granulocytosis	G-CSF, GM-CSF, IL-6	Lung cancer, gastrointestinal cancer, ovarian cancer, genitourinary cancer, Hodgkin's disease
Thrombocytosis	IL-6	Lung cancer, gastrointestinal cancer, breast cancer, ovarian cancer, lymphoma
Eosinophilia	IL-5	Lymphoma, leukemia, lung cancer
Thrombophlebitis	Unknown	Lung cancer, pancreatic cancer, gastrointestinal cancer, breast cancer, genitourinary cancer, ovarian cancer, prostate cancer, lymphoma

Abbreviations: G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin.

count. Approximately 3% of patients with renal cell cancer, 10% of patients with hepatoma, and 15% of patients with cerebellar hemangioblastomas have erythrocytosis. In most cases, the erythrocytosis is asymptomatic.

Patients with erythrocytosis due to a renal cell cancer, hepatoma, or CNS cancer should have measurement of red cell mass. If the red cell mass is elevated, the serum erythropoietin level should be measured. Patients with a cancer that has been associated with erythrocytosis, elevated erythropoietin levels, and no other explanation for erythrocytosis (e.g., hemoglobinopathy that causes increased O₂ affinity; Chaps. 63 and 98) have the paraneoplastic syndrome.

TREATMENT

Erythrocytosis

Successful resection of the cancer usually resolves the erythrocytosis. If the tumor cannot be resected or treated effectively with radiation therapy or chemotherapy, phlebotomy may control any symptoms or risk related to erythrocytosis.

■ GRANULOCYTOSIS

Approximately 30% of patients with solid tumors have granulocytosis (granulocyte count >8000/ μ L). In about half of patients with granulocytosis and cancer, the granulocytosis has an identifiable nonparaneoplastic etiology (e.g., infection, tumor necrosis, glucocorticoid administration). The other patients have proteins in urine and serum that stimulate the growth of bone marrow cells. Tumors and tumor cell lines from patients with lung, ovarian, and bladder cancers have been documented to produce granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and/or interleukin 6 (IL-6). However, the etiology of granulocytosis has not been characterized in most patients.

Patients with granulocytosis are nearly all asymptomatic, and the differential white blood cell count does not have a shift to immature forms of neutrophils. Granulocytosis occurs in 40% of patients with lung and gastrointestinal cancers, 20% of patients with breast cancer, 30% of patients with brain tumors and ovarian cancers, 20% of patients with Hodgkin's disease, and 10% of patients with renal cell carcinoma. Patients with advanced-stage disease are more likely to have granulocytosis than are those with early-stage disease.

Paraneoplastic granulocytosis does not require treatment. The granulocytosis resolves when the underlying cancer is treated.

■ THROMBOCYTOSIS

Some 35% of patients with thrombocytosis (platelet count >400,000/ μ L) have an underlying diagnosis of cancer. IL-6, a candidate molecule for the etiology of paraneoplastic thrombocytosis, stimulates the production of platelets *in vitro* and *in vivo*. Some patients with cancer and thrombocytosis have elevated levels of IL-6 in plasma. Another candidate molecule is thrombopoietin, a peptide hormone that stimulates megakaryocyte proliferation and platelet production. The etiology of thrombocytosis has not been established in most cases.

Patients with thrombocytosis are nearly all asymptomatic. Thrombocytosis is not clearly linked to thrombosis in patients with cancer. Thrombocytosis is present in 40% of patients with lung and gastrointestinal cancers; 20% of patients with breast, endometrial, and ovarian cancers; and 10% of patients with lymphoma. Patients with thrombocytosis are more likely to have advanced-stage disease and have a poorer prognosis than do patients without thrombocytosis. In ovarian cancer, IL-6 has been shown to directly promote tumor growth. Paraneoplastic thrombocytosis does not require treatment other than treatment of the underlying tumor.

■ EOSINOPHILIA

Eosinophilia is present in ~1% of patients with cancer. Tumors and tumor cell lines from patients with lymphomas or leukemia may

produce IL-5, which stimulates eosinophil growth. Activation of IL-5 transcription in lymphomas and leukemias may involve translocation of the long arm of chromosome 5, to which the genes for IL-5 and other cytokines map.

Patients with eosinophilia are typically asymptomatic. Eosinophilia is present in 10% of patients with lymphoma, 3% of patients with lung cancer, and occasional patients with cervical, gastrointestinal, renal, and breast cancer. Patients with markedly elevated eosinophil counts (>5000/ μ L) can develop shortness of breath and wheezing. A chest radiograph may reveal diffuse pulmonary infiltrates from eosinophil infiltration and activation in the lungs.

TREATMENT

Eosinophilia

Definitive treatment is directed at the underlying malignancy. Tumors should be resected or treated with radiation or chemotherapy. In most patients who develop shortness of breath related to eosinophilia, symptoms resolve with the use of oral or inhaled glucocorticoids. IL-5 antagonists exist but have not been evaluated in this clinical setting.

■ THROMBOPHLEBITIS AND DEEP VENOUS THROMBOSIS

Deep venous thrombosis and pulmonary embolism are the most common thrombotic conditions in patients with cancer. Migratory or recurrent thrombophlebitis may be the initial manifestation of cancer. Nearly 15% of patients who develop deep venous thrombosis or pulmonary embolism have a diagnosis of cancer (Chap. 117). The coexistence of peripheral venous thrombosis with visceral carcinoma, particularly pancreatic cancer, is called *Trousseau's syndrome*.

Pathogenesis Patients with cancer are predisposed to thromboembolism because they are often at bed rest or immobilized, and tumors may obstruct or slow blood flow. Postoperative deep venous thrombosis is twice as common in cancer patients who undergo surgery. Chronic IV catheters also predispose to clotting. In addition, clotting may be promoted by release of procoagulants or cytokines from tumor cells or associated inflammatory cells or by platelet adhesion or aggregation. The specific molecules that promote thromboembolism have not been identified.

Chemotherapeutic agents, particularly those associated with endothelial damage, can induce venous thrombosis. The annual risk of venous thrombosis in patients with cancer receiving chemotherapy is about 11%, sixfold higher than the risk in the general population. Bleomycin, -asparaginase, nitrogen mustard, thalidomide analogues, cisplatin-based regimens, and high doses of busulfan and carmustine are all associated with an increased risk.

In addition to cancer and its treatment causing secondary thrombosis, primary thrombophilic diseases may be associated with cancer. For example, the antiphospholipid antibody syndrome is associated with a wide range of pathologic manifestations (Chap. 357). About 20% of patients with this syndrome have cancers. Among patients with cancer and antiphospholipid antibodies, 35–45% develop thrombosis.

Clinical Manifestations Patients with cancer who develop deep venous thrombosis usually develop swelling or pain in the leg, and physical examination reveals tenderness, warmth, and redness. Patients who present with pulmonary embolism develop dyspnea, chest pain, and syncope, and physical examination shows tachycardia, cyanosis, and hypotension. Some 5% of patients with no history of cancer who have a diagnosis of deep venous thrombosis or pulmonary embolism will have a diagnosis of cancer within 1 year. The most common cancers associated with thromboembolic episodes include lung, pancreatic, gastrointestinal, breast, ovarian, and genitourinary cancers; lymphomas; and brain tumors. Patients with cancer who undergo

surgical procedures requiring general anesthesia have a 20–30% risk of deep venous thrombosis.

Diagnosis The diagnosis of deep venous thrombosis in patients with cancer is made by impedance plethysmography or bilateral compression ultrasonography of the leg veins. Patients with a noncompressible venous segment have deep venous thrombosis. If compression ultrasonography is normal and there is a high clinical suspicion for deep venous thrombosis, venography should be done to look for a luminal filling defect. Elevation of D-dimer is not as predictive of deep venous thrombosis in patients with cancer as it is in patients without cancer; elevations are seen in people over age 65 years without concomitant evidence of thrombosis, probably as a consequence of increased thrombin deposition and turnover in aging.

Patients with symptoms and signs suggesting a pulmonary embolism should be evaluated with a chest radiograph, electrocardiogram, arterial blood gas analysis, and ventilation-perfusion scan. Patients with mismatched segmental perfusion defects have a pulmonary embolus. Patients with equivocal ventilation-perfusion findings should be evaluated as described above for deep venous thrombosis in their legs. If deep venous thrombosis is detected, they should be anticoagulated. If deep venous thrombosis is not detected, they should be considered for a pulmonary angiogram.

Patients without a diagnosis of cancer who present with an initial episode of thrombophlebitis or pulmonary embolus need no additional tests for cancer other than a careful history and physical examination. In light of the many possible primary sites, diagnostic testing in asymptomatic patients is wasteful. However, if the clot is refractory to standard treatment or is in an unusual site, or if the thrombophlebitis is migratory or recurrent, efforts to find an underlying cancer are indicated.

TREATMENT

Thrombophlebitis and Deep Venous Thrombosis

Patients with cancer and a diagnosis of deep venous thrombosis or pulmonary embolism should be treated initially with IV unfractionated heparin or low-molecular-weight heparin for at least 5 days, and warfarin should be started within 1 or 2 days. The warfarin dose should be adjusted so that the international normalized ratio (INR) is 2–3. Patients with proximal deep venous thrombosis and a relative contraindication to heparin anticoagulation (hemorrhagic brain metastases or pericardial effusion) should be considered for placement of a filter in the inferior vena cava (Greenfield filter) to prevent pulmonary embolism. Warfarin should be administered for 3–6 months. An alternative approach is to use low-molecular-weight heparin for 6 months. The new oral anticoagulants (factor Xa and thrombin inhibitors) are attractive because they do not require close monitoring of the prothrombin time and are not affected by dietary factors. Oral apixaban (10 mg bid for 7 days followed by 5 mg bid for 6 months) is noninferior to dalteparin in the treatment of cancer patients who develop deep vein thrombosis or pulmonary embolism. Patients with cancer who undergo a major surgical procedure should be considered for heparin prophylaxis or pneumatic boots. Breast cancer patients undergoing chemotherapy and patients with implanted catheters should be considered for prophylaxis. Guidelines recommend that hospitalized patients with cancer and patients receiving a thalidomide analogue receive prophylaxis with low-molecular-weight heparin or low-dose aspirin. Use of prophylaxis routinely during chemotherapy is controversial. Risk is affected by type of cancer, type of therapy, blood counts, and body mass index (all taken into account in the Khorana risk score; Table 93-3). Studies of Khorana high-risk patients with cancer using rivaroxaban and apixaban as clot prophylaxis have resulted in a 50% reduction in risk with a level of bleeding of about 5%. However,

TABLE 93-3 Khorana Risk Score for Venous Thromboembolism in Cancer Patients

PATIENT CHARACTERISTICS	RISK SCORE POINTS	
Site of cancer		
Very high risk (stomach, pancreas)	2	
High risk (lung, lymphoma, gynecologic, genitourinary excluding prostate)	1	
Prechemotherapy platelet count $\geq 350,000/\mu\text{L}$	1	
Hemoglobin level $< 10 \text{ g/dL}$ or use of red cell growth factors	1	
Prechemotherapy leukocyte count $> 11,000/\mu\text{L}$	1	
BMI $\geq 35 \text{ kg/m}^2$	1	
RISK SCORE (POINTS)	RISK CATEGORY	RATES OF sVTE ACCORDING TO SCORES (%)
0	Low	0.3–0.8
1–2	Intermediate	1.8–2.0
≥ 3	High	6.7–7.1

Abbreviations: BMI, body mass index; sVTE, symptomatic venous thromboembolism.

Source: AJ Muñoz Martín et al: Clinical guide SEOM on venous thromboembolism in cancer patients. Clin Transl Oncol 16:1079, 2014.

prophylaxis is not routinely recommended by the American Society of Clinical Oncology.

MISCELLANEOUS REMOTE EFFECTS OF CANCER

Patients with cancer can develop paraneoplastic autoimmune disorders (e.g., thrombocytopenia) and dysfunction of organs not directly invaded or involved with the cancer (rheumatologic and renal abnormalities are among the most frequent). The pathogenesis of these disorders is undefined, but often, the conditions reverse if the tumor is removed or successfully treated.

Cutaneous paraneoplastic syndromes are discussed in Chap. 58. Neurologic paraneoplastic syndromes are discussed in Chap. 94.

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94

Paraneoplastic Neurologic Syndromes and Autoimmune Encephalitis

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Paraneoplastic neurologic disorders (PNDs) are cancer-related syndromes that can affect any part of the nervous system (Table 94-1). They are caused by mechanisms other than metastasis or by any of the complications of cancer such as coagulopathy, stroke, metabolic and nutritional conditions, infections, and side effects of cancer therapy. In 60% of patients, the neurologic symptoms precede the cancer diagnosis. Clinically disabling PNDs occur in 0.5–1% of all cancer patients, but they affect 2–3% of patients with neuroblastoma or small-cell lung cancer (SCLC) and 30–50% of patients with thymoma.

PATHOGENESIS

Most PNDs are mediated by immune responses triggered by neuronal proteins ectopically expressed by tumors (e.g., SCLC and other cancers) or as a result of altered immunologic responses caused by some types of tumors such as thymomas or lymphomas. In PNDs of the central nervous system (CNS), many antibody-associated immune responses have been identified (Table 94-2). These antibodies react with neurons and the patient's tumor, and their detection in serum or cerebrospinal fluid (CSF) usually predicts the presence of cancer. When the antigens are intracellular, most syndromes are associated with extensive infiltrates of CD4+ and CD8+ T cells, microglial activation, gliosis, and variable neuronal loss. The infiltrating T cells are often in close contact with neurons undergoing degeneration, suggesting a primary pathogenic role. T-cell-mediated cytotoxicity may contribute directly to cell death in these PNDs and probably underlies the resistance of many of these conditions to therapy.

In contrast to the predominant role of cytotoxic T-cell mechanisms in PND associated with antibodies against intracellular antigens, those associated with antibodies to antigens expressed on the neuronal cell surface of the CNS or at the neuromuscular junction are mediated by

TABLE 94-1 Paraneoplastic Syndromes of the Nervous System

CLASSIC SYNDROMES: USUALLY OCCUR WITH CANCER ASSOCIATION	NONCLASSIC SYNDROMES: MAY OCCUR WITH AND WITHOUT CANCER ASSOCIATION
Encephalomyelitis	Brainstem encephalitis
Limbic encephalitis	Stiff-person syndrome
Cerebellar degeneration (adults)	Progressive encephalomyelitis with rigidity and myoclonus
Opsoclonus-myoclonus	Necrotizing myopathy
Subacute sensory neuronopathy	Motor neuron disease
Gastrointestinal paresis or pseudo-obstruction	Guillain-Barré syndrome
Dermatomyositis (adults)	Subacute and chronic mixed sensory-motor neuropathies
Lambert-Eaton myasthenic syndrome	Neuropathy associated with plasma cell dyscrasias and lymphoma
Cancer- or melanoma-associated retinopathy	Vasculitis of nerve
	Pure autonomic neuropathy
	Acute necrotizing myopathy
	Polymyositis
	Optic neuropathy
	BDUMP
	Peripheral nerve hyperexcitability (neuromyotonia)
	Myasthenia gravis

Abbreviation: BDUMP, bilateral diffuse uveal melanocytic proliferation.

TABLE 94-2 Antibodies to Intracellular Antigens, Syndromes, and Associated Cancers

ANTIBODY	ASSOCIATED NEUROLOGIC SYNDROME(S)	TUMORS
Anti-Hu (ANNA1)	Encephalomyelitis, subacute sensory neuronopathy	SCLC
Anti-Yo (PCA1)	Cerebellar degeneration	Ovary, breast
Anti-Ri (ANNA2)	Cerebellar degeneration, opsoclonus, brainstem encephalitis	Breast, gynecologic, SCLC
Anti-CRMP5 (CV2)	Encephalomyelitis, chorea, optic neuritis, uveitis, peripheral neuropathy	SCLC, thymoma, other
Anti-Ma proteins	Limbic, hypothalamic, brainstem encephalitis	Testicular (Ma2), other (Ma)
Anti-Kelch-like protein 11	Brainstem encephalitis, ataxia, hearing loss, diplopia	Seminoma, germ-cell tumor, teratoma
Anti-amphiphysin ^a	Stiff-person syndrome, encephalomyelitis	Breast, SCLC
Recoverin, bipolar cell antibodies, others ^b	Cancer-associated retinopathy (CAR), melanoma-associated retinopathy (MAR)	SCLC (CAR), melanoma (MAR)
Anti-GAD	Stiff-person, cerebellar syndromes, limbic encephalitis	Infrequent tumor association (thymoma and several cancers)

^aAmphiphysin is likely exposed to the cell surface during synaptic vesicle endocytosis. ^bA variety of target antigens have been identified.

Abbreviations: CRMP, collapsin response-mediator protein; SCLC, small-cell lung cancer.

direct antibody effects on the target antigens and are more responsive to immunotherapy (Table 94-3, Fig. 94-1). These disorders occur with and without a cancer association and may affect children and young adults. Some disorders are triggered by viral encephalitis such as herpes simplex virus encephalitis or Japanese encephalitis, leading to autoimmune encephalitis.

In patients with cancer, the use of immune checkpoint inhibitors is associated in rare instances with immune-related adverse events accompanied by neuronal antibodies, which are indistinguishable from paraneoplastic neurologic syndromes.

Other PNDs are likely immune-mediated, although their antigens are unknown. The best example is opsoclonus-myoclonus syndrome associated with neuroblastoma or SCLC. For still other PNDs, the cause remains quite obscure. These include, among others, several neuropathies that occur in the terminal stages of cancer and a number of neuropathies associated with plasma cell dyscrasias or lymphoma without evidence of tumor infiltration or deposits of immunoglobulin, cryoglobulin, or amyloid.

APPROACH TO THE PATIENT

Paraneoplastic Neurologic Disorders

Three key concepts are important for the diagnosis and management of PNDs. First, it is common for symptoms to appear before the presence of a tumor is known; second, the neurologic syndrome usually develops rapidly, producing severe deficits in a short period of time; and third, there is evidence that prompt tumor control improves the neurologic outcome. Therefore, the major concern of the physician is to recognize a disorder promptly as paraneoplastic and to identify and treat the tumor.

PND OF THE CENTRAL NERVOUS SYSTEM AND DORSAL ROOT GANGLIA

When symptoms involve brain, spinal cord, or dorsal root ganglia, the suspicion of PND is usually based on a combination of clinical, radiologic, and CSF findings. Presence of antineuronal antibodies

TABLE 94-3 Antibodies to Cell Surface or Synaptic Antigens, Syndromes, and Associated Tumors

ANTIBODY	NEUROLOGIC SYNDROME	TUM OR TYPE WHEN ASSOCIATED
Anti-NMDAR ^a	Anti-NMDAR encephalitis	Teratoma in young women (children and men rarely have tumors)
Anti-AMPAR ^b	Limbic encephalitis with relapses	SCLC, thymoma, breast, in ~70% of the patients
Anti-GluK2 ^a	Encephalitis, cerebellar ataxia, cerebellitis	No tumor, rarely teratoma
Anti-GABA _A R ^b	Encephalitis with prominent seizures and status epilepticus	Thymoma in ~30% of the patients
Anti-GABA _B R ^b	Limbic encephalitis with early and prominent seizures	SCLC in ~50% of the patients
Glycine receptor ^a	PERM, stiff-person syndrome	Rarely, thymoma, lung, Hodgkin's
Anti-mGluR5 ^a	Autoimmune encephalitis without distinctive features	Hodgkin's lymphoma, or no tumor
Anti-dopamine-2R ^b	Basal ganglia encephalitis	No cancer association
Anti-LGI1 ^{a,c}	Limbic encephalitis, hyponatremia, facioabdominal dystonic seizures	Rarely thymoma
Anti-Caspr2 ^{a,c}	Limbic encephalitis, ataxia, peripheral nerve hyperexcitability, neuropathy, Morvan's syndrome	~20% thymoma. In cases of Morvan syndrome: ~40% thymoma
Anti-DPPX ^a	Agitation, myoclonus, tremor, seizures, hyperekplexia, encephalomyelitis with rigidity	No cancer, but frequent diarrhea or cachexia suggesting paraneoplasia
Anti-neurexin 3 α ^b	Autoimmune encephalitis without distinctive features	No cancer association
IgLON5 ^a	NREM and REM sleep disorder, brainstem dysfunction, movement disorder, obstructive sleep apnea, stridor	No tumor association
Anti-mGluR1 ^a	Cerebellar syndrome	Hodgkin's lymphoma, or no tumor
Anti-mGluR2	Cerebellar syndrome	Small-cell neuroendocrine tumor, rhabdomyosarcoma
Anti-Tr (DNER)	Cerebellar syndrome	Hodgkin's lymphoma, or no tumor
Anti-Sez6l2	Cerebellar ataxia, postural instability, frequent falls, dysarthria, extrapyramidal symptoms	No cancer association
Anti-MOG	ADEM, optic neuritis, myelitis, cortical encephalitis	No cancer association
Anti-AChR (muscle) ^a	Myasthenia gravis	Thymoma
Anti-AChR (neuronal) ^a	Autonomic ganglionopathy	SCLC
Anti-VGCC ^a	LEMS, cerebellar degeneration	SCLC

^aA direct pathogenic role of these antibodies has been demonstrated in cultured neurons or animal models. ^bThese antibodies are strongly suspected to be pathogenic. ^cPreviously named voltage-gated potassium channel antibodies (VGKC); currently included under the term VGKC-complex proteins. Of note, the significance of antibodies to VGKC-complex proteins other than LGI1 and Caspr2 is uncertain (the antigens are unknown, and the response to immunotherapy is variable).

Abbreviations: AChR, acetylcholine receptor; ADEM, acute disseminated encephalomyelitis; AMPAR, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; Caspr2, contactin-associated protein-like 2; DNER, delta/notch-like epidermal growth factor-related receptor; DPPX, dipeptidyl-peptidase-like protein-6; GABA_AR, γ -aminobutyric acid B receptor; GAD, glutamic acid decarboxylase; GluK2, glutamate receptor ionotropic kainate 2; mGluR, metabotropic glutamate receptor; LEMS, Lambert-Eaton myasthenic syndrome; LGI1, leucine-rich glioma-inactivated 1; MOG, myelin oligodendrocyte glycoprotein; NMDAR, N-methyl-D-aspartate receptor; NREM, non-rapid eye movement; PERM, progressive encephalomyelitis with rigidity and myoclonus; REM, rapid eye movement; SCLC, small-cell lung cancer; Sez6l2, Seizure-related 6 homolog like 2; VGCC, voltage-gated calcium channel.

(Tables 94-2 and 94-3) may help in the diagnosis, but only 60–70% of PNDs of the CNS and <20% of those involving the peripheral nervous system have neuronal or neuromuscular junction antibodies that can be used as diagnostic tests.

Magnetic resonance imaging (MRI) and CSF studies are important to rule out neurologic complications due to the direct spread of cancer, particularly metastatic and leptomeningeal disease. In most PNDs, the MRI findings are nonspecific. Paraneoplastic limbic encephalitis is usually associated with characteristic MRI abnormalities in the mesial temporal lobes (see below), but similar findings can occur with other disorders (e.g., nonparaneoplastic autoimmune limbic encephalitis and human herpesvirus type 6 [HHV-6] encephalitis) (Fig. 94-2A). The CSF profile of patients with PND of the CNS or dorsal root ganglia typically consists of mild to moderate pleocytosis (<200 mononuclear cells, predominantly lymphocytes), an increase in the protein concentration, and a variable presence of oligoclonal bands. There are no specific electrophysiologic tests that are diagnostic of PND. Moreover, a biopsy of the affected tissue is often difficult to obtain, and although useful to rule out other disorders (e.g., metastasis), the pathologic findings are not specific for PND.

PND OF NERVE AND MUSCLE

If symptoms involve peripheral nerve, neuromuscular junction, or muscle, the diagnosis of a specific PND is usually established on clinical, electrophysiologic, and pathologic grounds. The clinical history, accompanying symptoms (e.g., anorexia, weight loss), and type of syndrome dictate the studies and degree of effort needed to demonstrate a neoplasm. For example, the frequent association of Lambert-Eaton myasthenic syndrome (LEMS) with SCLC should lead to a chest and abdomen computed tomography (CT) or body positron emission tomography (PET) scan and, if negative, periodic tumor screening for at least 3 years after the neurologic diagnosis. In contrast, the weak association of polymyositis with cancer calls into question the need for repeated cancer screenings in this situation. Serum and urine immunofixation studies should be considered in patients with peripheral neuropathy of unknown cause; detection of a monoclonal gammopathy suggests the need for additional studies to uncover a B-cell or plasma-cell malignancy. In paraneoplastic neuropathies, diagnostically useful antineuronal antibodies are limited to CRMP5 (CV2) and Hu (ANNA1).

For any type of PND, if antineuronal antibodies are negative, the diagnosis relies on the demonstration of cancer and the exclusion of other cancer-related or independent neurologic disorders. Combined CT and PET scans often uncover tumors undetected by other tests. For germ cell tumors of the testis and teratomas of the ovary, ultrasound (testicular, transvaginal, or pelvic) and MRI or CT of the abdomen and pelvis may reveal tumors undetectable by PET.

SPECIFIC PARANEOPLASTIC NEUROLOGIC SYNDROMES

■ PARANEOPLASTIC ENCEPHALOMYELITIS AND FOCAL ENCEPHALITIS WITH ANTIBODIES AGAINST INTRACELLULAR NEURONAL PROTEINS

The term *encephalomyelitis* describes an inflammatory process with multifocal involvement of the nervous system, including brain, brainstem, cerebellum, and spinal cord. It is often associated with dorsal root ganglia and autonomic dysfunction. For any given patient, the clinical manifestations are determined by the areas predominantly involved, but pathologic studies almost always reveal abnormalities beyond the symptomatic regions. Several clinicopathologic syndromes may occur alone or in combination: (1) *cortical encephalitis*, which may present as “epilepsia partialis continua”; (2) *limbic encephalitis*, characterized by confusion, depression, agitation, anxiety, severe deficit forming new memories (“short-term memory deficit”), and temporal lobe or generalized seizures (the MRI usually shows unilateral or bilateral medial temporal lobe abnormalities, best seen with T2 and fluid-attenuated

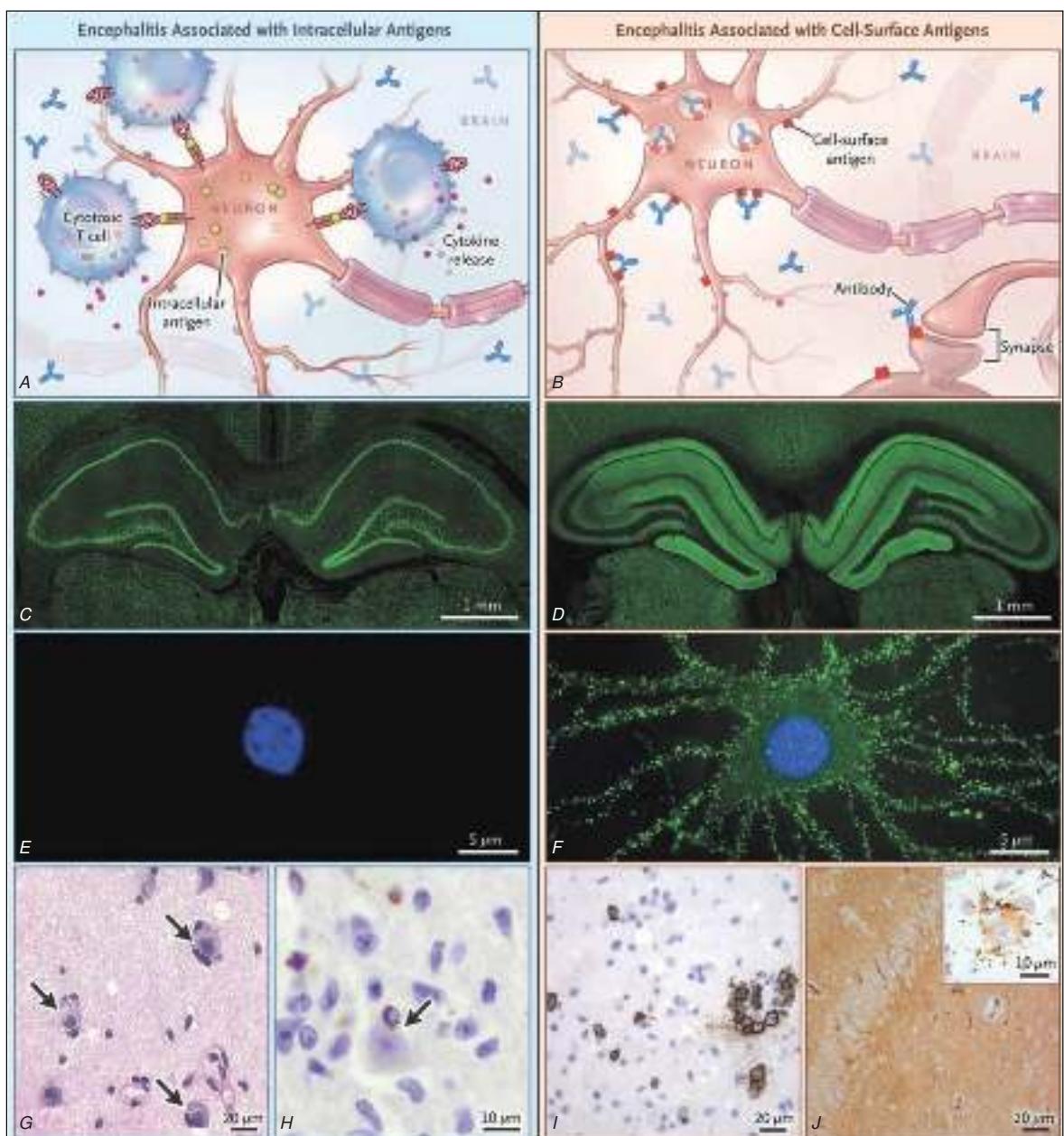


FIGURE 94-1 Antibody reactivity and pathologic findings in patients with antibodies against intracellular antigens compared with those of patients with antibodies against neuronal surface antigens. In encephalitis associated with antibodies against intracellular antigens, the antibodies cannot reach the intracellular epitopes and cytotoxic T-cell mechanisms are predominantly involved (A), whereas in encephalitis with antibodies against surface antigens, the antibodies have access to the epitopes and can potentially alter the structure and function of the antigen (B). The Hu antibodies (C, E) are shown here to exemplify the group of antibodies against intracellular antigens, and the NMDAR antibodies (D, F) are shown to exemplify the group of antibodies against cell-surface antigens. In rodent brain immunofluorescence with tissue permeabilization to allow entry of antibodies, the Hu antibodies produce a discrete pattern of cellular immunolabeling (C), whereas the NMDAR antibodies produce a pattern of neuropil-like immunolabeling (D). In contrast, with live cultured neurons, only the NMDAR antibodies have access to the target antigen showing intense immunolabeling (F), whereas the Hu antibodies cannot reach the intracellular antigen showing no immunolabeling (E). In autopsy studies, patients with encephalitis associated with antibodies to intracellular antigens (Hu or other) have extensive neuronal loss and inflammatory infiltrates (not shown); the T cells show direct contact with neurons (arrows in G) likely contributing to neuronal degeneration via perforin and granzyme mechanisms (arrow in H). In contrast, patients with antibodies against cell-surface antigens (NMDAR shown here, and probably applicable to other antigens) have moderate brain inflammatory infiltrates along with plasma cells (brown cells in I), deposits of IgG (diffuse brown staining in J), and microglial proliferation (inset in J), without evidence of predominant T-cell-mediated neuronal loss (not shown). All human tissue sections (G-J) were obtained from hippocampus. (From J Dalmau: Antibody mediated encephalitis. *N Engl J Med* 378:840, 2018. Copyright © 2018 Massachusetts Medical Society. Reprinted with permission.)

inversion recovery [FLAIR] sequences); (3) *brainstem encephalitis*, resulting in eye movement disorders (nystagmus, opsoclonus, supranuclear or nuclear paresis), cranial nerve paresis, dysarthria, dysphagia, unsteady gait, and central autonomic dysfunction; (4) *cerebellar gait and limb ataxia*; (5) *myelitis*, which may cause lower or upper motor

neuron symptoms, myoclonus, muscle rigidity, spasms, sensory deficits, and sphincter dysfunction; and (6) *autonomic dysfunction* as a result of involvement of the neuraxis at multiple levels, including hypothalamus, brainstem, and autonomic nerves (see Paraneoplastic Peripheral Neuropathies, below). Cardiac arrhythmias, postural

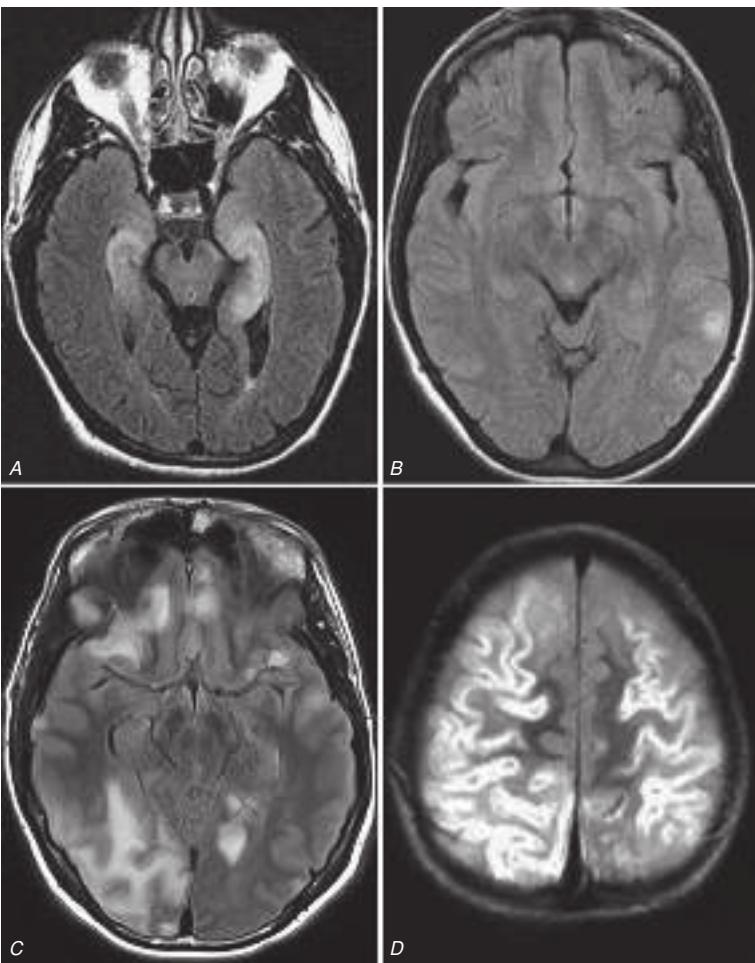


FIGURE 94-2 Brain MRI findings in paraneoplastic and autoimmune encephalitis. Representative MRI studies of patients with several types of autoimmune encephalitides. *A*, Limbic encephalitis (LE) may result from several different immune responses (Hu, Ma2, AMPAR, GABA_AR, LGI1, Caspr2) and typically manifests with unilateral or bilateral medial temporal lobe increased FLAIR signal. *B*, Anti-NMDAR encephalitis often occurs with normal MRI findings or mild FLAIR signal abnormalities. *C*, In contrast, anti-GABA_AR encephalitis usually occurs with multiple cortical-subcortical increased FLAIR signal changes. *D*, Cortical encephalitis can occur in patients with myelin oligodendrocyte glycoprotein (MOG) antibodies, as shown in this T2-weighted MRI image from a 3-year-old boy who presented with extensive cortical abnormalities with mild enhancement (not shown here) suggesting cortical necrosis. (*Panels A-C* from J Dalmau: *Antibody mediated encephalitis*. *N Engl J Med* 378:840, 2018. Copyright © 2018 Massachusetts Medical Society. Reprinted with permission. *Panel D* from T Armangue: *Associations of paediatric demyelinating and encephalitic syndromes with myelin oligodendrocyte glycoprotein antibodies: A multicentre observational study*. *Lancet Neurol* 19:234, 2020.)

hypotension, and central hypoventilation can be the cause of death in patients with encephalomyelitis.

Paraneoplastic encephalomyelitis and focal encephalitis are usually associated with SCLC, but many other cancers have been implicated. Patients with SCLC and these syndromes usually have Hu antibodies in serum and CSF. CRMP5 antibodies occur less frequently; some of these patients may develop chorea, uveitis, or optic neuritis. Antibodies to Ma proteins are associated with limbic, hypothalamic, and brainstem encephalitis and occasionally with cerebellar symptoms; some patients develop hypersomnia, cataplexy, and severe hypokinesia. MRI abnormalities are frequent, including those described with limbic encephalitis and variable involvement of the hypothalamus, basal ganglia, or upper brainstem. Kelch-like protein 11 antibodies are predominantly associated with brainstem encephalitis and seminomas, germ cell tumors, and teratomas. Amphiphysin antibodies usually are associated with paraneoplastic stiff-person syndrome, but in some patients, they can occur with paraneoplastic encephalomyelitis or isolated myelitis.

The oncologic associations of these antibodies are shown in Table 94-2.

Most types of paraneoplastic encephalitis and encephalomyelitis in which the antigens are intracellular respond poorly to treatment. Stabilization of symptoms or partial neurologic improvement may occur, particularly if there is a satisfactory response of the tumor to treatment. Controlled trials of therapy are lacking, but many reports and the opinion of experts suggest that therapies aimed to remove the antibodies against intracellular antigens, such as intravenous immunoglobulin (IVIg) or plasma exchange, usually fail. The main concern should be to treat the tumor and consider immunotherapies aimed at cytotoxic T-cell responses. Approximately 30% of patients with anti-Ma2-associated encephalitis respond to treatment of the tumor (usually a germ cell neoplasm of the testis) and immunotherapy.

■ ENCEPHALITIDES WITH ANTIBODIES TO CELL SURFACE OR SYNAPTIC PROTEINS TABLE 94-3

These disorders are important for four reasons: (1) they can occur with and without tumor association; less frequently, they develop after a viral encephalitis (herpes simplex or Japanese encephalitis); (2) some syndromes predominate in young individuals and children; (3) despite the severity of the symptoms, patients usually respond to treatment of the tumor, if found, and immunotherapy (e.g., glucocorticoids, IVIg, plasma exchange, rituximab, or cyclophosphamide); and (4) for many of these disorders, the antibody pathogenicity has been demonstrated in models using cultures of neurons or passive transfer of patients' antibodies to animals (Fig. 94-3).

Encephalitis with N-methyl- -aspartate (NMDA) receptor antibodies usually occurs in young women and children, but men and older patients of both sexes can be affected. The disorder has a characteristic pattern of symptom progression that often includes a prodrome resembling a viral process, followed in a few days by the onset of severe psychiatric symptoms, sleep dysfunction (usually insomnia), reduced verbal output, memory loss, seizures, decreased level of consciousness, abnormal movements (orofacial, limb, and trunk dyskinesias, dystonic postures), autonomic instability, and frequent hypoventilation. Monosymptomatic episodes, such as pure psychosis, occur in about 5% of patients. Clinical relapses occur in 12–24% of patients (12% during the first 2 years after initial presentation). Most patients have intrathecal synthesis of antibodies, likely by infiltrating plasma

cells in brain and meninges (Fig. 94-1*J*). In about 65% of patients, the brain MRI is normal; in the other 35%, it shows FLAIR abnormalities that can affect cortical and subcortical regions, usually mild and transient, and rarely the presence of contrast enhancement (Fig. 94-2*B*). The syndrome may be misdiagnosed as a viral or idiopathic encephalitis, neuroleptic malignant syndrome, or encephalitis lethargica, and some patients are initially evaluated by psychiatrists with the suspicion of acute psychosis as the presentation of a primary psychiatric disease. The detection of an associated teratoma is dependent on age and gender: 46% of female patients 12 years or older have uni- or bilateral ovarian teratomas, whereas <7% of girls younger than 12 have a teratoma (Fig. 94-4*A*). In young male patients, the detection of a tumor is rare. Patients older than 45 years are more frequently male; about 20% of these patients have tumors (e.g., cancer of the breast, ovary, or lung). Prompt diagnosis and treatment with immunotherapy (and tumor removal when it applies) improve outcome. Overall, about 85–90% of patients have substantial neurologic improvement or full recovery.

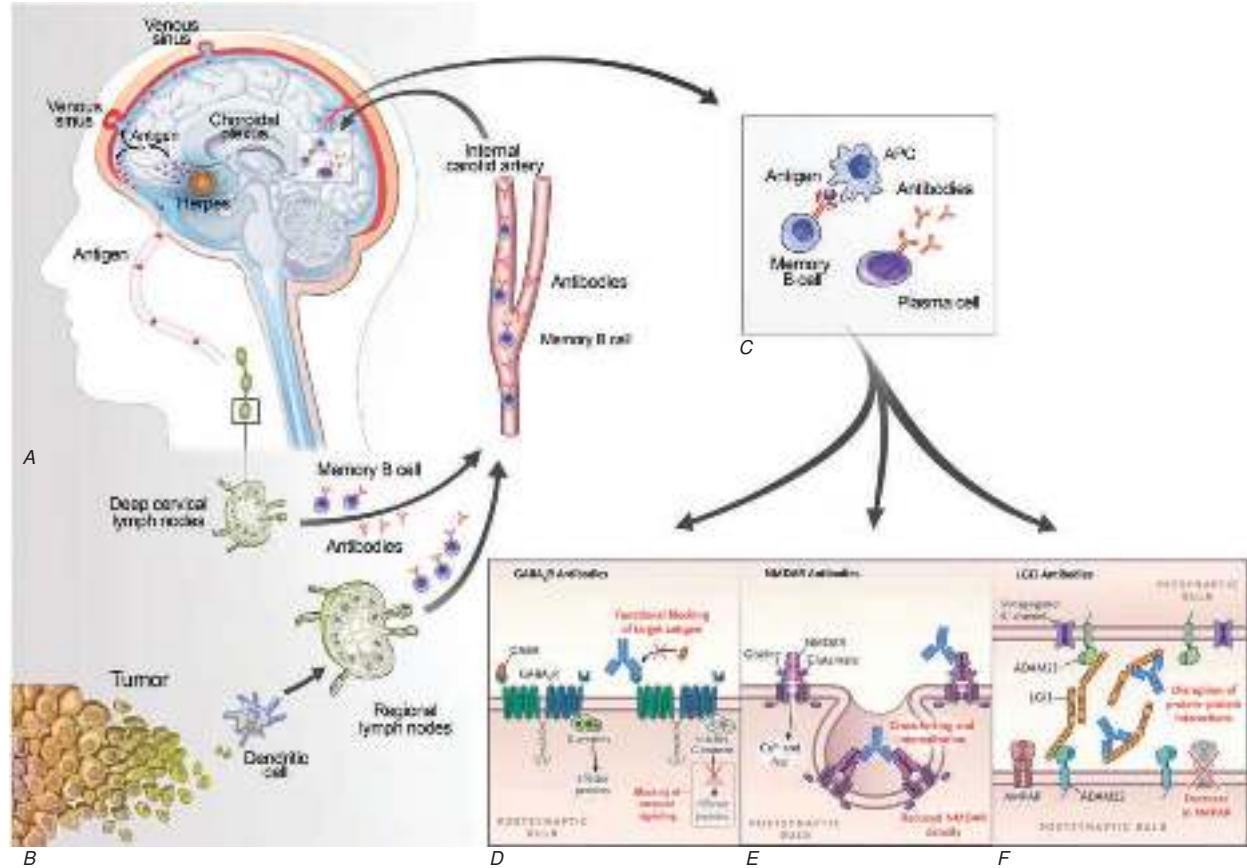


FIGURE 94-3 Proposed mechanisms of disease and functional interactions of autoantibodies with neuronal proteins. The graph shows a multistep process that results in antibody-mediated neuronal dysfunction; some of the steps have been demonstrated in reported studies, whereas others are based on proposed hypotheses. Two well-known triggers of autoimmune encephalitides are represented: herpes simplex encephalitis (A) and systemic tumors (B); the genetic susceptibility of some autoimmune encephalitides and unknown immunologic triggers are not depicted. It is postulated that antigens released by viral-induced neuronal destruction or apoptotic tumor cells are loaded into antigen-presenting cells (APCs; dendritic cells) and transported to regional lymph nodes. In the lymph nodes, naïve B cells exposed to the processed antigens, with cooperation of CD4+ T cells, become antigen-experienced and differentiate into antibody-producing plasma cells. After entering the brain, memory B cells undergo restimulation, antigen-driven affinity maturation, clonal expansion, and differentiation into antibody-producing plasma cells (C). The contribution of systemically produced antibodies to the pool of antibodies present in the brain is unclear and may depend on systemic antibody titers and integrity of the blood-brain barrier. Based on experimental models with cultured neurons, the presence of antibodies in the brain may lead to neuronal dysfunction by different mechanisms, including functional blocking of the target antigen (GABA_A R antibodies; D), receptor crosslinking and internalization (NMDAR antibodies; E), and disruption of protein-protein interaction, leading to downstream effects on receptors (LG1 leading to a decrease of Kv1 potassium channels and AMPAR; F). These mechanisms are influenced by the type of antibodies; for example, whereas IgG1 antibodies frequently crosslink and internalize the target antigen, IgG4 antibodies are less effective at crosslinking the target and more often alter protein-protein interactions. (Panels D-F J Dalmau: Antibody mediated encephalitis. *N Engl J Med* 378:840, 2018. Copyright © 2018 Massachusetts Medical Society. Reprinted with permission.)

Deficits of attention, memory, and executive functions may recover slowly over many months, sometimes a few years.

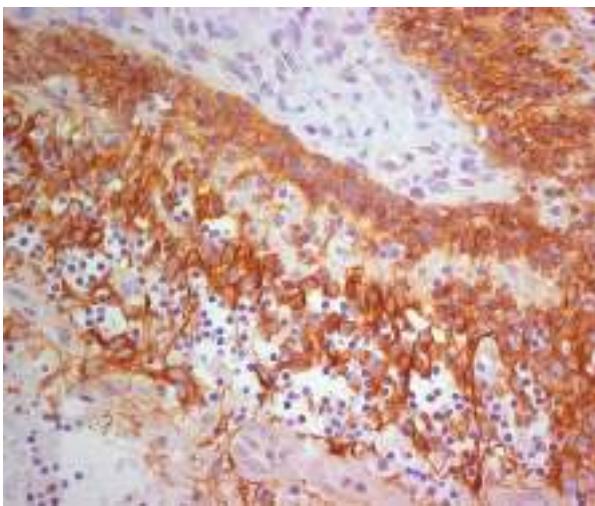
Approximately 25% of patients with herpes simplex encephalitis develop a form of autoimmune encephalitis that usually is associated with abnormal movements (choreoathetosis after herpes simplex encephalitis) in children and with cognitive and psychiatric symptoms in adults. This disorder develops a few weeks after the viral infection has resolved, is associated with new synthesis of antibodies against the NMDA receptor and other neuronal cell surface proteins, and is usually less responsive to immunotherapy than anti-NMDA receptor encephalitis (idiopathic or teratoma-associated).

Encephalitis with α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antibodies affects middle-aged women, who develop acute limbic dysfunction or, less frequently, prominent psychiatric symptoms; 70% of patients have an underlying tumor in the lung, breast, or thymus (Fig. 94-4B). In about 50% of cases, the brain MRI shows typical features of limbic encephalitis (similar to Fig. 94-2A). Neurologic relapses may occur; these also respond to immunotherapy and are not necessarily associated with tumor recurrence.

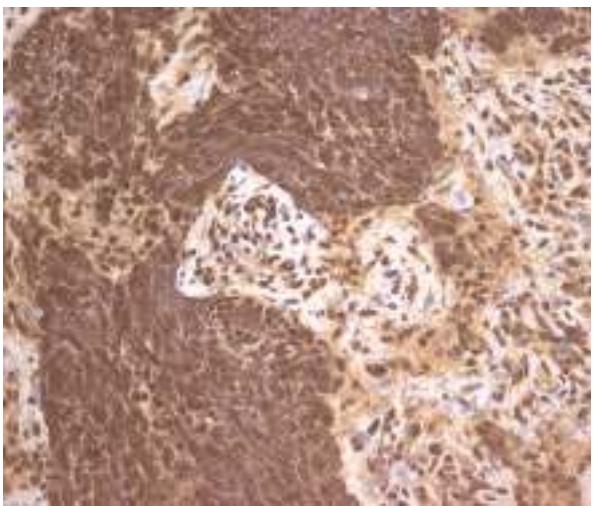
Encephalitis with GluK2 antibodies can affect children and adults and is associated with rapidly progressive encephalopathy with cerebellar ataxia or cerebellitis. Symptoms of encephalopathy may include impairment of memory and level of consciousness and motor alterations such as dyskinesias, choreoathetosis, bradykinesia, and spastic paraparesis. Some patients develop intracranial hypertension. In one patient, the symptoms were associated with teratoma.

Encephalitis with γ-aminobutyric acid type A (GABA_A) receptor antibodies may affect children and adults and is associated with prominent seizures and status epilepticus often requiring a pharmacologically induced coma. In approximately 80% of patients, the brain MRI shows multifocal, asynchronous, cortical-subcortical T2/FLAIR abnormalities predominantly involving temporal and frontal lobes, but also basal ganglia and other regions (Fig. 94-2C). Most patients do not have an underlying tumor, but some, usually of Japanese ethnicity, may have thymoma.

Encephalitis with GABA_B receptor antibodies is usually associated with limbic encephalitis and seizures. In >50% of cases, the MRI shows increased medial temporal lobe FLAIR changes characteristic of limbic encephalitis (similar to Fig. 94-2A). In rare instances, patients



A



B

FIGURE 94-4 Immunopathological studies in tumors of patients with autoimmune encephalitis. A. Neurons and neuronal processes (brown cells; stained with MAP2) in the teratoma of a patient with anti-NMDA receptor encephalitis; these neurons express NMDA receptors (not shown). B. Lung cancer from a patient with anti- α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor encephalitis showing expression of AMPA receptors by the neoplastic cells (brown cells). (Panel B from M Lai et al: AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. Ann Neurol 65:424, 2009.)

develop cerebellar symptoms and opsoclonus. Fifty percent of patients have SCLC or a neuroendocrine tumor of the lung. Patients may have additional antibodies to glutamic acid decarboxylase (GAD), which are of unclear significance. Other antibodies to nonneuronal proteins are often found in these patients as well as in patients with AMPA receptor antibodies, indicating a general tendency to autoimmunity.

Encephalitis with glycine receptor (GlyR) antibodies usually manifests with a syndrome characterized by progressive encephalomyelitis with rigidity and myoclonus (PERM) or stiff-person spectrum of symptoms. The disease usually occurs in adults and rarely in children. About 20% of adult patients have a concurrent underlying tumor (thymoma, B-cell lymphoma, breast or lung cancer) or past history of cancer (thymoma, breast, Hodgkin lymphoma, melanoma).

Encephalitis with metabotropic glutamate receptor 5 (mGluR5) antibodies is characterized by nonspecific clinical features of encephalitis (confusion, agitation, memory loss, delusions, paranoid ideation, hallucinations, psychosis, or seizures) without distinctive MRI changes and frequent association with Hodgkin's lymphoma (Ophelia syndrome). The encephalitis is highly responsive to immunotherapy and treatment of the tumor.

Encephalitis with antibodies against dopamine-2 receptor has been reported in children with basal ganglia encephalitis manifesting with abnormal movements (coarse tremor, parkinsonism, chorea, oculogyric crises) along with psychiatric features, lethargy, drowsiness, brainstem dysfunction, or ataxia. The disorder is extremely rare and is not associated with cancer.

Encephalitis with leucine-rich glioma-inactivated 1 (LGII) antibodies predominates in patients older than 50 years (65% male) and frequently presents with short-term memory loss and seizures (limbic encephalopathy), along with hyponatremia and sleep dysfunction. The MRI often shows increased FLAIR signal in one or both medial temporal lobes. In about 40% of patients, these symptoms are preceded by faciobrachial dystonic seizures, which consist of sudden, short-lasting, mainly distal muscle contractions involving the arm, face, or leg. These are unilateral but can independently affect both sides and occur multiple times during the day or night. About 15% of patients present with rapidly progressive cognitive decline, resembling a rapidly progressive dementia. Less than 5% of patients have thymoma. An association with the human leukocyte antigen (HLA) haplotypes DRB1 07:01, DQB1 02:02, DQA1 02:01, and DRB4 has been identified in non-paraneoplastic cases. All symptoms, including faciobrachial dystonic seizures, respond to immunotherapy, although about two-thirds of patients are left with memory or cognitive deficits.

Encephalitis with contactin-associated protein-like 2 (Caspr2) antibodies predominates in patients older than 50 years and is associated with a form of encephalitis with three or more of the following core symptoms: encephalopathy, cerebellar symptoms, peripheral nervous system hyperexcitability, dysautonomia, insomnia, neuropathic pain, and weight loss. Patients with Morvan's syndrome, which includes clinical features of encephalitis (confusion, hallucinations, prominent sleep dysfunction, or "agrypnia excitata"), autonomic alterations, and peripheral nerve hyperexcitability or neuromyotonia, usually have Caspr2 antibodies. About 20% of patients with Caspr2 antibody-associated syndromes have thymoma; this percentage is higher (~40%) in patients with Morvan's syndrome. An association of Caspr2 antibody-associated syndromes with HLA DRB1 *11.01 has been reported.

Encephalitis with dipeptidyl-peptidase-like protein-6 (DPPX) antibodies is usually preceded or develops concurrently with diarrhea, other gastrointestinal symptoms, and substantial loss of weight that often suggest the presence of a gastrointestinal disease. Neurologic symptoms include agitation, hallucinations, paranoid delusions, and features of CNS hyperexcitability such as tremor, myoclonus, nystagmus, seizures, or hyperekplexia. Some patients develop a clinical picture similar to progressive encephalomyelitis with rigidity and myoclonus. The few patients reported with an associated tumor all had B-cell neoplasms.

Encephalitis with antibodies against neurexin 3 alpha does not have distinctive clinical features; the experience is limited, and the disorder does not appear to be associated with cancer.

Anti-IgLON5 disease is a chronic or subacute encephalopathy that characteristically is associated with rapid eye movement (REM) and non-REM (NREM) parasomnia that may be preceded or accompanied by bulbar symptoms, gait abnormalities, movement disorders (chorea, distal myoclonus, tremor, dystonia, or spasms), oculomotor dysfunction, and, in less than half of cases, cognitive decline. The median age of the patients is in the early 60s, and men and women are equally affected. The sleep disorder is characterized by abnormal sleep initiation with undifferentiated NREM sleep associated with frequent vocalizations and quasi-purposeful movements. Examination of the CSF and MRI is unrevealing or demonstrates minor changes of unclear clinical relevance. It is not associated with cancer but shows a strong association with HLA-DRB1 10:01 and HLA-DQB1 05:01. The response to immunotherapy is poor. Neuropathologic studies often show a neuronal tauopathy predominantly involving the hypothalamus and tegmentum of the brainstem.

With the exception of patients with anti-IgLON5 disease, who rarely respond to treatment, most patients with autoimmune or

paraneoplastic encephalopathies associated with antibodies against cell-surface or synaptic proteins respond to immunotherapy and treatment of the tumor (if appropriate). Although there are no specific standardized treatment protocols, the most frequent approach consists of progressive escalation of immunotherapy using first a combination of glucocorticoids, IVIg, and plasma exchange, and then, if there is no response, rituximab or cyclophosphamide.

Encephalitis with myelin oligodendrocyte glycoprotein (MOG) antibodies can present with a clinical picture suggestive of autoimmune encephalitis related to neuronal antibodies. Most patients with MOG antibody-associated syndromes are children and young adults who present with optic neuritis, myelitis, or acute disseminated encephalomyelitis (ADEM). About 85% of patients with these syndromes respond to immunotherapy, although relapses occur in about 30% of cases. Besides these syndromes, there is a small group of adults and children that present with unilateral or bilateral cortical encephalitis, and their response to treatment is variable. In children, two phenotypes of poor prognosis include ADEM-like relapses progressing to leukodystrophy-like features and extensive cortical encephalitis evolving to atrophy (Fig. 94-2D). In general, MOG antibody syndromes are not associated with tumors.

■ PARANEOPLASTIC CEREBELLAR DEGENERATION

This disorder is often preceded by a prodrome that may include dizziness, oscillopsia, blurry or double vision, nausea, and vomiting. A few days or weeks later, patients develop dysarthria, gait and limb ataxia, and variable dysphagia. The examination usually shows downbeating nystagmus and, rarely, opsoclonus. Brainstem dysfunction, upgoing toes, or a mild neuropathy may occur. Early in the course, MRI studies are usually normal; later, the MRI reveals cerebellar atrophy. The disorder results from extensive degeneration of Purkinje cells, with variable involvement of other cerebellar cortical neurons, deep cerebellar nuclei, and spinocerebellar tracts. The tumors more frequently involved are SCLC, cancer of the breast and ovary, and Hodgkin's lymphoma.

Anti-Yo (PCA1) antibodies in patients with breast or gynecologic cancers typically are associated with prominent or pure cerebellar degeneration. A variable degree of cerebellar dysfunction can be associated with virtually any of the antibodies and PND of the CNS shown in Table 94-2. A number of single case reports have described neurologic improvement after tumor removal, plasma exchange, IVIg, cyclophosphamide, rituximab, or glucocorticoids. However, most patients with paraneoplastic cerebellar degeneration and any of the antibodies shown in Table 94-2 do not improve with treatment.

A cerebellar syndrome can also occur with antibodies against cell-surface or synaptic proteins, including P/Q-type voltage-gated calcium channels (VGCC), Tr (DNER), mGluR2, or Sez6l2 (Table 94-3). The frequency and type of tumor association vary with the type of antibody. The cerebellar syndrome of patients with mGluR1 antibodies is highly responsive to treatment of the tumor and immunotherapy, whereas the syndrome of patients with Tr or VGCC antibodies is less treatment responsive. The experience with mGluR2 and Sez6l2 is limited to a few patients, but mGluR2 antibody-associated cerebellar symptoms seem to be highly responsive to treatment. Patients with GluK2 antibodies can present with cerebellitis and posterior fossa edema with compression of the 4th ventricle; the syndrome is potentially treatable with immunotherapy.

■ PARANEOPLASTIC OPSOCLONUS MYOCLONUS SYNDROME

Opsoclonus is a disorder of eye movement characterized by involuntary, chaotic saccades that occur in all directions of gaze; it is frequently associated with myoclonus and ataxia. Opsoclonus-myoclonus may be cancer-related or idiopathic. When the cause is paraneoplastic, the tumors involved are usually cancer of the lung and breast in adults, neuroblastoma in children, and ovarian teratoma in adolescents and young women. The pathologic substrate of opsoclonus-myoclonus is unclear, but studies suggest that disinhibition of the fastigial nucleus of the cerebellum is involved. Most patients do not have antineuronal antibodies. A small subset of patients with ataxia, opsoclonus,

and other eye-movement disorders develop Ri antibodies; these patients may also develop muscle rigidity, laryngeal spasms, and autonomic dysfunction. The tumors most frequently involved in anti-Ri-associated syndromes are breast, ovarian, and lung cancers. If the tumor is not successfully treated, the syndrome in adults often progresses to encephalopathy, coma, and death. In addition to treating the tumor, symptoms may respond to immunotherapy (glucocorticoids, plasma exchange, and/or IVIg).

At least 50% of children with opsoclonus-myoclonus have an underlying neuroblastoma. Hypotonia, ataxia, behavioral changes, and irritability are frequent accompanying symptoms. Neurologic symptoms often improve with treatment of the tumor and glucocorticoids, adrenocorticotrophic hormone (ACTH), plasma exchange, IVIg, rituximab, or cyclophosphamide. Many patients are left with psychomotor retardation and behavioral and sleep problems.

■ PARANEOPLASTIC SYNDROMES OF THE SPINAL CORD

The number of reports of paraneoplastic spinal cord syndromes, such as *subacute motor neuronopathy* and *acute necrotizing myopathy*, has decreased in recent years. This may represent a true decrease in incidence, due to improved and prompt oncologic interventions, or the identification of nonparaneoplastic etiologies. Some patients with cancer or lymphoma develop *upper or lower motor neuron dysfunction* or both, resembling amyotrophic lateral sclerosis. It is unclear whether these disorders have a paraneoplastic etiology or simply coincide with the presence of cancer.

Paraneoplastic myelitis may present with upper or lower motor neuron symptoms, segmental myoclonus, sensory deficits, sphincter dysfunction, and neurogenic pruritus and can be the first manifestation of encephalomyelitis. The spine MRI usually shows longitudinally extensive, symmetric tract or gray matter abnormalities in the spinal cord. It is mainly associated with breast and lung carcinomas and with CRMP5 or amphiphysin antibodies. The prognosis is poor. *Neuromyelitis optica (NMO) with aquaporin 4 antibodies* may occur in rare instances as a paraneoplastic manifestation of a cancer. NMO is discussed in detail in Chap. 445.

■ PARANEOPLASTIC STIFF PERSON SYNDROME

This disorder is characterized by progressive muscle rigidity, stiffness, and painful spasms triggered by auditory, sensory, or emotional stimuli. Rigidity mainly involves the lower trunk and legs, but it can affect the upper extremities and neck. Sometimes, only one extremity is affected (*stiff-limb syndrome*). Symptoms improve with sleep and general anesthetics. Electrophysiologic studies demonstrate continuous motor unit activity. The associated antibodies target proteins (GAD, amphiphysin) involved in the function of inhibitory synapses using γ -aminobutyric acid (GABA) or glycine as neurotransmitters. The presence of amphiphysin antibodies usually indicates a paraneoplastic etiology related to SCLC and breast cancer. By contrast, GAD antibodies may occur in some cancer patients but are much more frequently present in the nonparaneoplastic disorder. GlyR antibodies may occur in some patients with stiff-person syndrome; these antibodies are more frequently detectable in patients with PERM (Fig. 94-5).

Optimal treatment of stiff-person syndrome requires therapy of the underlying tumor, glucocorticoids, and symptomatic use of drugs that enhance GABAergic transmission (diazepam, baclofen, sodium valproate, tiagabine, vigabatrin). IVIg and plasma exchange are transiently effective in some patients, and there are reports of responses to rituximab in patients who did not respond to other treatments.

■ PARANEOPLASTIC SENSORY NEURONOPATHY OR DORSAL ROOT GANGLIONOPATHY

This syndrome is characterized by sensory deficits that may be symmetric or asymmetric, painful dysesthesias, radicular pain, and decreased or absent reflexes. All modalities of sensation and any part of the body including face and trunk can be involved. Special senses such as taste and hearing can also be affected. Electrophysiologic studies show decreased or absent sensory nerve potentials with normal or

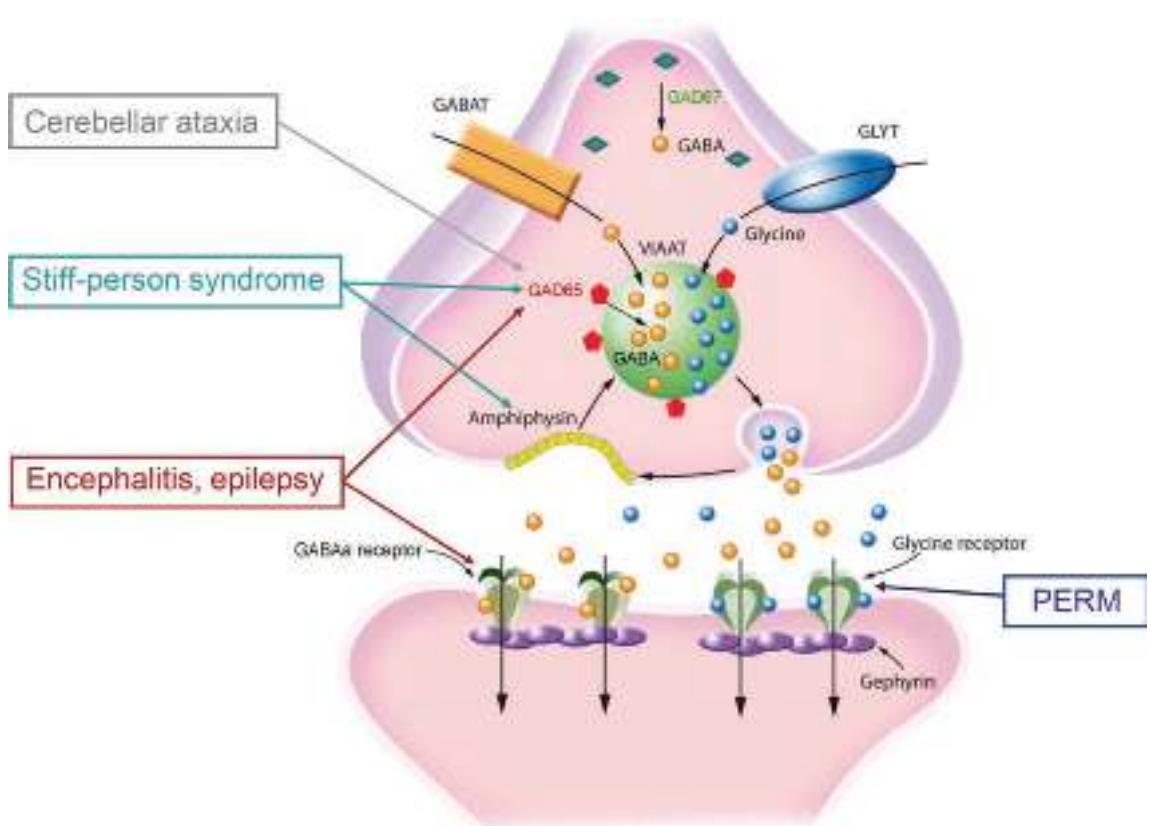


FIGURE 94-5 Schematic representation of an inhibitory synapse showing the main autoimmune targets (GAD, amphiphysin, GABA receptor, and glycine receptor) and the corresponding neurologic disorders. GAD antibodies predominantly occur in stiff-person syndrome (SPS), cerebellar ataxia, and epilepsy, sometimes in the setting of encephalitis. Amphiphysin antibodies are markers of paraneoplastic SPS and breast cancer, GlyR antibodies often associate with progressive encephalomyelitis with rigidity and myoclonus (PERM), and GABA_A receptor antibodies occur in a form of autoimmune encephalitis that is frequently associated with refractory seizures and status epilepticus. (Modified from FGraus et al: *Nat Rev Neurol* 16:353, 2020)

near-normal motor conduction velocities. Symptoms result from an inflammatory, likely immune-mediated, process that targets the dorsal root ganglia, causing neuronal loss and secondary degeneration of the posterior columns of the spinal cord. The dorsal and, less frequently, the anterior nerve roots and peripheral nerves may also be involved. This disorder often precedes or is associated with encephalomyelitis and autonomic dysfunction and has the same immunologic and oncologic associations (Hu antibodies, SCLC).

As with anti-Hu-associated encephalomyelitis, the therapeutic approach focuses on prompt treatment of the tumor and cytotoxic T-cell-mediated mechanisms. Glucocorticoids occasionally produce clinical stabilization or improvement. The benefit of IVIg and plasma exchange is not proven.

■ PARANEOPLASTIC PERIPHERAL NEUROPATHIES

These disorders may develop any time during the course of the neoplastic disease. Neuropathies occurring at late stages of cancer or lymphoma usually cause mild to moderate sensorimotor deficits due to axonal degeneration of unclear etiology. These neuropathies are often masked by concurrent neurotoxicity from chemotherapy and other cancer therapies. In contrast, the neuropathies that develop in the early stages of cancer frequently show a rapid progression, sometimes with a relapsing and remitting course, and evidence of inflammatory infiltrates and axonal loss or demyelination. If demyelinating features predominate (Chaps. 446 and 447), IVIg, plasma exchange, or glucocorticoids may improve symptoms. Occasionally, CRMP5 antibodies are present; detection of anti-Hu suggests concurrent dorsal root ganglionitis.

Guillain-Barré syndrome (Chap. 447) and *brachial plexitis* (Chap. 446) have occasionally been reported in patients with

Hodgkin's lymphoma, but there is no clear evidence of a paraneoplastic association.

Diseases associated with monoclonal gammopathies such as multiple myeloma, osteosclerotic myeloma, cryoglobulinemia, amyloidosis, Waldenström's macroglobulinemia, or POEMS (polyneuropathy, organomegaly, endocrinopathy, M-protein spike, and skin manifestations) syndrome, among others, may cause neuropathy by a variety of mechanisms, including compression of roots and plexuses by metastasis to vertebral bodies and pelvis, by deposits of amyloid in peripheral nerves, or through a direct interaction of the abnormal immunoglobulin with peripheral nerve antigens. In other patients, the mechanisms underlying the neuropathy remain unknown and paraneoplastic immune-mediated mechanisms have not been ruled out. Neuropathies more often occur with IgM gammopathies followed by IgG and IgA. The phenotype of the neuropathy and likelihood of improvement with successful treatment of the gammopathy are dependent on the underlying hematologic disorder (Chap. 447).

Vasculitis of the nerve and muscle causes a painful symmetric or asymmetric distal axonal sensorimotor neuropathy with variable proximal weakness. It predominantly affects elderly men and is associated with an elevated erythrocyte sedimentation rate and increased CSF protein concentration. SCLC and lymphoma are the primary tumors involved. Glucocorticoids and cyclophosphamide often result in neurologic improvement.

Peripheral nerve hyperexcitability (*neuromyotonia*, or *Isaacs' syndrome*) is characterized by spontaneous and continuous muscle fiber activity of peripheral nerve origin. Clinical features include cramps, muscle twitching (fasciculations or myokymia), stiffness, delayed muscle relaxation (pseudomyotonia), and spontaneous or evoked

carpal or pedal spasms. The involved muscles may be hypertrophic, and some patients develop paresthesias and hyperhidrosis. The electromyogram (EMG) shows fibrillations; fasciculations; and doublet, triplet, or multiplet single-unit (myokymic) discharges that have a high intraburst frequency. Some patients have Caspr2 antibodies usually in the context of Morvan's syndrome, but most patients with isolated neuromyotonia are antibody negative. The disorder often occurs without cancer; if paraneoplastic, benign and malignant thymomas and SCLC are the usual tumors. Phenytoin, carbamazepine, and plasma exchange improve symptoms.

Paraneoplastic autonomic neuropathy usually develops as a component of other disorders, such as LEMS and encephalomyelitis. It may rarely occur as a pure or predominantly autonomic neuropathy with cholinergic or adrenergic dysfunction at the pre- or postganglionic levels. Patients can develop several life-threatening complications, such as gastrointestinal paresis with pseudo-obstruction, cardiac dysrhythmias, and postural hypotension. Other clinical features include abnormal pupillary responses, dry mouth, anhidrosis, erectile dysfunction, and problems in sphincter control. The disorder occurs in association with several tumors, including SCLC, cancer of the pancreas or testis, carcinoid tumors, and lymphoma. Because autonomic symptoms can be the presenting feature of encephalomyelitis, serum Hu and CRMP5 antibodies should be sought. Antibodies to ganglionic (α 3-type) neuronal acetylcholine receptors are the cause of autoimmune autonomic ganglionopathy, a disorder that frequently occurs without cancer association (Chap. 440).

LAMBERT EATON MYASTHENIC SYNDROME

LEMS is discussed in Chap. 448.

MYASTHENIA GRAVIS

Myasthenia gravis is discussed in Chap. 448.

POLYMYOSITIS DERMATOMYOSITIS

Polymyositis and dermatomyositis are discussed in detail in Chap. 365.

IMMUNE MEDIATED NECROTIZING MYOPATHY

Patients with this syndrome develop myalgias and rapid progression of weakness involving the extremities, neck, pharyngeal, respiratory, and sometimes cardiac muscles. Serum muscle enzymes are elevated, and muscle biopsy shows extensive necrosis with minimal or absent inflammation and sometimes deposits of complement. The disorder may occur without cancer association (sometimes as a result of statin exposure, connective tissue disease, or HIV) or with cancer association. Patients with antibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and seronegative patients are more likely to have an underlying cancer than those with antibodies against signal recognition particle. No specific type of cancer has been found to be predominant. Successful treatment of the tumor and aggressive immunotherapy (steroids, IVIg, and steroid-sparing immunosuppressants) may lead to complete or substantial recovery. Immune-mediated necrotizing myopathy is discussed in Chap. 365.

PARANEOPLASTIC VISUAL SYNDROMES

This group of disorders involves the retina and, less frequently, the uvea and optic nerves. The term *cancer-associated retinopathy* is used to describe paraneoplastic cone and rod dysfunction characterized by photosensitivity, progressive loss of vision and color perception, central or ring scotomas, night blindness, and attenuation of photopic and scotopic responses in the electroretinogram (ERG). The most commonly associated tumor is SCLC. Melanoma-associated retinopathy affects patients with metastatic cutaneous melanoma. Patients develop acute onset of night blindness and shimmering, flickering, or pulsating photopsias that often progress to visual loss. The ERG shows reduced b-waves with normal dark adapted a-waves. Paraneoplastic optic neuritis and uveitis can develop in association with encephalomyelitis. Patients with paraneoplastic uveitis and optic neuritis may harbor CRMP5 antibodies.

Some paraneoplastic retinopathies are associated with serum antibodies that specifically react with the subset of retinal cells undergoing degeneration, supporting an immune-mediated pathogenesis (Table 94-2). Paraneoplastic retinopathies rarely show substantial improvement after treatment of the tumor and immunotherapy; however, stabilization of symptoms and partial responses to a variety of immunotherapies (glucocorticoids, plasma exchange, IVIg, rituximab, or alemtuzumab) have been reported.

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Cancer Survivorship and the Long-Term Impact of Cancer and Its Treatment

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The impact of cancer extends well past initial diagnosis. Patients are significantly affected by cancer and treatment-related toxicities often extending beyond the initial treatment period. Adult survivors of childhood, adolescent, and young adult cancer face special health consequences of cancer treatment related to premature physiologic aging and frailty. More than 40% of these patients will experience a severe, disabling, or life-threatening condition or die of a chronic condition. Long-term effects include toxicities that emerge during therapy and continue beyond treatment, while late effects include toxicities that may not emerge for months or years after treatment. Significant improvements in cancer treatments have enabled more people to survive once-deadly diseases, leading to more cancer survivors subjected to the potential long-term impact of cancer treatment (Table 95-1 lists potential long-term and late effects of cancer therapy by organ system). The direct causality of emerging treatments may not be immediately evident, and pharmacovigilance remains critical after treatments first become approved.

TABLE 95-1 Organ Systems at Risk for Long-Term and Late Effects of Cancer Treatment

ORGAN SYSTEM	LONG-TERM EFFECTS	LATE EFFECTS
Cardiovascular	Congestive heart failure	Congestive heart failure
	Arrhythmias	Arrhythmias
	Pericardial disease	Coronary artery disease
	Myocarditis	Peripheral vascular disease
	Hypertension	Cardiac valvular disease
Pulmonary	Pneumonitis	Pulmonary fibrosis Radiation recall pneumonitis
Immunologic	Opportunistic infections	Second malignancies
	Autoimmune disease	Myelodysplasia Recurrent infections Autoimmune disease
Endocrine	Fractures	Gonadal dysfunction/infertility
	Hypopituitarism	Premature ovarian insufficiency
	Avascular necrosis	Sarcopenia
	Diabetes insipidus	Diabetes
		Osteoporosis
		Thyroid disorders
Neurologic	Peripheral sensorimotor neuropathy Myelopathy Hearing loss	Cognitive impairment
Gastrointestinal	Malabsorption Colitis Chronic liver disease	Chronic diarrhea Small bowel obstruction Gastrointestinal stricture
Genitourinary	Chronic renal failure	Hemorrhagic cystitis Ureteral stricture
Psychological	Anxiety Depression	Mood disorders Posttraumatic stress disorder Sexual dysfunction Substance abuse disorders Financial hardship Psychosocial dysfunction

In the United States, the number of cancer survivors may increase from 17 million to nearly 26 million by the year 2040, and the number of patients who survive at least 5 years after initial diagnosis is expected to increase by 35% over the next decade. Improvements in cancer treatments for children and adolescents have led to modern 5-year survival rates of approximately 80% or greater. Despite the magnitude of the growing problem, the core issues related to cancer survivorship remain understudied and the research is often concentrated in highly prevalent cancers. Most studies are observational and descriptive with fewer studies focused on the prevention and treatment of complications. Cancer survivorship remains an area that is ripe for further discovery; a deeper understanding of the biological basis and/or the influence of genetics on host susceptibility and the long-term effects of cancer therapy is needed.

Our primary understanding of the long-term impact of cancer treatment originated from survivors of childhood malignancies who are often cured after treatment with a combination of chemotherapy, radiation, and surgery. Treatment paradigms for cancer continuously evolve, however, and newer treatments including targeted agents and immunotherapy have introduced unique long-term effects, particularly when these agents are administered indefinitely (Table 95-2 lists the potential long-term effects of specific cancer treatments). Improvements in the tolerability of cancer therapy and in supportive care have allowed a greater number of patients with comorbid conditions and advanced age to receive treatment, which has increased the rate of

chronic morbidity. Approximately 60% of current cancer survivors are older than age 65. Indeed, the cause-specific mortality following initial treatment of Hodgkin lymphoma includes nearly 40% of deaths attributed to nonlymphoma causes. Indeed, more patients diagnosed with Hodgkin lymphoma die from treatment-related late toxicity than from Hodgkin lymphoma. Individuals aged 60–74 have a disproportionate excess of deaths related to heart disease, lung disease, infections, and adverse effects of drugs. The complexities of cancer survivorship and the importance of longitudinal care of cancer patients are recognized as vital components to comprehensive cancer care, and model systems have been specifically developed for this purpose. Still, the primary goal of cancer therapy remains long-term disease control, and the treating physician must maintain proper perspective when considering these relative risks. The fear of long-term complications should not prevent the application of effective cancer treatment, particularly when delivered with curative intent. In a sense, managing long-term effects is a privilege only afforded those fortunate enough to overcome the initial threat to life represented by the cancer diagnosis.

CARDIOVASCULAR DYSFUNCTION

The excess risks of cardiovascular disease after anthracycline chemotherapy and radiation that involves the mediastinum are well characterized and include arrhythmias, cardiac ischemia, congestive heart failure (CHF), pericardial disease, myocarditis, and peripheral vascular disease. It is estimated that one in eight childhood cancer survivors will experience a life-threatening cardiovascular event within 30 years after initial exposure, and cardiovascular disease is the most common cause of noncancer death in this population. Newer targeted agents and immunotherapy have introduced additional cardiovascular risks that extend into older populations as well. A new discipline of cardio-oncology has been developed to better characterize individuals at high risk for treatment-related cardiac toxicities, develop surveillance strategies, and improve the management of long-term effects.

Radiation therapy that includes the heart can cause interstitial myocardial fibrosis, acute and chronic pericarditis, valvular disease, and accelerated premature atherosclerotic coronary artery disease. Repeated or high (>6000 cGy) radiation doses are associated with greater risk, as is concomitant cardiotoxic cancer chemotherapy exposure. Symptoms of acute pericarditis peak about 9 months after treatment and include dyspnea, chest pain, and fever. Chronic constrictive pericarditis may develop 5–10 years following radiation therapy. Cardiac valvular disease includes aortic insufficiency from fibrosis or papillary muscle dysfunction resulting in mitral regurgitation. Extensive radiation fields are associated with accelerated coronary artery disease and peripheral vascular disease and a markedly increased risk of fatal myocardial infarction or thromboembolic stroke. Three-dimensional conformal techniques and newer particles including proton beams may more precisely target the tumor and spare normal tissue, but these are not widely available and the degree to which they will decrease long-term cardiovascular effects is unknown. In recognition of the risks of radiation, careful planning procedures are performed before treatment designed to limit the field of radiation to the greatest extent possible.

The myocardial toxicity of anthracyclines is dose-dependent and is associated with the pathognomonic finding of myofibrillar dropout on endomyocardial biopsy. Anthracycline cardiotoxicity occurs through a root mechanism of chemical free radical damage. Fe³⁺-doxorubicin complexes damage DNA, nuclear and cytoplasmic membranes, and mitochondria. These cardiotoxic effects may also be mediated by topoisomerase IIB. Approximately 5% of patients receiving >450–550 mg/m² total dose of doxorubicin will develop CHF, but it can also develop at substantially lower doses in some patients. Anthracycline-related CHF is often irreversible and carries a high mortality rate, making prevention crucial. Genome-wide association studies have identified multiple genetic polymorphisms associated with a higher risk of cardiotoxicity, but our ability to risk-stratify is limited. The risk of cardiac failure appears to be related to the route of administration, and regimens that use continuous infusion of doxorubicin or liposomally encapsulated doxorubicin are associated with less cardiotoxicity. Baseline assessment of cardiac function with multigated acquisition scan (MUGA) or

TABLE 95-2 Long-Term Effects of Cancer Treatment by Type of Therapy

THERAPY TYPE	LONG-TERM EFFECT
Radiation	Second malignancies Coronary artery disease Pericardial disease Peripheral vascular disease Cardiac valvular disease Neurocognitive impairment Hypopituitarism and infertility Hypothyroidism and reduced bone density Gastrointestinal stricture Hepatic venoocclusive disease
Chemotherapy and Hormonal Agents	
Anthryacyclines, trastuzumab, cyclophosphamide	Congestive heart failure
Bleomycin, oxaliplatin, 5-fluorouracil	Pulmonary fibrosis
Nitrosoureas, methotrexate, fludarabine, brentuximab	Pneumonitis
Alkylating agents, anthracyclines, tamoxifen, bendamustine, platinum agents	Second malignancies/myelodysplasia
Bendamustine, alkylating agents, anthracyclines	Immune dysfunction and recurrent infections
Alkylating agents	Infertility
Cyclophosphamide, Ifosfamide	Hemorrhagic cystitis
Platinum agents	Renal tubular dysfunction
Vinca alkaloids, taxanes, platinum agents	Neuropathy
Cytarabine	Ataxia
Aromatase inhibitors	Vasomotor symptoms
Antiandrogens	Sexual dysfunction
Immunotherapy Agents	
Immune checkpoint inhibitors	Autoimmune conditions/autoimmune hepatitis
Immune checkpoint inhibitors	Pericarditis and fulminant myocarditis
CAR-T therapy	Congestive heart failure
CAR-T therapy	Arrhythmias
CAR-T therapy, bi-specific monoclonal antibodies, immune checkpoint inhibitors	Peripheral neuropathy
CAR-T therapy, bi-specific monoclonal antibodies	B-cell aplasia
Targeted Agents	
Immunomodulatory agents	Second cancers/leukemia
Proteasome inhibitors	Peripheral neuropathy
Anti-CD20 agents, immunomodulatory agents	Neutropenia
BTK inhibitors, ALK inhibitors	Atrial and ventricular arrhythmias
PI3K inhibitors, CDK inhibitors	Hepatitis, colitis
Gemtuzumab	Sinusoidal obstruction syndrome
EGFR inhibitors, anti-VEGF agents, BCR-ABL inhibitors, MEK inhibitors, proteasome inhibitors	Congestive heart failure
BCR-ABL inhibitors	Pleural effusions, pancreatitis
BCR-ABL inhibitors, PI3K inhibitors, BTK inhibitors, immunomodulatory agents, EGFR inhibitors	Chronic diarrhea
BCR-ABL inhibitors	Impaired growth and stature
Anti-VEGF agents, BCR-ABL inhibitors	Thyroid dysfunction
PI3K inhibitors, mTOR inhibitors, BRAF inhibitors	Hyperglycemia
FLT3 inhibitors, anti-VEGF agents, PI3K inhibitors	Systemic hypertension
Anti-VEGF agents, BCR-ABL inhibitors	Pulmonary hypertension

Abbreviation: CAR-T, chimeric antigen receptor T cell.

transthoracic echocardiograms is commonly performed, and a patient who develops symptoms suggestive of CHF should be tested immediately while therapy is held. Periodic surveillance testing during therapy is often done in asymptomatic patients with preexisting risk factors.

Trastuzumab is a monoclonal antibody targeting human epidermal growth factor receptor 2 (HER2) that is also associated with CHF. It is used in combination with chemotherapy as both adjuvant therapy and as treatment of metastatic breast cancer, and it is sometimes combined

with anthracyclines, which is believed to result in additive or possibly synergistic toxicity. In contrast to anthracyclines, cardiotoxicity is not dose related, is usually reversible, is not associated with pathologic changes on cardiac myofibrils, and has a different biochemical mechanism inhibiting intrinsic cardiac repair mechanisms. Monitoring for cardiac toxicity is typically performed every three or four doses using functional cardiac testing, and treatment is interrupted when ejection fractions significantly decline from baseline. Other potentially

cardiotoxic chemotherapy agents include phosphoramido mustards (cyclophosphamide) at high doses and ifosfamide.

Small-molecule inhibitors, including tyrosine kinase inhibitors, are novel classes of molecularly targeted anticancer agents that have become routinely applicable across a variety of malignancies. Although the overall tolerability of these drugs is often better than chemotherapy, they are frequently administered indefinitely, which introduces new notions of cumulative long-term effects. These agents also carry risks of cardiovascular toxicities including CHF, atrial and ventricular arrhythmias, prolongation of the QT interval, and pulmonary and systemic hypertension. New anticancer agents often become available for use on accelerated approvals before a full understanding of the long-term toxicity profile is known. Two illustrative examples of this are lapatinib and ponatinib, which both had their approvals updated with black box cardiovascular toxicity warnings a median of 4 years after initial drug approval. Other small-molecule inhibitors that have been associated with CHF include bosutinib, dasatinib, nilotinib, pazopanib, axitinib, trametinib, sunitinib, carfilzomib, and sorafenib. Systemic hypertension is commonly associated with agents targeting vascular endothelial growth factor or its receptors (e.g., bevacizumab, cabozantinib, lenvatinib, nintedanib), ponatinib (an Abl inhibitor), and trametinib (a MEK inhibitor), whereas dasatinib (an Abl inhibitor) has a well-documented association with pulmonary hypertension. Ibrutinib (a BTK inhibitor) has been associated with atrial fibrillation as well as ventricular arrhythmias. As more of these small-molecule inhibitors become approved for use and their indications broaden, additional monitoring for long-term and late effects should be incorporated into the routine surveillance of oncologists, cardiologists, and primary care providers.

Immunotherapy agents have also emerged as effective anticancer therapies that have substantially improved clinical outcomes in a variety of cancers. Immune checkpoint inhibitors have been associated with a number of important cardiovascular toxicities including pericardial disease, vasculitis, and fulminant myocarditis. The mechanism of these toxicities is T cell mediated, and the toxicities often respond to early institution of glucocorticoids, but they can be severe or fatal if not recognized promptly. Combinations of multiple immune checkpoint inhibitors increase the risk of these immune-related toxicities, and no clear pattern of which patients are most susceptible has emerged. Chimeric antigen receptor T-cell (CAR-T) therapies are associated with cytokine release syndromes (CRS), which can be severe and associated with arrhythmias or decompensated heart failure. Interleukin 6 receptor blockers may decrease these risks, and supportive care guidelines recommend early institution of these agents in severe CRS.

The management of treatment-associated cardiovascular disease is essentially the same as for cardiac disease unrelated to cancer treatment. Discontinuation of the offending agent is the first step. Diuretics, fluid and sodium restriction, and antiarrhythmic agents are often useful for acute symptoms. Afterload reduction with angiotensin-converting enzyme inhibitors or β -adrenergic blockers may improve systolic function over time, and digitalis may improve symptoms. Routine screening for asymptomatic systolic dysfunction is currently recommended for survivors at high risk for cardiomyopathy including those with an anthracycline exposure $\geq 250 \text{ mg/m}^2$, $\geq 35 \text{ Gy}$ of chest radiation, or combined therapy with both anthracyclines and radiation. Echocardiography is the recommended screening modality, and surveillance should begin no later than 2 years after exposure and should be repeated a minimum of every 5 years thereafter.

PULMONARY DYSFUNCTION

Radiation-induced lung injury presents in early phases as acute pneumonitis at 4 weeks following treatment, but it can evolve into pulmonary fibrosis in late phases. Risk factors for radiation pneumonitis include advanced age, smoking, poor performance status, preexisting compromised pulmonary function, and radiation volume and dose. It occurs most commonly in patients with lung cancer, mediastinal lymphoma, and breast cancer, and the incidence is decreasing due to advances in radiation delivery techniques. The dose “threshold” is thought to be in the range of 5–20 Gy. Hypoxemia and dyspnea on

exertion are characteristic, and the severity of symptoms may be out of proportion to the lung volume irradiated. Fine, high-pitched “Velcro rales” may be an accompanying physical finding, and fever, cough, and pleuritic chest pain are common symptoms. The diffusion capacity of the lungs for carbon dioxide (D_{CO}) is the most sensitive measure of pulmonary functional impairment, and ground-glass infiltrates often correspond with relatively sharp edges to the irradiated volume, although the pneumonitis may progress beyond the field and even occasionally involve the contralateral unirradiated lung. The mechanism of lung injury is a direct effect of radiation that leads to increased capillary permeability and pulmonary edema. Damage to type I and II pneumocytes leads to surfactant loss and the transudation of serum proteins into the alveoli. Cytokines including tumor necrosis factor α are released from the damaged lung cells and attract inflammatory cells to the alveoli and interstitial space. The late phases of injury are caused by reactive oxygen species that stimulate collagen production and lead to fibrosis but do not occur in all cases. Transforming growth factor β ($TGF-\beta$) is particularly important in stimulating collagen synthesis and may represent a therapeutic target to prevent pulmonary fibrosis.

Bleomycin generates activated free radical oxygen species and causes pneumonitis associated with a radiographic or interstitial ground-glass appearance diffusely throughout both lungs, often worse in the lower lobes. A nonproductive cough with or without fever may be an early sign. This toxicity is dose-related and dose-limiting. The D_{CO} is a sensitive measure of toxicity and recovery, and a baseline value is generally obtained for future comparison before administering bleomycin therapy. Doses are reduced or stopped if the baseline D_{CO} falls 25% or more. Additive or synergistic risk factors include age, prior lung disease, and concomitant use of other chemotherapy, lung irradiation, and high concentrations of inspired oxygen. Other chemotherapeutic agents notable for pulmonary toxicity include mitomycin, nitrosoureas, doxorubicin with radiation, gemcitabine combined with weekly docetaxel, methotrexate, and fludarabine. High-dose alkylating agents, cyclophosphamide, ifosfamide, and melphalan are frequently used in the hematopoietic stem cell transplant setting, often with whole-body radiation. This therapy may result in severe pulmonary fibrosis and/or pulmonary venoocclusive disease.

Radiation-induced lung injury and chemotherapy-induced pneumonitis are generally glucocorticoid responsive, except in the case of nitrosoureas. Prednisone 1 mg/kg is often used to control acute symptoms and prevent pulmonary dysfunction with a slow taper over 12 weeks. Prolonged glucocorticoid therapy requires gastrointestinal protection with proton pump inhibitors, management of hyperglycemia, heightened infection management, and prevention or treatment of steroid-induced osteoporosis. Antibiotics, bronchodilators, oxygen in only lowest necessary doses, and diuretics may all play an important role in management of pneumonitis, and consultation with a pulmonologist should be routinely undertaken. Relapses can occur after an initial response to glucocorticoids and may respond to agents such as azathioprine or cyclosporine. Amifostine is a free radical scavenger and radioprotective agent that reduces the rate of pneumonitis, but it is associated with severe nausea and hypotension that limit its use. No effective therapy exists for pulmonary fibrosis, and the treatment is primarily supportive with supplemental oxygen. Targeted anti-inflammatory agents are being tested to reduce the incidence of pulmonary fibrosis, but they remain experimental.

Pulmonary toxicity resulting from targeted anticancer agents including small-molecule inhibitors and immunotherapy agents is uncommon but can be potentially life-threatening and often lead to drug discontinuation. Noninfectious pneumonitis is associated with cough, dyspnea, and infiltrates on chest imaging and has been reported to be associated with sunitinib, sorafenib, epidermal growth factor receptor (EGFR) inhibitors (cetuximab, afatinib), crizotinib (ALK inhibitor), phosphoinositide 3-kinase (PI3K) inhibitors (idelalisib, copanlisib), and mammalian target of rapamycin (mTOR) inhibitors (everolimus, temsirolimus). The antibody-drug conjugate brentuximab vedotin may also cause severe pulmonary toxicity when used in combination with other chemotherapy agents, particularly bleomycin. The onset of drug-induced pneumonitis can be rapid, and prompt use

of glucocorticoids is important once infectious causes are excluded. Severe pneumonitis is typically a reason to discontinue the offending drug permanently.

IMMUNE SYSTEM DYSFUNCTION

A significant risk of most anticancer treatment is hematologic toxicity with cumulative effects on the host immune system leading to a higher risk of second malignancies and impaired long-term immune health. Second malignancies in cancer survivors are a major cause of death, and survivors of childhood cancers have a twofold increased risk of solid tumors beyond the age of 40 years compared to the general population. The induction of second malignancies is governed by the complex interplay of age, sex, environmental exposures, genetic susceptibility, and specific cancer treatments. Often, the events that led to the primary cancer remain, and a risk of a second malignancy persists. Patients with a history of lung cancer remain at increased risk of other cancers that are associated with tobacco use including esophageal, head and neck, kidney, and bladder cancers. Patients with breast cancer are at increased risk of breast cancer in the opposite breast. Patients with Hodgkin lymphoma are at risk for other B-cell non-Hodgkin lymphomas. Genetic cancer syndromes (e.g., multiple endocrine neoplasia or Li-Fraumeni, Lynch's, Cowden's, and Gardner's syndromes) are examples of genetically based second malignancies of specific types. Cancer treatment itself does not appear to be responsible for the risk of these secondary malignancies. Genetic disorders that result in DNA repair deficiencies including ataxia-telangiectasia, Bloom's syndrome, and Fanconi's anemia greatly increase the lifetime risk of cancers as well as the risks associated with DNA-damaging agents. Importantly, the risk of treatment-related second malignancies is at least additive and often synergistic with combined chemotherapy and radiation therapy, and hence for such combined-therapy treatment approaches, it is important to establish the necessity of each component in the treatment program. These patients require indefinite surveillance and prophylactic surgery in some cases.

Patients receiving radiation have an increasing and lifelong risk of second malignancies that is 1–2% in the second decade following treatment but increases to >25% after 25 years. The risk of second malignancies from radiation is dose-dependent and often occurs within or near the treatment field. Common radiation-related solid tumors include central nervous system (CNS), breast, lung, thyroid, skin, and bone cancers and sarcomas, which are often aggressive and have a poor prognosis. An example of an organ-, age-, and sex-dependent radiation-induced secondary malignancy is breast cancer, in which the risk is small with radiation in women aged older than 30 years but increases about twentyfold over baseline in women aged younger than 30 years. A 25-year-old woman treated with mantle radiation for Hodgkin lymphoma has a 29% actuarial risk of developing breast cancer by age 55.

Chemotherapy is significantly associated with two fatal second malignancies: acute leukemia and myelodysplastic syndromes. Two types of secondary leukemia have been described; in patients treated with chronic alkylating agents (especially combined with radiation therapy), acute myeloid leukemia is associated with deletions in chromosome 5 or 7 and complex karyotypes and often is preceded by myelodysplasia. The lifetime risk is about 1–5%, is increased by radiation therapy, and increases with age. The incidence of these leukemias peaks at 5–8 years, with risk returning close to baseline at 10 years. The other type of acute myeloid leukemia is related to therapy with topoisomerase inhibitors, is associated with chromosome 11q23 translocations, has an incidence of <1%, generally occurs 1.5–3 years after treatment, and is rarely preceded by myelodysplasia. Both of these acute myeloid leukemias are largely refractory to treatment and have a high mortality. The development of myelodysplastic syndromes is increased following chemotherapy, and these cases are often associated with leukemic progression and a dismal prognosis. A fraction of the population develops clonal hematopoiesis not related to prior cancer treatment, and the percentage increases with age. In such patients, the hematopoietic stem cells carry mutations that are associated with myeloid malignancy despite normal blood counts. It is thought that the presence of these genetic lesions may predispose patients to develop

myeloid malignancies, but evidence is greater that clonal hematopoiesis increases the risk of lymphoma and atherosclerotic heart disease.

Other cytotoxic agents are associated with long-term alterations in immunity beyond the initial treatment period and neutrophil recovery. Bendamustine has significant effects on both B-cell and T-cell subpopulations that can persist for years, and long-term studies of lymphoma patients treated with bendamustine-based regimens show higher rates of death from second malignancies compared to other chemotherapy regimens. Purine analogues like cladribine and pentostatin also produce long-term T-cell suppression. The risk of solid tumors is also increased after chemotherapy, and alkylating agents increase the risk of thyroid, lung, breast, and bladder cancers and sarcomas. Cyclophosphamide increases the risk of both sarcoma and breast cancer in a dose-dependent manner. Other chemotherapy agents, including procarbazine and platinum agents, have been associated with gastrointestinal malignancies. Treatment of breast cancer with tamoxifen for 5 years or longer is associated with a 1–2% risk of endometrial cancer. Surveillance is generally effective at finding these cancers at an early stage. The risk of mortality from tamoxifen-induced endometrial cancer is low compared to the benefit of tamoxifen as adjuvant therapy for breast cancer. Treatment of multiple myeloma with the immunomodulatory agent lenalidomide is associated with a significantly increased risk of second hematologic malignancies including lymphomas and leukemias. These risks are highest after prior use of the alkylating agent melphalan.

Given the high risk of second malignancies in cancer survivors, patients need indefinite surveillance. Guidelines for breast cancer surveillance in survivors exposed to chest radiation recommend that patients be screened with mammograms and/or breast MRI beginning at age 25 years or 8 years after treatment, whichever occurs later. Any organ in the treatment field is susceptible to developing a cancer; e.g., radiation to the chest may increase the risk of gastric or esophageal cancer. Patients exposed to abdominal or pelvic radiation should have annual colonoscopies starting at age 35 years or 10 years after exposure. For patients treated with neck radiation, no formal surveillance is recommended, but fine-needle aspiration should be performed on any palpable thyroid nodules and thyroid-stimulating hormone (TSH) levels should be monitored periodically.

Combination chemotherapy is also associated with impaired immune health and increases the risk of opportunistic infections, autoimmune complications, and impaired host protection from infections. Survivors of lymphoma have elevated risks of developing autoimmune hemolytic anemia, viral or fungal pneumonias, meningitis, or other infections, and these risks remain high decades after treatment. Agents or treatment regimens that result in significant T-cell depletion, including antithymocyte globulin or antibodies targeting cell surface proteins on T cells, increase the risk of Epstein-Barr virus-associated B-cell lymphoproliferative disorders. Discontinuing immunosuppressive therapy, if possible, is often associated with complete disease regression. Anti-CD20 monoclonal antibodies, CAR-T therapy, bi-specific monoclonal antibodies targeting both B cells and T cells, and immune checkpoint inhibitors have all been associated with long-term B-cell aplasia that often requires intravenous immunoglobulin replacement and persistent vigilance for recurrent sinopulmonary infections. Rituximab and immunomodulatory agents have both been associated with late-onset neutropenia that can occur months after exposure to the drug and often requires growth factor support. Given these risks of impaired immune system function, all cancer survivors should undergo annual influenza vaccination and should be considered for pneumococcal vaccination depending on age and immune health status.

REPRODUCTIVE AND ENDOCRINE DYSFUNCTION

Endocrine complications are prevalent in childhood cancer survivors. Nearly half of all survivors will have at least one hormonal disorder in their lifetime, and these most commonly present as late effects. Radiation to the head, neck, or pelvis is associated with the greatest risk of endocrine dysfunction. Testicles and ovaries in prepubertal patients are sensitive to radiation damage in a dose-related fashion;

spermatogenesis is affected by low doses of radiation, and complete azoospermia occurs at 600–700 cGy. Leydig cell dysfunction, in contrast, occurs at <2000 cGy, and hence, endocrine function is lost at much higher radiation doses than spermatogenesis. Erectile dysfunction occurs in up to 80% of men treated with external-beam radiation therapy for prostate cancer. Sildenafil may be useful in reversing erectile dysfunction. Ovarian function damage with radiation is age-related and occurs at doses of 150–500 cGy. Hormone replacement therapy is often contraindicated (as in estrogen receptor-positive breast cancer). Attention must be paid to maintenance of bone mass with calcium and vitamin D supplements and oral bisphosphonates, and bone mass should be monitored using bone density determinations. Paroxetine, clonidine, pregabalin, and other drugs may be useful in symptomatically controlling hot flashes. Long-term survivors of childhood cancer who have received cranial radiation may have altered leptin biology and growth hormone deficiency, leading to obesity and reduced strength, exercise tolerance, and bone density. Radiation therapy to the neck may lead to hypothyroidism, Graves' disease, thyroiditis, and thyroid malignancies. TSH is followed routinely in such patients to prevent hypothyroidism and to suppress persistently elevated levels of TSH, which may cause or drive thyroid cancer. Cranial radiation may also be associated with an array of endocrine abnormalities with disruption of normal pituitary-hypothalamic axis function, and a high index of suspicion needs to be maintained to identify and treat this toxicity. Efforts to eliminate unnecessary radiation such as prophylactic CNS irradiation may decrease some of the late endocrine effects. Patients who have received abdominal radiation should receive annual screens for obesity and diabetes mellitus with height and weight measurements along with a hemoglobin A_{1c} at least every 2 years.

Alkylating agents are the chemotherapy agents associated with the highest rates of male and female infertility, which is directly dependent on age, dose, and duration of treatment. The age at treatment is an important determinant of fertility outcome, with prepubertal patients having the highest tolerance. Ovarian failure is age related, and females who resume menses after treatment are still at increased risk for premature menopause. Males generally have reversible azoospermia during lower intensity alkylator chemotherapy, and long-term infertility is associated with doses of cyclophosphamide >9 g/m² and with high-intensity therapy, such as that used in hematopoietic stem cell transplantation. All patients should be counseled on the potential impact on future reproduction, and timely referral for established interventions such as sperm cryopreservation, oocyte preservation, and embryo preservation should be offered when feasible and appropriate. Assisted reproductive technologies can be helpful to couples with chemotherapy-induced infertility.

Combination chemotherapy can impair bone health, and older patients may be more susceptible to these effects. Due to the combined risk of age-related osteoporosis and the effect of therapy, the risk of fractures in patients over the age of 70 years may be as high as 5–10% within a few years of finishing therapy. The risk of low bone mineral density is highest in certain high-risk groups including survivors of pediatric acute lymphoblastic leukemia and CNS tumors and those who have undergone hematopoietic stem cell transplant.

Immune checkpoint inhibitors that target CTLA-4 and PD-1 have led to serious chronic toxicities including the breaking of self-tolerance and the autoimmune destruction of certain endocrine organs, particularly the thyroid and adenohypophysis (anterior pituitary). Hypophysitis is more commonly reported in association with CTLA-4 inhibitors, whereas thyroid dysfunction is more common with PD-1 inhibitors. Most immune-related toxicities occur within 8–12 weeks of starting treatment, but they can occur at any time during treatment or even after therapy has stopped. Patients with autoimmune thyroiditis or hypophysitis require lifelong hormone replacement, and early recognition is important.

Tyrosine kinase inhibitors such as imatinib have been associated with growth deceleration in children, particularly when treatment is initiated before puberty. The mechanism is postulated to be related to disruptions in growth hormone signaling or inhibition of the insulin-like growth factor 1 (IGFR-1) receptor. Other BCR-ABL inhibitors,

including nilotinib and dasatinib, have been associated with both hyper- and hypothyroidism. Endocrine effects that have been reported to be associated with other tyrosine kinase inhibitors include alterations in bone remodeling, reduced calcium and vitamin D levels, thyroid dysfunction, gonadal dysfunction, adrenal dysfunction, altered glucose metabolism, and secondary hyperparathyroidism. Thyroid function tests should be monitored periodically while patients are on these targeted agents, and replacement hormones and/or vitamins should be prescribed as necessary.

NEUROLOGIC DYSFUNCTION

Neurologic dysfunction from cancer treatment is increasing in both incidence and severity as a result of improved supportive care that enables more aggressive regimens, an expanded number of older patients receiving treatment, extended durations of therapy, and longer periods of cancer survivorship. Direct effects on myelin, glial cells, and neurons have all been implicated, with alterations in cellular cytoskeleton, axonal transport, and cellular metabolism as potential mechanisms. Telomere shortening that occurs with normal aging may be accelerated by radiation. Survivors of CNS tumors are at the greatest risk of late-onset neurocognitive impairment that includes impaired intelligence and slower processing speeds along with deficits in executive function, memory, and attention. These toxicities are reported after treatment with both radiation and chemotherapy in childhood survivors.

Acute radiation CNS toxicity occurs within weeks and is characterized by nausea, drowsiness, hypersomnia, and ataxia, which typically recover over time. Early delayed toxicity occurring weeks to 3 months following therapy is associated with similar symptoms as acute toxicity and is pathologically associated with reversible demyelination. Chronic, late radiation injury occurs 9 months to up to 10 years following therapy, and dysfunction increases over time. Radiation-associated spinal cord injury (myelopathy) is highly dose-dependent and rarely occurs with modern radiation therapy. An early, self-limited form involving electric sensations down the spine on neck flexion (Lhermitte's sign) is seen 6–12 weeks after treatment and generally resolves over weeks. Peripheral nerve toxicity is quite rare owing to relative radiation resistance. Diffuse radiation injury is associated with global CNS neurologic dysfunction and diffuse white matter changes on computed tomography (CT) or MRI. Pathologically, small vessel changes are prominent and focal necrosis is common. Necrotizing encephalopathy is the most severe form of radiation injury and almost always is associated with concurrent use of chemotherapy, notably methotrexate. Prophylactic cranial irradiation in both childhood and adult leukemias/lymphomas has largely been abandoned due to the acute and long-term effects of therapy. Glucocorticoids may be symptomatically useful for acute toxicities but do not alter the course. Psychostimulants such as methylphenidate may improve attention and executive functioning in childhood survivors.

In children and adolescent cancers, younger age, higher cranial irradiation dose, larger brain volumes irradiated, and longer treatment times are associated with worse neurocognitive outcomes. In adult cancers, patients over the age of 60 who receive whole-brain radiation therapy are at high risk for neurocognitive impairment after therapy. Genetic polymorphisms may be associated with an increased risk of neurocognitive problems, and emerging evidence suggests polymorphisms in the folate pathway, oxidative stress genes, and enzymes that regulate both catecholamines and deamination of amines are associated with individual risk.

Vinca alkaloids produce a characteristic "stocking-glove" neuropathy with numbness and tingling advancing to loss of motor function, which is highly dose related. Distal sensorimotor polyneuropathy prominently involves loss of deep tendon reflexes with initially loss of pain and temperature sensation, followed by proprioceptive and vibratory loss. This requires careful patient history and physical examination by experienced oncologists to decide when the drug must be stopped or reduced to prevent permanent damage. Milder toxicity often slowly completely resolves after treatment discontinuation. Vinca alkaloids may sometimes be associated with jaw claudication,

hoarseness, autonomic neuropathy, ileus, cranial nerve palsies, and, in severe cases, encephalopathy, seizures, and coma. Cisplatin is associated with sensorimotor neuropathy and hearing loss, especially at doses $>400 \text{ mg/m}^2$, requiring audiometry in patients with preexisting hearing compromise. Carboplatin is often substituted in such cases given its lesser effect on hearing. Many of the agents that target kinase enzymes in tumor cells and 5-fluorouracil congeners produce dysesthesias and painful hands and feet known as hand-foot syndrome or palmar-planter erythrodynesthesia. Symptoms usually abate when the agent is stopped. Methotrexate alone may cause acute leukoencephalopathy characterized by somnolence and confusion that is often reversible. Acute toxicity is dose-related, especially at doses $>3 \text{ g/m}^2$, with younger patients being at greater risk. Subacute methotrexate toxicity occurs weeks after therapy and is often ameliorated with glucocorticoid therapy. Chronic methotrexate toxicity (leukoencephalopathy) develops months or years after treatment and is characterized clinically as progressive loss of cognitive function and focal neurologic signs, which are irreversible, promoted by synchronous or metachronous radiation therapy, and more pronounced at a younger age. Neurocognitive decline following chemotherapy alone occurs notably in breast cancer patients receiving adjuvant chemotherapy with anthracyclines, taxanes, or cyclophosphamide; this has been referred to as "chemo brain." It is clinically associated with impaired memory, learning, attention, and speed of information processing. There is no clear mechanistic explanation for its cause and no clearly effective therapy, although regular exercise is associated with improved symptoms. Most symptoms improve within a year of therapy, but symptoms can persist in 10–20% of patients for extended periods of time.

Newer molecularly targeted agents and immunotherapy have also been associated with neurologic dysfunction and may exacerbate persistent neuropathy from prior therapy. Proteasome inhibitors are associated with neuropathic pain and motor neuropathy that can occur immediately or be delayed in onset. The proposed mechanism is through enhanced oxidative stress on neural cells. Subcutaneous administration of these agents is associated with less peripheral neuropathy than intravenous infusions. Immunotherapy agents such as CAR-T therapies and bi-specific monoclonal antibodies targeting both T cells and B cells are associated with significant acute neurotoxicity including confusion, encephalopathy, seizures, and cerebellar symptoms. These symptoms are hypothesized to be related to the CRS associated with these therapies and often are time limited. However, a minority of patients treated with these agents will have persistent central and neurologic dysfunction for which management is mainly supportive. Glucocorticoids may be useful in the short term, but treatment with interleukin 6 receptor antagonists that are effective for decreasing the severity of CRS is largely ineffective at preventing neurologic toxicities. Progressive multifocal leukoencephalopathy (PML) is a rare but serious brain infection that is caused by the JC virus and has been reported as a rare complication of treatment with rituximab, ibritumomab, and PI3K inhibitors. The inflammatory response to the virus presents as unifocal or multifocal hyperintense lesions involving the subcortical white matter that are best seen on T2/fluid-attenuated inversion recovery (FLAIR) images on MRI. Treatment is supportive and includes removal of the offending agent. Other targeted agents that may be associated with neurologic dysfunction when given for extended durations of treatment include dasatinib, thalidomide, and lenalidomide.

Antibody-drug conjugates (ADCs) are novel therapies in which a monoclonal antibody targeted to a tumor antigen is attached to a potent anticancer agent via a chemical linker. Often, these agents are associated with significant central and peripheral neurotoxicity, as has been described with agents such as brentuximab vedotin and pertuzumab. These neuropathies often emerge during treatment similar to those seen with chemotherapy and are dose-dependent. Early recognition of treatment-emergent neuropathy induced by ADCs mandates dose interruption or modification to less frequent dosing schedules in order for patients to remain on therapy. Immune checkpoint inhibitors have been associated with autoimmune complications and unique neurologic manifestations such as optic neuritis that may be reversible after glucocorticoids.

HEPATIC AND GASTROINTESTINAL DYSFUNCTION

Long-term hepatic damage from standard chemotherapy regimens is rare. Long-term methotrexate or high-dose chemotherapy alone or with radiation therapy, for example, in preparative regimens for bone marrow transplantation, may result in venoocclusive disease of the liver. This potentially lethal complication classically presents with anicteric ascites, elevated alkaline phosphatase, and hepatosplenomegaly. Pathologically, there is venous congestion, epithelial cell proliferation, and hepatocyte atrophy progressing to frank fibrosis. Frequent monitoring of liver function tests during any chemotherapy is necessary to avoid both idiosyncratic and expected toxicities. Certain nucleoside drugs have been associated with hepatic dysfunction; however, this complication is rare in oncology. Hepatic radiation damage depends on dose, volume, fractionation, preexisting liver disease, and synchronous or metachronous chemotherapy. In general, radiation doses to the liver $>1500 \text{ cGy}$ can produce hepatic dysfunction with a steep dose-injury curve. Radiation-induced liver disease closely mimics hepatic venoocclusive disease.

Novel targeted agents including immunotherapy agents have introduced a number of gastrointestinal toxicities that can occur late in the course of treatment including hepatitis, colitis, malabsorption, and chronic diarrhea. Early signs of serious liver injury should lead to discontinuation of the offending agent as the effect does not appear to be dose-related and dose reductions do not reliably reduce further liver injury. The mechanisms for colitis or hepatitis associated with these targeted agents are not completely understood but are hypothesized to be T cell-mediated, and the risk is highest when used in targeted agent combinations. Diarrhea with or without severe colitis can be associated with virtually all targeted agents including PI3K inhibitors, BCR-ABL inhibitors, BTK inhibitors, EGFR inhibitors, MEK inhibitors, CDK inhibitors, and immunomodulatory agents. Even if the diarrhea is not severe, the impact associated with indefinite treatment greatly interferes with the quality of life and often leads to discontinuation of targeted therapy if not managed effectively. Immunomodulatory agents including lenalidomide are associated with late onset of diarrhea that is caused by bile acid malabsorption and often responds to bile acid sequestrants. Immune checkpoint inhibitors are associated with colitis and hepatitis that may be responsive to prompt initiation of glucocorticoids that may require a long taper for resolution.

RENAL AND BLADDER DYSFUNCTION

Cisplatin produces reversible decrements in renal function but may also produce severe irreversible toxicity in the presence of renal disease and may predispose to accentuated damage with subsequent renal insults. Cyclophosphamide and ifosfamide are prodrugs primarily activated in the liver with cleavage products (acrolein) that can produce hemorrhagic cystitis. This can be prevented with the free radical scavenger MESNA (mercaptoethane sulfonate), which is required for ifosfamide administration. Hemorrhagic cystitis caused by these agents may predispose to bladder cancer.

Targeted agents generally do not carry significant acute nephrotoxic risks, but a number of agents, including PI3K inhibitors, anti-VEGF agents, and FLT3 inhibitors, are associated with systemic hypertension, which can lead to late effects or a progressive decline in renal function. Renal dysfunction following immunotherapy is uncommon, but acute interstitial nephritis can occur. Similar to other immune-related toxicities, this acute toxicity requires prompt use of glucocorticoids to avoid long-term effects on renal function.

PSYCHOLOGICAL DYSFUNCTION AND SOCIOECONOMIC IMPACT OF SURVIVORSHIP

The diagnosis and treatment of cancer can introduce long-term and late psychological effects that continue throughout life. Cancer survivors are at increased risk for anxiety, depression, attention problems, and posttraumatic stress syndromes. Many cancer patients experience intrusive or debilitating concerns about cancer recurrence following

successful therapy. In addition, these patients may experience socio-economic stressors that affect employment, insurance, relationships and lead to financial and/or sexual difficulties. Survivors of childhood cancers are less likely to graduate from college or gain full-time employment than their peers and are more likely to engage in risky health behaviors such as substance abuse and excessive alcohol use. The long-term psychosocial effects of treatment are greatest in patients who undergo CNS-directed therapies including radiation and intensive combination chemotherapy regimens. Oncologists should ask about and address these issues explicitly with patients and provide appropriate counseling or support systems. The overall risk of suicidal ideation and suicide is low but is greater in cancer patients and survivors than age-matched controls. Tailored cognitive-behavioral therapy may improve the anxiety and posttraumatic stress associated with cancer survivorship.

CANCER SURVIVORSHIP CARE PLANS

Survivorship starts at the time of diagnosis and continues indefinitely. Many guidelines recommend that every patient be provided with a survivorship care plan unique to their situation, but the evidence that these improve health outcomes is limited and sufficient resources to implement recommendations are often lacking. Focused surveillance plans for late effects are critical for early detection and implementation of interventions but also must include risk stratification to avoid unnecessary surveillance testing that wastes resources and leads to overdiagnosis and/or psychological distress. Survivorship care has traditionally been performed by oncologists, but the scope of the problem mandates that primary care physicians, midlevel providers, and preventive medicine specialists be trained in the follow-up of treated cancer patients in remission. All former cancer patients should undergo surveillance for recurrence and second malignancies and be monitored for long-term effects of treatment; however, as a practical matter, nearly all recurrences are detected because of symptoms. Health promotion and disease prevention with age- and sex-specific routine screening tests (e.g., colonoscopy, Pap smears, mammography, human papillomavirus vaccination, dual-energy x-ray absorptiometry scans) should be a focus of survivorship care along with psychosocial well-being. Annual mammography should start no later than 10 years after breast radiation. Patients receiving radiation fields encompassing thyroid tissue should have regular thyroid examinations and TSH testing. Localized pain or palpable abnormality in a previously radiated field should prompt radiographic evaluation. Patients treated with alkylating agents or topoisomerase inhibitors should have a complete blood count every 6–12 months, and cytopenias, abnormal cells on peripheral smear, or macrocytosis should be evaluated with bone marrow biopsy and aspirate and include cytogenetics, flow cytometry, or fluorescence in situ hybridization (FISH) studies as appropriate.

As the population of cancer survivors increases and patients live longer, cancer survivorship has become increasingly important, and the Institute of Medicine and National Research Council have published a monograph entitled *From Cancer Patient to Cancer Survivor: Lost in Transition*. The monograph proposes a plan that would inform clinicians caring for cancer survivors of the complete details of patients' previous treatments, complications thereof, signs and symptoms of late effects, and recommended screening and follow-up procedures.

OUTLOOK

Survivorship care is one of the most challenging problems facing oncologists today. The challenge is to develop cancer treatments that utilize the most effective combination of surgery, chemotherapy, radiation, targeted agents, or immunotherapy that is required to cure disease or effect long-term disease control with the least amount of toxicity. As cancer treatments continue to improve, the need for cancer care increases due to more cancer survivors with increasing life expectancy. Clearly, much work remains to elucidate the pathophysiology of cancer treatment-related effects and identification of patient characteristics associated with an increased vulnerability to adverse effects. Clinical management strategies focused on the clinical management of acute toxicities and prevention of long-term effects after therapy are

necessary. Finally, research initiatives should recognize that as treatment paradigms continue to evolve, the nature and biologic basis for toxicities will change. Advances in genomic medicine may add depth to our understanding of toxicities and allow for more personalized surveillance strategies. Longitudinal monitoring of the health of cancer survivors is required since the incidence of late effects of treatment does not appear to plateau over time.

A

We would like to acknowledge the contribution of Carl E. Freter who coauthored a previous version of this chapter; material from his chapter was retained in this version.

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Section 2 Hematopoietic Disorders

96

Hematopoietic Stem Cells

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All of the cell types in the peripheral blood and some cells in every tissue of the body are derived from hematopoietic (*hemo*: blood; *poiesis*: creation) stem cells. If the hematopoietic stem cell is damaged and can no longer function (e.g., due to a nuclear accident), a person would survive 2–4 weeks in the absence of extraordinary support measures. With the clinical use of hematopoietic stem cells, tens of thousands of lives are saved each year (Chap. 114). Stem cells produce hundreds of billions of blood cells daily from a stem cell pool that is estimated to be only 100,000. How stem cells do this, how they persist for many decades despite the production demands, and how they may be better used in clinical care are important issues in medicine.

The study of blood cell production has become a paradigm for how other tissues may be organized and regulated. Basic research in hematopoiesis includes defining stepwise molecular changes accompanying functional changes in maturing cells, aggregating cells into functional subgroups, and demonstrating hematopoietic stem cell

regulation by a specialized microenvironment; these concepts are worked out in hematopoiesis and offer models for other tissues. Moreover, these concepts may not be restricted to normal tissue function but extend to malignancy. Stem cells are rare cells among a heterogeneous population of cell types, and their behavior is assessed mainly in experimental animal models involving reconstitution of hematopoiesis. Thus, much of what we know about stem cells is imprecise and based on inferences from genetically manipulated animals.

CARDINAL FUNCTIONS OF HEMATOPOIETIC STEM CELLS

All stem cell types have two cardinal functions: self-renewal and differentiation (Fig. 96-1). Stem cells exist to generate, maintain, and repair tissues. They function successfully if they can replace a wide variety of shorter-lived mature cells over prolonged periods. The process of self-renewal (see below) assures that a stem cell population can be sustained over time. Without self-renewal, the stem cell pool would become exhausted and tissue maintenance would not be possible. The process of differentiation leads to production of the effectors of tissue function: mature cells. Without proper differentiation, the integrity of tissue function would be compromised and organ failure or neoplasia would ensue.

In the blood, mature cells have variable average life spans, ranging from hours for mature neutrophils to a few months for red blood cells to many years for memory lymphocytes. However, the stem cell pool is the central, durable source of all blood and immune cells, maintaining a capacity to produce a broad range of cells from a single cell source, yet keeping itself vigorous over decades of life. As an individual stem cell divides, it has the capacity to accomplish one of three division outcomes: two stem cells, two cells destined for differentiation, or one stem cell and one differentiating cell. The former two outcomes are the result of symmetric cell division, whereas the latter indicates a different outcome for the two daughter cells—an event termed asymmetric cell division. The relative balance for these types of outcomes may change during development and under particular kinds of demands on the stem cell pool.

DEVELOPMENTAL BIOLOGY OF HEMATOPOIETIC STEM CELLS

During development, blood cells are produced at different sites. Initially, the yolk sac provides oxygen-carrying red blood cells and many of the macrophage-like cells that are resident in tissues: cells like microglia in the brain. The placenta and several sites of intraembryonic blood cell production then become involved in sequential order. These

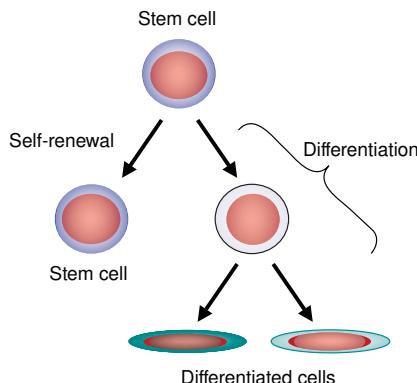


FIGURE 96-1 Signature characteristics of the stem cell. Stem cells have two essential features: the capacity to differentiate into a variety of mature cell types and the capacity for self-renewal. Intrinsic factors associated with self-renewal include expression of *Bmi-1*, *Gli-1*, *PTEN*, *STAT5*, *Tel/Atv6*, *p21*, *p18*, *MCL-1*, *Mel-18*, *RAE28*, and *HoxB4*. Extrinsic signals for self-renewal include Notch, Wnt, SHH, angiogenin, and Tie2/Ang-1. Based mainly on murine studies, hematopoietic stem cells express the following cell surface molecules: CD34, Thy-1 (CD90), c-Kit receptor (CD117), CD133, CD164, and c-Mpl (CD110, also known as the thrombopoietin receptor).

move from the genital ridge at a site where the aorta, gonadal tissue, and mesonephros are emerging to the fetal liver and then, in the second trimester, to the bone marrow and spleen. As the location of stem cells changes, the cells they produce also change. The yolk sac provides red cells expressing embryonic hemoglobins and tissue-resident macrophages. Intraembryonic sites of hematopoiesis generate stem cells, red cells, platelets, and the circulating cells of innate immunity. The production of the cells of adaptive immunity occurs then as well but becomes robust as the thymus forms and the bone marrow is colonized in the second trimester. Stem cell proliferation remains high, even in the bone marrow, until shortly after birth, when it appears to dramatically decline. The cells in the bone marrow are thought to arrive by the bloodborne transit of cells from the fetal liver after calcification of the long bones has begun. The presence of stem cells in the circulation is not unique to a time window in development, however, as hematopoietic stem cells circulate throughout life. The time that stem cells spend freely circulating appears to be brief (measured in minutes in the mouse), but the stem cells that do circulate are functional and can be used for transplantation. The number of stem cells that circulate can be increased in a number of ways to facilitate harvest and transfer to the same or a different host.

MOBILITY OF HEMATOPOIETIC STEM CELLS

Cells entering and exiting the bone marrow do so through a series of molecular interactions. Circulating stem cells (through CD162 and CD44) engage the lectins (carbohydrate binding proteins) P- and E-selectin on the endothelial surface to slow the movement of the cells to a rolling phenotype. Stem cell integrins are then activated and accomplish firm adhesion between the stem cell and vessel wall, with a particularly important role for stem cell VCAM-1 engaging endothelial VLA-4. The chemokine CXCL12 (SDF1) interacting with stem cell CXCR4 receptors and ionic calcium interacting with the calcium-sensing receptor appear to be important in the process of stem cells getting from the circulation to where they engraft in the bone marrow. This is particularly true in the developmental move from fetal liver to bone marrow.

In the adult, the role for CXCR4 is in retention of stem cells in the bone marrow as well as getting them there. Interrupting that retention process through specific molecular blockers of the CXCR4/CXCL12 interaction, cleavage of CXCL12, or downregulation of the CXCR4 receptor can result in the release of stem cells into the circulation. This process is an increasingly important aspect of recovering stem cells for therapeutic use as it has permitted the harvesting process to be done by leukapheresis rather than bone marrow punctures in the operating room. Granulocyte colony-stimulating factor and plerixafor, a macrocyclic compound that can block CXCR4, are both used clinically to mobilize marrow hematopoietic stem cells for transplant. Refining our knowledge of how stem cells get into and out of the bone marrow may improve our ability to obtain stem cells and make them more efficient at finding their way to the specific sites for blood cell production, the so-called stem cell niche.

HEMATOPOIETIC STEM CELL MICROENVIRONMENT

The concept of a specialized microenvironment, or stem cell niche, was first proposed to explain why cells derived from the bone marrow of one animal could be used in transplantation and again be found in the bone marrow of the recipient. This niche is more than just a housing site for stem cells, however. It is an anatomic location where regulatory signals are provided that allow the stem cells to thrive, to expand if needed, and to provide varying amounts of descendant daughter cells. In addition, unregulated growth of stem cells may be problematic based on their undifferentiated state and self-renewal capacity. Thus, the niche must also regulate the number of stem cells produced. In this manner, the niche has the dual function of serving as a site of nurture but imposing limits for stem cells: in effect, acting as both a nutritive and constraining home.

The niche for blood stem cells changes with each of the sites of blood production during development, but for most of human life, it

is located in the bone marrow. Within the bone marrow, the perivascular space particularly in regions of trabecular bone serves as a niche. The mesenchymal and endothelial cells of the marrow microvessels produce kit ligand and CXCL12, both known to be important for hematopoietic stem cells. Other cell types, such as sympathetic neurons, nonmyelinating Schwann cells, macrophages, megakaryocytes, osteoclasts, and osteoblasts, have been shown to regulate stem cells, some by direct and others by indirect effects. Extracellular matrix proteins like osteopontin and heparan sulfates also affect stem cell function. The endosteal region appears to be particularly important for transplanted cells, in part because many of the mesenchymal cells and sinusoidal blood vessels of the central marrow are disrupted by the conditioning regimens used to prepare a patient for transplantation. The functioning of the niche as a supportive context for stem cells is of obvious importance for maintaining hematopoiesis and in transplantation. An active area of study involves determining whether the niche is altered in disease as experimental models have shown that mutations in niche cells can lead to myeloid malignancies. It logically follows that targeting of niche functions is a potential therapeutic strategy for both malignant and normal hematopoiesis.

■ EXCESS CAPACITY OF HEMATOPOIETIC STEM CELLS

In the absence of disease, one never runs out of hematopoietic stem cells. Indeed, serial transplantation studies in mice suggest that sufficient stem cells are present to reconstitute several animals in succession, with each animal having normal blood cell production. The fact that allogeneic stem cell transplant recipients also never run out of blood cells in their life span, which can extend for decades, argues that even the limiting numbers of stem cells provided to them are sufficient. How stem cells respond to different conditions to increase or decrease their mature cell production remains poorly understood. Clearly, negative feedback mechanisms affect the level of production of most of the cells, leading to the normal tightly regulated blood cell counts. However, many of the regulatory mechanisms that govern production of more mature progenitor cells do not apply or apply differently to stem cells. Similarly, most of the molecules shown to be able to change the size of the stem cell pool have little effect on more mature blood cells. For example, the growth factor erythropoietin, which stimulates red blood cell production from precursor cells, has no effect on stem cells. Similarly, granulocyte colony-stimulating factor drives the rapid proliferation of granulocyte precursors but has little or no effect on the cell cycling of stem cells. Rather, it changes the location of stem cells by indirect means, altering molecules such as CXCL12 that tether stem cells to their niche. Molecules shown to be important for altering the proliferation, self-renewal, or survival of stem cells, such as cyclin-dependent kinase inhibitors, transcription factors like Bmi-1, microRNA-processing enzymes like Dicer, or even metabolic regulators like pyruvate kinase isoforms, have little or different effects on progenitor cells. Hematopoietic stem cells have governing mechanisms that are distinct from the cells they generate.

■ HEMATOPOIETIC STEM CELL DIFFERENTIATION

Hematopoietic stem cells sit at the base of a branching hierarchy of cells culminating in the many mature cell types that compose the blood and immune system (Fig. 96-2). The maturation steps leading to terminally differentiated and functional blood cells take place both as a consequence of intrinsic changes in gene expression and niche-directed and cytokine-directed changes in the cells. Our knowledge of the details remains incomplete. As stem cells mature to progenitors, precursors, and, finally, mature effector cells, they undergo a series of functional changes. These include the obvious acquisition of functions defining mature blood cells, such as phagocytic capacity or hemoglobin synthesis. They also include the progressive loss of plasticity (i.e., the ability to become other cell types). For example, the myeloid progenitor can make all cells in the myeloid series but none in the lymphoid series. As common myeloid progenitors mature, they become precursors for either monocytes and granulocytes or erythrocytes and megakaryocytes, but not both. Some amount of reversibility of this

process may exist early in the differentiation cascade, but that is lost beyond a distinct stage in normal physiologic conditions. With genetic interventions, however, blood cells, like other somatic cells, can be reprogrammed to become a variety of cell types.

As cells differentiate, they may also lose proliferative capacity (Fig. 96-3). Mature granulocytes are incapable of proliferation and only increase in number by increased production from precursors. The exceptions to the rule are some tissue-resident macrophages, which appear capable of proliferation, and lymphoid cells. Lymphoid cells retain the capacity to proliferate but have linked their proliferation to the recognition of particular proteins or peptides by specific antigen receptors on their surface. Like many tissues with short-lived mature cells such as the skin and intestine, blood cell proliferation is largely accomplished by a more immature progenitor population. In general, cells within the highly proliferative progenitor cell compartment are also relatively short-lived, making their way through the differentiation process in a defined molecular program involving the sequential activation of particular sets of genes. For any particular cell type, the differentiation program is difficult to speed up. The time it takes for hematopoietic progenitors to become mature cells is ~10–14 days in humans, evident clinically by the interval between cytotoxic chemotherapy and blood count recovery in patients.

Although hematopoietic stem cells are generally thought to have the capacity to form all cells of the blood, it is becoming clear that individual stem cells may not be equal in their differentiation potential. That is, some stem cells are “biased” to become mature cells of a particular type. In addition, the general concept of cells having a binary choice of lymphoid or myeloid differentiation is not entirely accurate. A cell population with limited megakaryocytic and erythroid or myeloid (monocyte and granulocyte) and lymphoid potential is now added to the commitment steps stem cells may undergo.

■ SELF RENEWAL

The hematopoietic stem cell must balance its three potential fates: apoptosis, self-renewal, and differentiation. The proliferation of cells is generally not associated with the ability to undergo a self-renewing division except among memory T and B cells and among stem cells. Self-renewal capacity has generally been regarded as giving way to differentiation as the only option after cell division when cells leave the stem cell compartment, unless they become memory lymphocytes. However, emerging data suggest that some myeloid committed progenitors may also have self-renewing potential *in vivo*, providing long-term production of cells. Stem cells all have self-renewing capacity by definition, and they have an additional feature characterizing their proliferation machinery. Stem cells in many mature adult tissues are heterogeneous with some being deeply quiescent, serving as a deep reserve, whereas others are more proliferative and replenish the short-lived progenitor population. In the hematopoietic system, stem cells are generally cytokine-resistant, remaining dormant even when cytokines drive bone marrow progenitors to proliferation rates measured in hours. Stem cells, in contrast, are thought to divide at far longer intervals, measured in months to years, for the most quiescent cells. This quiescence is difficult to overcome *in vitro*, limiting the ability to effectively expand human hematopoietic stem cells. The process may be controlled by particularly high levels of cyclin-dependent kinase inhibitors like p57 or CDKN1c that restrict entry of stem cells into the cell cycle, blocking the G₁-S transition. Exogenous signals from the niche also appear to enforce quiescence, including angiogenin, interleukin 18, and perhaps angiopoietin 1.

The regulation of stem cell proliferation also appears to change with age. Both cell intrinsic features like the cyclin-dependent kinase inhibitor p16INK4a and bone marrow microenvironment features like declining sympathetic innervation are implicated in age-related stem cell changes. Either lowering expression of p16INK4a or stimulating beta-3 adrenergic receptors in older animals improves stem cell cycling and capacity to reconstitute hematopoiesis in adoptive hosts, making them similar to younger animals. Mature cell numbers are unaffected. Therefore, molecular events governing the specific functions of stem cells are being gradually made clear and offer the potential of new

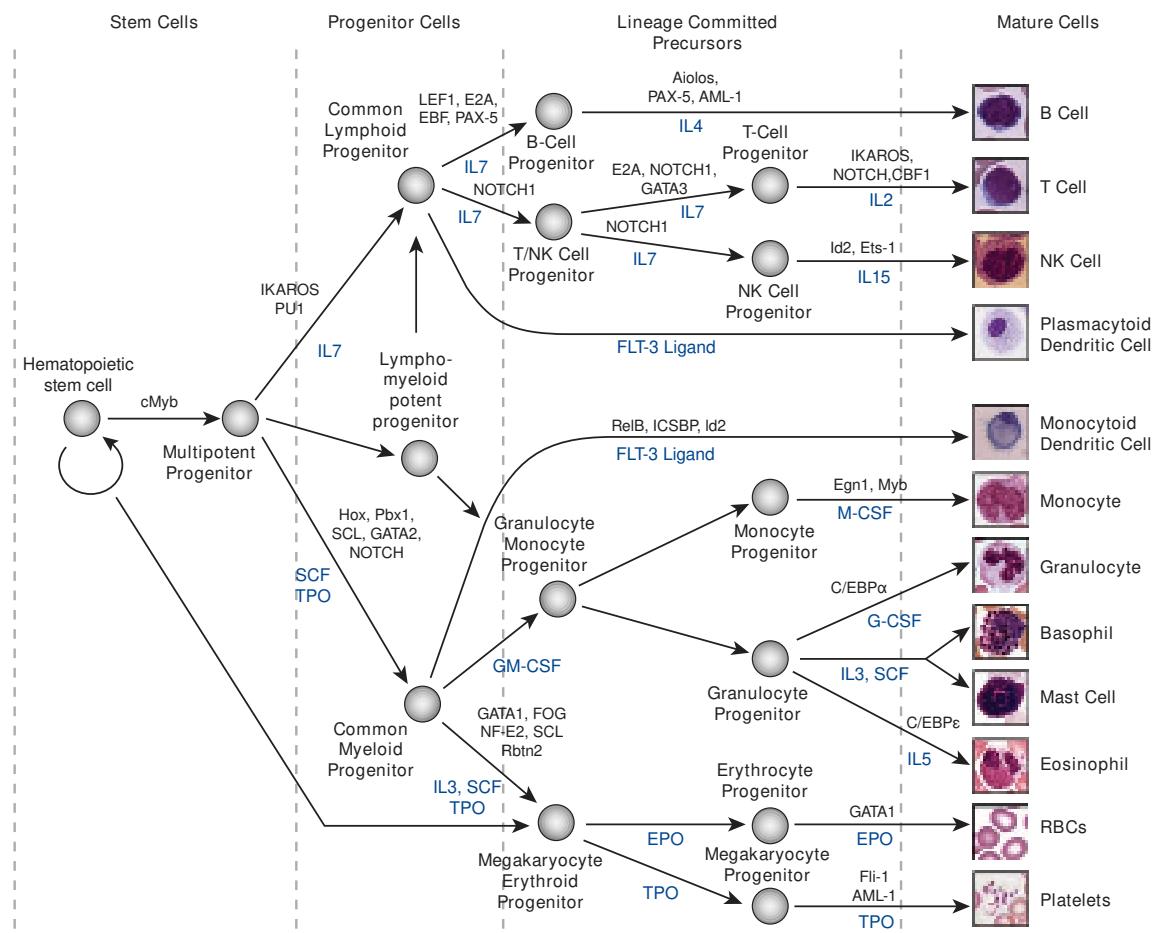


FIGURE 96-2 Hierarchy of hematopoietic differentiation. *Stem cells* are multipotent cells that are the source of all descendant cells and have the capacity to provide either long-term (measured in years) or short-term (measured in months) cell production. *Progenitor cells* have a more limited spectrum of cells they can produce and are generally a shorter-lived, highly proliferative population also known as transient amplifying cells. *Precursor cells* are cells committed to a single blood cell lineage but with a continued ability to proliferate; they do not have all the features of a fully mature cell. *Mature cells* are the terminally differentiated product of the differentiation process and are the effector cells of specific activities of the blood and immune system. Progress through the pathways is mediated by alterations in gene expression. The regulation of the differentiation by soluble factors and cell-cell communications within the bone marrow niche are still being defined. The transcription factors that characterize particular cell transitions are illustrated on the arrows; the soluble factors that contribute to the differentiation process are in blue. This picture is a simplification of the process. Active research is revealing multiple discrete cell types in the maturation of B cells and T cells and has identified cells that are biased toward one lineage or another (rather than uncommitted) in their differentiation. EPO, erythropoietin; RBC, red blood cell; SCF, stem cell factor; TPO, thrombopoietin.

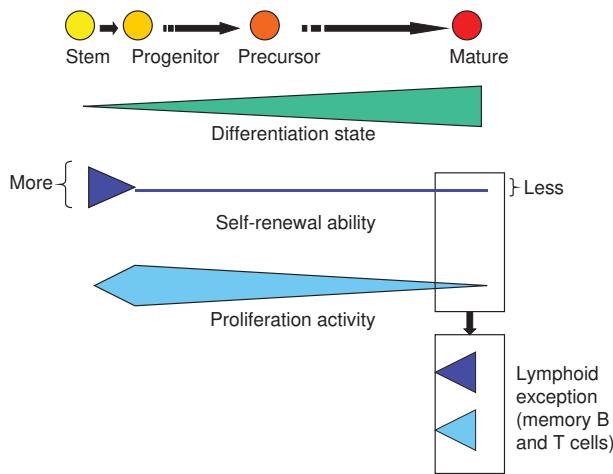


FIGURE 96-3 Relative function of cells in the hematopoietic hierarchy. The boxes represent distinct functional features of cells in the myeloid (upper box) versus lymphoid (lower box) lineages.

therapeutic approaches to changing stem cell functions. One critical stem cell function that remains poorly defined is the molecular regulation of self-renewal.

For medicine, self-renewal is perhaps the most important function of stem cells because it is critical in regulating the number of stem cells. Stem cell number is a key limiting parameter for both autologous and allogeneic stem cell transplantation. Were we to have the ability to use fewer stem cells or expand limited numbers of stem cells ex vivo, it might be possible to reduce the morbidity and expense of stem cell harvests, enable use of other stem cell sources, and improve the potential for gene-modified stem cell transplant. For example, umbilical cord blood is a rich source of stem cells but generally has an inadequate number of stem cells for use in transplantation in adults. These cells have two advantages over other stem cell sources: there is a lower incidence of graft-versus-host disease, and cord blood banks have representation of populations underrepresented in adult donor registries. Hematopoietic reconstitution from cord blood is slow, however, in part due to cell number. Expansion might improve this; however, advances in haploidentical donor cell transplantation have reduced cord blood use.

Gene-modified stem cells are increasingly being tested and have been found to offer great promise for genetic blood diseases like congenital immunodeficiencies and hemoglobinopathies such as sickle cell

disease. However, the complexity and cost of modifying enough stem cells for transplantation is problematic. Expanding a small number of gene-modified stem cells may mitigate that issue. Therefore, understanding self-renewal offers the potential to facilitate development of an important new area of stem cell-based medicine. Some limited understanding of self-renewal exists and, intriguingly, implicates gene products that are associated with the chromatin state, a high-order organization of chromosomal DNA that influences transcription. These include members of the polycomb family, a group of zinc finger-containing transcriptional regulators that interact with the chromatin structure, contributing to the accessibility of groups of genes for transcription. One member, Bmi-1, is important in enabling hematopoietic stem cell self-renewal through modification of cell cycle regulators such as the cyclin-dependent kinase inhibitors. In the absence of Bmi-1 or of the transcriptional regulator, Gfi-1, hematopoietic stem cells decline in number and function. In contrast, dysregulation of Bmi-1 has been associated with leukemia; it may promote leukemic stem cell self-renewal when it is overexpressed. The same is true for the polycomb gene, *AxrlI*, that is commonly mutated in myelodysplasia and leukemia. Other transcription regulators have also been associated with self-renewal, particularly homeobox, or "hox," genes. These transcription factors are named for their ability to govern large numbers of genes, including those determining body patterning in invertebrates. HoxB4 is capable of inducing extensive self-renewal of stem cells through its DNA-binding motif. Other members of the hox family of genes have been noted to affect normal stem cells, but they are also associated with leukemia. Epigenetic modifiers such as the DNA methyl transferase DNMT3a or the dioxygenase involved in DNA demethylation, Tet2, also play a role in stem cell regulation. Like *AxrlI*, mutations of these genes are associated with clonal outgrowth of stem cells bearing the mutations. These mutations are not sufficient for malignancy, but they enable clones bearing them to gain dominance and predispose cells to malignant transformation. They are often referred to as "founder mutations" because myelodysplastic and leukemic cells appear to evolve from them by DNA sequencing analysis.

CANCER IS SIMILAR TO AN ORGAN WITH SELF RENEWING CAPACITY

The relationship of stem cells to cancer is an important dimension of adult stem cell biology. Cancer may share principles of organization with normal tissues. Cancer cells are heterogeneous even within a given patient and may have a hierarchical organization of cells with a base of stem-like cells capable of the signature stem cell features: self-renewal and differentiation. These stem-like cells might be the basis for perpetuation of the tumor and represent a slowly dividing, rare population with distinct regulatory mechanisms, including a relationship with a specialized microenvironment. A subpopulation of self-renewing cells has been defined for some, but not all, cancers. These include myeloid leukemias where founder mutations appear to enable clones of cells to expand. With additional mutations, these can serve as the initiating or stem cells of a cancer, and eliminating them may be necessary for curing the patient. Understanding the hierarchical cell organization within cancers and whether eliminating cancer stem cell equivalents can improve cure rates is an area of active investigation.

Does the concept of cancer stem cells provide insight into the cellular origin of cancer? The fact that some cells within a cancer have stem cell-like properties does not necessarily mean that the cancer arose in the stem cell itself. Rather, more mature cells could have acquired the self-renewal characteristics of stem cells. Any single genetic event is unlikely to be sufficient to enable full transformation of a normal cell to a frankly malignant one. Rather, cancer is a multistep process, and for the multiple steps to accumulate, the cell of origin must be able to persist for prolonged periods. It must also be able to generate large numbers of daughter cells. The normal stem cell has these properties and, by virtue of its having intrinsic self-renewal capability, may be more readily converted to a malignant phenotype. This hypothesis has been tested experimentally in the hematopoietic system. Taking advantage of the cell-surface markers that distinguish hematopoietic cells of varying maturity, stem cells, progenitors, precursors, and mature cells

can be isolated. Powerful transforming gene constructs were placed in these cells, and it was found that the cell with the greatest potential to produce a malignancy was dependent on the transforming gene. In some cases, it was the stem cell, but in others, the progenitor cell functioned to initiate and perpetuate the cancer. This shows that cells can acquire stem cell-like properties in malignancy.

WHAT ELSE CAN HEMATOPOIETIC STEM CELLS DO?

Some experimental data have suggested that hematopoietic stem cells or other bone marrow cells are capable of playing a role in healing the vascular and tissue damage associated with stroke and myocardial infarction. These data are controversial, and the applicability of a stem cell approach to nonhematopoietic conditions remains experimental. However, reprogramming technology offers the potential for using readily obtained hematopoietic cells as a source for cells with other capabilities. Active areas of investigation are to use reprogrammed cells to generate mature lymphoid cells for immuno-oncology applications or red cells and platelets to overcome dependency on blood donors.

STEM CELLS AS TARGETS OF GENE THERAPY

Tools to alter gene sequence, expression, and regulation are becoming increasingly feasible. The hematopoietic stem cell is a target for a wide range of interventions. Lentiviral, retroviral, and adenoviral vectors are being used to replace defective genes (e.g., in primary immunodeficiency diseases). Antisense technology is being applied to block gene expression (e.g., blocking the *Bcl11a* repression of fetal globin expression in sickle cell disease and thalassemia). CRISPR/Cas technology is being applied to repair abnormal gene sequences. Precision genetic manipulations are expanding, and the hematopoietic system is central to it.

In sum, the stem cell has tremendous healing capacity and is essential for life. However, if dysregulated, it can threaten the life it maintains. Understanding how stem cells function, the signals that modify their behavior, and the tissue niches that modulate stem cell responses to injury and disease is critical for more effectively developing stem cell-based medicines.

■ FURTHER READING

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97

Iron Deficiency and Other Hypoproliferative Anemias

John W. Adamson

Anemias associated with normocytic and normochromic red cells and an inappropriately low reticulocyte response (reticulocyte index <2–2.5) are *hypoproliferative anemias*. This category includes early iron deficiency (before hypochromic microcytic red cells develop), acute and chronic inflammation (including many malignancies), renal disease, hypometabolic states such as protein malnutrition and endocrine

TABLE 97-1 Body Iron Distribution

	IRON CONTENT, mg	
	ADULT MALE, 80 kg	ADULT FEMALE, 60 kg
Hemoglobin	2500	1700
Myoglobin/enzymes	500	300
Transferrin iron	3	3
Iron stores	600–1000	0–300

deficiencies, and anemias from marrow damage. Marrow damage states are discussed in [Chap. 102](#).

Hypoproliferative anemias are the most common anemias, and in the clinic, iron deficiency anemia is the most common of these followed by the anemia of inflammation. The anemia of inflammation, similar to iron deficiency, is related in part to abnormal iron metabolism. The anemias associated with renal disease, inflammation, cancer, and hypometabolic states are characterized by a suboptimal erythropoietin response to the anemia.

IRON METABOLISM

Iron is a critical element in the function of all cells, although the amount of iron required by individual tissues varies during development. At the same time, the body must protect itself from free iron, which is highly toxic in that it participates in chemical reactions that generate free radicals such as singlet O₂ or OH⁻. Consequently, elaborate mechanisms have evolved that allow iron to be made available for physiologic functions while at the same time conserving this element and handling it in such a way that toxicity is avoided.

The major role of iron in mammals is to carry O₂ as part of hemoglobin. O₂ is also bound by myoglobin in muscle. Iron is a critical element in iron-containing enzymes, including the cytochrome system in mitochondria. Iron distribution in the body is shown in [Table 97-1](#). Without iron, cells lose their capacity for electron transport and energy metabolism. In erythroid cells, hemoglobin synthesis is impaired, resulting in anemia and reduced O₂ delivery to tissue.

THE IRON CYCLE IN HUMANS

[Figure 97-1](#) outlines the major pathways of internal iron exchange in humans. Iron absorbed from the diet or released from stores circulates

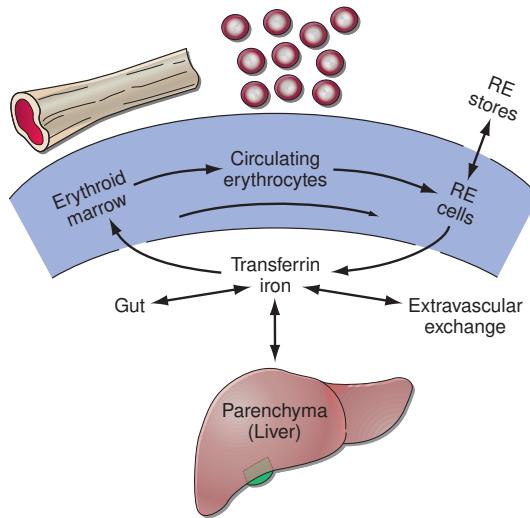


FIGURE 97-1 Internal iron exchange. Normally 80% of iron passing through the plasma transferrin pool is recycled from senescent red cells. Absorption of 1 mg/d is required from the diet in men, and 1.4 mg/d in women to maintain homeostasis. As long as transferrin saturation is maintained between 20 and 60% and erythropoiesis is not increased, use of iron stores is not required. However, in the event of blood loss, dietary iron deficiency, or inadequate iron absorption, up to 40 mg/d of iron can be mobilized from stores. RE, reticuloendothelial.

in the plasma bound to *transferrin*, the iron transport protein. Transferrin is a bilobed glycoprotein with two iron-binding sites. Transferrin that carries iron exists in two forms—*monoferric* (one iron atom) or *diferric* (two iron atoms). The turnover (half-clearance time) of transferrin-bound iron is very rapid—typically 60–90 min. Because almost all of the iron transported by transferrin is delivered to the erythroid marrow, the clearance time of transferrin-bound iron from the circulation is affected most by the plasma iron level and the erythroid marrow activity. When erythropoiesis is markedly stimulated, the pool of erythroid cells requiring iron increases, and the clearance time of iron from the circulation decreases. The half-clearance time of iron in the presence of iron deficiency is as short as 10–15 min. With suppression of erythropoiesis, the plasma iron level typically increases, and the half-clearance time may be prolonged to several hours. Normally, the iron bound to transferrin turns over 6–8 times per day. Assuming a normal plasma iron level of 80–100 µg/dL, the amount of iron passing through the transferrin pool is 20–24 mg/day.

The iron-transferrin complex circulates in the plasma until it interacts with specific *transferrin receptors* on the surface of marrow erythroid cells. Diferric transferrin has the highest affinity for transferrin receptors; apotransferrin (not carrying iron) has very little affinity. Although transferrin receptors are found on cells in many tissues within the body—and all cells at some time during development will display transferrin receptors—the cell having the greatest number of receptors (300,000–400,000/cell) is the developing erythroblast.

Once the iron-bearing transferrin interacts with its receptor, the complex is internalized via clathrin-coated pits and transported to an acidic endosome, where the iron is released at the low pH. The iron is then made available for heme synthesis while the transferrin-receptor complex is recycled to the surface of the cell, where the bulk of the transferrin is released back into circulation and the transferrin receptor re-anchors into the cell membrane. At this point, a certain amount of the transferrin receptor protein may be released into circulation and can be measured as soluble transferrin receptor protein. Within the erythroid cell, iron in excess of the amount needed for hemoglobin synthesis binds to a storage protein, *apoferritin*, forming *ferritin*. This mechanism of iron exchange also takes place in other cells of the body expressing transferrin receptors, especially liver parenchymal cells where the iron can be incorporated into heme-containing enzymes or stored. The iron incorporated into hemoglobin subsequently enters the circulation as new red cells are released from the bone marrow. The iron is then part of the red cell mass and will not become available for reutilization until the red cell dies.

In a normal individual, the average red cell life span is 120 days. Thus, 0.8–1% of red cells are replaced each day. At the end of its life span, the red cell is recognized as senescent by the cells of the *reticuloendothelial (RE)* system, and the red cell undergoes phagocytosis. Once within the RE cell, the ingested hemoglobin is broken down, the globin and other proteins are returned to the amino acid pool, and the iron is shuttled back to the surface of the RE cell, where it is presented to circulating transferrin via the iron export channel, ferroportin. It is the efficient and highly conserved recycling of iron from senescent red cells that supports steady-state (and even accelerated) erythropoiesis.

Because each milliliter of red cells contains 1 mg of elemental iron, the amount of iron needed to replace those red cells lost through senescence amounts to 20 mg/d (assuming an adult with a red cell mass of 2 L). Any additional iron required for daily red cell production comes from the diet. Normally, an adult male will need to absorb at least 1 mg of elemental iron daily to meet needs, while females in the child-bearing years will need to absorb an average of 1.4 mg/d. However, to achieve a maximum proliferative erythroid marrow response to anemia, additional iron must be available. With markedly stimulated erythropoiesis, demands for iron are increased by as much as six- to eightfold. With extravascular hemolytic anemia, the rate of red cell destruction is increased, but the iron recovered from the red cells is efficiently reutilized for hemoglobin synthesis. In contrast, with intravascular hemolysis or blood loss anemia, the rate of red cell production is limited by the amount of iron that can be mobilized from stores. Typically, the rate of mobilization under these circumstances will not

support red cell production more than 2.5 times normal. If the delivery of iron to the stimulated marrow is suboptimal, the marrow's proliferative response is blunted, and hemoglobin synthesis is impaired. The result is a hypoproliferative marrow accompanied by microcytic, hypochromic anemia.

Whereas blood loss or hemolysis places a demand on the iron supply, inflammatory conditions interfere with iron release from stores and can result in a rapid decrease in the serum iron (see below).

NUTRITIONAL IRON BALANCE

The balance of iron in humans is tightly controlled and designed to conserve iron for reutilization. There is no regulated excretory pathway for iron, and the only mechanisms by which iron is lost are blood loss (via gastrointestinal bleeding, menses, or other forms of bleeding) and the loss of epithelial cells from the skin, gut, and genitourinary tract. Normally, the only route by which iron comes into the body is via absorption from food or from medicinal iron taken orally. Iron may also enter the body through red cell transfusions or injection of iron complexes. The margin between the amount of iron available for absorption and the requirement for iron in growing infants and the adult female is narrow; this accounts for the great prevalence of iron deficiency worldwide—currently estimated at more than one billion people.

The amount of iron required from the diet to replace losses averages 10% of body iron content a year in men and 15% in women of childbearing age. Dietary iron content is closely related to total caloric intake (6 mg of elemental iron per 1000 calories). Iron bioavailability is affected by the nature of the foodstuff, with heme iron (e.g., red meat) being most readily absorbed. In the United States, the average iron intake in an adult male is 15 mg/d with 6% absorption; for the average female, the intake is 11 mg/d with 12% absorption. An individual with iron deficiency can increase iron absorption to 20% of the iron present in a meat-containing diet but only 5–10% of the iron in a vegetarian diet. As a result, one-third of the female population in the United States has virtually no iron stores. Vegetarians are at an additional disadvantage because certain foodstuffs that include phytates and phosphates reduce iron absorption by 50%. When ionizable iron salts are given together with food, the amount of iron absorbed is reduced. When the percentage of iron absorbed from individual food items is compared with the percentage for an equivalent amount of ferrous salt, iron in vegetables is only about one-twentieth as available, egg iron one-eighth, liver iron one-half, and heme iron one-half to two-thirds.

Infants, children, and adolescents may be unable to maintain normal iron balance because of the demands of body growth and lower dietary intake of iron. During the last two trimesters of pregnancy, daily iron requirements increase to 5–6 mg, and iron supplements are strongly recommended for pregnant women in developed countries.

Iron absorption takes place largely in the duodenum and proximal small intestine and is a carefully regulated process. For absorption, iron must be taken up by the luminal cell. That process is facilitated by the acidic contents of the stomach, which maintains the iron in solution. At the brush border of the absorptive cell, the ferric iron is converted to the ferrous form by a ferrireductase. Transport across the membrane is accomplished by divalent metal transporter type 1 (DMT-1, also known as natural resistance macrophage-associated protein type 2 [Nramp 2] or DCT-1). DMT-1 is a general cation transporter. Once inside the gut cell, iron may be stored as ferritin or transported through the cell to be released at the basolateral surface to plasma transferrin through the membrane-embedded iron exporter, ferroportin. The function of ferroportin is negatively regulated by hepcidin, the principal iron regulatory hormone. In the process of release, iron interacts with another ferroxidase, hephaestin, which oxidizes the iron to the ferric form for transferrin binding. Hephaestin is similar to ceruloplasmin, the copper-carrying protein.

Iron absorption is influenced by a number of physiologic states. Erythroid hyperplasia stimulates iron absorption even in the face of normal or increased iron stores, and hepcidin levels are inappropriately low. Thus, patients with anemias associated with high levels of ineffective erythropoiesis absorb excess amounts of dietary iron. The molecular mechanism underlying this is the production of erythropherrone

(ERFE) by developing erythroblasts. ERFE suppresses hepcidin production, and over time, this may lead to iron overload and tissue damage. In iron deficiency, hepcidin levels are also low and iron is much more efficiently absorbed; the contrary is true in states of secondary iron overload. The normal individual can reduce iron absorption in situations of excessive intake or medicinal iron intake; however, while the percentage of iron absorbed goes down, the absolute amount goes up. This accounts for the acute iron toxicity occasionally seen when children ingest large numbers of iron tablets. Under these circumstances, the amount of iron absorbed exceeds the transferrin binding capacity of the plasma, resulting in free iron that affects critical organs such as cardiac muscle cells.

IRON DEFICIENCY ANEMIA

Iron deficiency is one of the most prevalent forms of malnutrition. Globally, 50% of anemia is attributable to iron deficiency and accounts for approximately nearly a million deaths annually worldwide. Africa and parts of Asia bear 71% of the global mortality burden; North America represents only 1.4% of the total morbidity and mortality associated with iron deficiency.

STAGES OF IRON DEFICIENCY

The progression to iron deficiency can be divided into three stages (Fig. 97-2). The first stage is *negative iron balance*, in which the demands for (or losses of) iron exceed the body's ability to absorb iron from the diet. This stage results from a number of physiologic mechanisms, including blood loss, pregnancy (in which the demands for red cell production by the fetus outstrip the mother's ability to provide iron), rapid growth spurts in the adolescent, or inadequate dietary iron intake. Blood loss in excess of 10–20 mL of red cells per day is greater than the amount of iron that the gut can absorb from a normal diet. Under these circumstances, the iron deficit must be made up by mobilization of iron from RE storage sites. During this period, iron stores—reflected by the serum ferritin level or the appearance of stainable iron on bone marrow aspirations—decrease. As long as iron stores are present and can be mobilized, the serum iron, total iron-binding

	Normal	Negative iron balance	Iron-deficient erythropoiesis	Iron-deficiency anemia
Iron stores				
Erythron iron				
Marrow iron stores	1-3+	0-1+	0	0
Serum ferritin (μg/L)	50-200	<20	<15	<15
TIBC (μg/dL)	300-360	>360	>380	>400
SI (μg/dL)	50-150	NL	<50	<30
Saturation (%)	30-50	NL	<20	<10
Marrow sideroblasts (%)	40-60	NL	<10	<10
RBC protoporphyrin (μg/dL)	30-50	NL	>100	>200
RBC morphology	NL	NL	NL	Microcytic/hypochromic

FIGURE 97-2 Laboratory studies in the evolution of iron deficiency. Measurements of marrow iron stores, serum ferritin, and total iron-binding capacity (TIBC) are sensitive to early iron-store depletion. Iron-deficient erythropoiesis is recognized from additional abnormalities in the serum iron (SI), percent transferrin saturation, the pattern of marrow sideroblasts, and the red blood cell (RBC) protoporphyrin level. Patients with iron-deficiency anemia demonstrate all the same abnormalities plus hypochromic microcytic anemia. (Based on RS Hillman, CA Finch: *The Red Cell Manual*, 7th ed. Philadelphia, F.A. Davis and Co, 1996.)

capacity (TIBC), and red cell protoporphyrin levels remain within normal limits. At this stage, red cell morphology and indices are normal.

When iron stores become depleted, the serum iron begins to fall. Gradually, the TIBC increases, as do red cell protoporphyrin levels. By definition, marrow iron stores are absent when the serum ferritin level is $<15 \mu\text{g/L}$. As long as the serum iron remains within the normal range, hemoglobin synthesis is unaffected despite the dwindling iron stores. Once the transferrin saturation falls to 15–20%, hemoglobin synthesis becomes impaired. This is a period of *iron-deficient erythropoiesis*. Careful evaluation of the peripheral blood smear reveals the first appearance of microcytic cells, and if the laboratory technology is available, one finds hypochromic reticulocytes in circulation. Gradually, the hemoglobin begins to fall, reflecting *iron-deficiency anemia*. The transferrin saturation at this point is $<10\text{--}15\%$.

When moderate anemia is present (hemoglobin 10–13 g/dL), the bone marrow remains hypoproliferative. With more severe anemia (hemoglobin 7–8 g/dL), hypochromia and microcytosis become more prominent, target cells and misshapen red cells (poikilocytes) appear on the blood smear as cigar- or pencil-shaped forms, and the erythroid marrow becomes increasingly ineffective. Consequently, with severe prolonged iron-deficiency anemia, erythroid hyperplasia of the marrow develops, rather than hypoproliferation.

■ CAUSES OF IRON DEFICIENCY

Conditions that increase demand for iron, increase iron loss, or decrease iron intake or absorption can produce iron deficiency (Table 97-2).

■ CLINICAL PRESENTATION OF IRON DEFICIENCY

Certain clinical conditions carry an increased likelihood of iron deficiency. Pregnancy, adolescence, periods of rapid growth, and an intermittent history of blood loss of any kind should alert the clinician to possible iron deficiency. A cardinal rule is that the appearance of iron deficiency in an adult male or postmenopausal female means gastrointestinal blood loss until proven otherwise. Signs related to iron deficiency depend on the severity and chronicity of the anemia in addition to the usual signs of anemia—fatigue, pallor, and reduced exercise capacity. *Cheilosis* (fissures at the corners of the mouth) and *koilonychia* (spooning of the fingernails) are signs of advanced tissue iron deficiency. The diagnosis of iron deficiency is typically based on laboratory results.

■ LABORATORY IRON STUDIES

Serum Iron and Total Iron-Binding Capacity The serum iron level represents the amount of circulating iron bound to transferrin. The TIBC is an indirect measure of the circulating transferrin. The normal range for the serum iron is 50–150 µg/dL; the normal range for TIBC is 300–360 µg/dL. Transferrin saturation, which is normally 25–50%, is obtained by the following formula: serum iron $\times 100 \div$

TABLE 97-2 Causes of Iron Deficiency

Increased Demand for Iron

Rapid growth in infancy or adolescence

Pregnancy

Erythropoietin therapy

Increased Iron Loss

Chronic blood loss

Menses

Acute blood loss

Blood donation

Phlebotomy as treatment for polycythemia vera

Decreased Iron Intake or Absorption

Inadequate diet

Malabsorption from disease (sprue, Crohn's disease)

Malabsorption from surgery (gastrectomy and some forms of bariatric surgery)

Acute or chronic inflammation

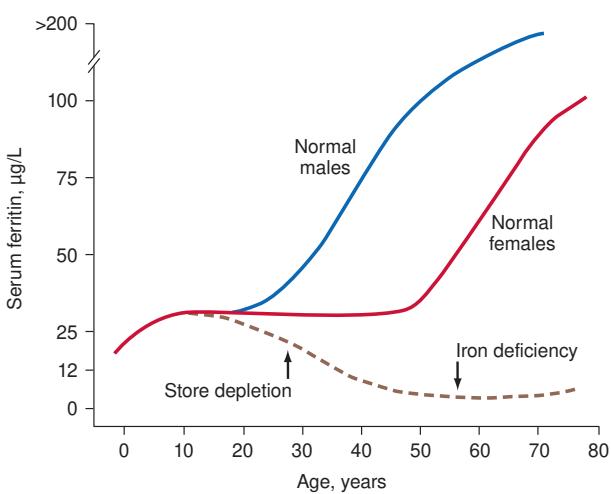


FIGURE 97-3 Serum ferritin levels as a function of sex and age. Iron store depletion and iron deficiency are accompanied by a decrease in serum ferritin level below 20 µg/L. (Reproduced with permission from RS Hillman: *Hematology in Clinical Practice*, 5th ed. New York, McGraw-Hill, 2011.)

TIBC. Iron-deficiency states are associated with saturation levels $<20\%$. There is a diurnal variation in the serum iron. A transferrin saturation $>50\%$ indicates that a disproportionate amount of the iron bound to transferrin is being delivered to nonerythroid tissues. If this persists for an extended time, tissue iron overload may occur.

Serum Ferritin Free iron is toxic to cells, and the body has established an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored complexed to protein as ferritin or hemosiderin. Apoferritin binds to free ferrous iron and stores it in the ferric state. As ferritin accumulates within cells of the RE system, protein aggregates are formed as hemosiderin. Iron in ferritin or hemosiderin can be extracted for release by the RE cells, although hemosiderin is less readily available. Under steady-state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin level is the most convenient laboratory test to estimate iron stores. The normal value for ferritin varies according to the age and gender of the individual (Fig. 97-3). Adult males have serum ferritin values averaging 100 µg/L, while adult females have levels averaging 30 µg/L. As iron stores are depleted, the serum ferritin falls to $<15 \mu\text{g/L}$. Such levels are diagnostic of absent body stores.

Evaluation of Bone Marrow Iron Stores Although RE iron stores can be estimated from the iron stain of a bone marrow aspirate or biopsy, the measurement of serum ferritin has largely supplanted these procedures for determination of storage iron (Table 97-3). The serum ferritin level is a better indicator of iron overload than the marrow iron stain. However, in addition to storage iron, the marrow iron stain provides information about the effective delivery of iron to developing erythroblasts. Normally, when the marrow smear is stained for iron, 20–40% of developing erythroblasts—called *sideroblasts*—will have visible ferritin granules in their cytoplasm. This represents iron in excess of that needed for hemoglobin synthesis. In states in which

TABLE 97-3 Iron Store Measurements

IRON STORES	MARROW IRON STAIN, 0–4+	SERUM FERRITIN, µg/L
0	0	<15
1–300 mg	Trace to 1+	15–30
300–800 mg	2+	30–60
800–1000 mg	3+	60–150
1–2 g	4+	>150
Iron overload	—	>500–1000

release of iron from storage sites is blocked, RE iron will be detectable, and there will be few or no sideroblasts. In the myelodysplastic syndromes, mitochondrial dysfunction can occur, and accumulation of iron in mitochondria appears in a necklace fashion around the nucleus of the erythroblast. Such cells are referred to as *ring sideroblasts*.

Red Cell Protoporphyrin Levels Protoporphyrin is an intermediate in the pathway to heme synthesis. Under conditions in which heme synthesis is impaired, protoporphyrin accumulates within the red cell. This reflects an inadequate iron supply to erythroid precursors to support hemoglobin synthesis. Normal values are <30 µg/dL of red cells. In iron deficiency, values >100 µg/dL are seen. The most common causes of increased red cell protoporphyrin levels are absolute or relative iron deficiency and lead poisoning.

Serum Levels of Transferrin Receptor Protein Because erythroid cells have the highest numbers of transferrin receptors of any cell in the body, and because transferrin receptor protein (TRP) is released by cells into the circulation, serum levels of TRP reflect the total erythroid marrow mass. Another condition in which TRP levels are elevated is absolute iron deficiency. Normal values are 4–9 µg/L determined by immunoassay. This laboratory test is becoming increasingly available and, along with the serum ferritin, has been proposed to distinguish between iron deficiency and the anemia of inflammation (see below).

DIFFERENTIAL DIAGNOSIS

Other than iron deficiency, only three conditions need to be considered in the differential diagnosis of a hypochromic microcytic anemia (**Table 97-4**). The first is an inherited defect in globin chain synthesis: the thalassemias. These are differentiated from iron deficiency most readily by serum iron values; normal or increased serum iron levels and transferrin saturation are characteristic of the thalassemias. In addition, the red blood cell distribution width (RDW) index is generally normal in thalassemia and elevated in iron deficiency.

The second condition is the anemia of inflammation (AI; also referred to as the anemia of chronic disease) with inadequate iron supply to the erythroid marrow. The distinction between true iron-deficiency anemia and AI is among the most common diagnostic problems encountered by clinicians (see below). Usually, AI is normocytic and normochromic. The iron values usually make the differential diagnosis clear, as the ferritin level is normal or increased and the percent transferrin saturation and TIBC are typically below normal.

Finally, the myelodysplastic syndromes represent the third and least common condition. Occasionally, patients with myelodysplasia have impaired hemoglobin synthesis with mitochondrial dysfunction, resulting in impaired iron incorporation into heme. The iron values again reveal normal stores and more than an adequate supply to the marrow, despite the microcytosis and hypochromia.

TREATMENT

Iron-Deficiency Anemia

The severity and cause of iron-deficiency anemia will determine the appropriate approach to treatment. As an example, symptomatic elderly patients with severe iron-deficiency anemia and

TABLE 97-5 Oral Iron Preparations

GENERIC NAME	TABLET (IRON CONTENT), mg	Elixir (iron content), mg in 5 mL
Ferrous sulfate	325 (65) 195 (39)	300 (60) 90 (18)
Extended release	525 (105)	
Ferrous fumarate	325 (107) 195 (64)	100 (33)
Ferrous gluconate	325 (39)	300 (35)
Polysaccharide iron	150 (150) 50 (50)	100 (100)

cardiovascular instability may require red cell transfusions. Younger individuals who have compensated for their anemia can be treated more conservatively with iron replacement. The foremost issue for the latter patient is the precise identification of the cause of the iron deficiency.

For the majority of cases of iron deficiency (pregnant women, growing children and adolescents, patients with infrequent episodes of bleeding, and those with inadequate dietary intake of iron), oral iron therapy will suffice. For patients with unusual blood loss or malabsorption, specific diagnostic tests and appropriate therapy take priority. Once the diagnosis of iron-deficiency anemia and its cause is made, there are three major therapeutic approaches.

RED CELL TRANSFUSION

Transfusion therapy is reserved for individuals who have symptoms of anemia, cardiovascular instability, and continued and excessive blood loss from whatever source and who require immediate intervention. The management of these patients is less related to the iron deficiency than it is to the consequences of the severe anemia. Not only do transfusions correct the anemia acutely, but the transfused red cells provide a source of iron for reutilization, assuming they are not lost through continued bleeding. Transfusion therapy will stabilize the patient while other options are reviewed.

ORAL IRON THERAPY

In the asymptomatic patient with established iron-deficiency anemia and an intact gastrointestinal tract, treatment with oral iron is usually adequate. Encouraging dietary intake of iron-rich foods is also useful. Such foods include oysters, kidney beans, beef liver, tofu, beef (chuck roast, lean ground beef), turkey leg, whole-wheat bread, tuna, eggs, shrimp, peanut butter, leg of lamb, brown rice, raisin bran (whole grain-enriched cereals), lentils, and beans. Multiple preparations of oral iron supplements are available, ranging from simple iron salts to complex iron compounds designed for sustained release throughout the small intestine (**Table 97-5**). Although the various preparations contain different amounts of iron, they are generally all absorbed well and are effective in treatment. Some come with other compounds designed to enhance iron absorption, such as ascorbic acid. It is not clear whether the benefits of such compounds justify their costs. Typically, for iron replacement therapy, up to 200 mg of elemental iron per day is given, usually

TABLE 97-4 Diagnosis of Microcytic Anemia

TESTS	IRON DEFICIENCY	INFLAMMATION	THALASSEMIA	SIDEROBLASTIC ANEMIA
Smear	Micro/hypo	Normal micro/hypo	Micro/hypo with targeting	Variable
Serum iron (µg/dL)	<30	<50	Normal to high	Normal to high
TIBC (µg/dL)	>360	<300	Normal	Normal
Percent saturation	<10	10–20	30–80	30–80
Ferritin (µg/L)	<15	30–200	50–300	50–300
Hemoglobin pattern on electrophoresis	Normal	Normal	Abnormal with β thalassemia; can be normal with α thalassemia	Normal

Abbreviation: TIBC, total iron-binding capacity.

as three or four iron tablets (each containing 50–65 mg elemental iron) given over the course of the day. Ideally, oral iron preparations should be taken on an empty stomach, since food may inhibit iron absorption. Some patients with gastric disease or prior gastric surgery require special treatment with iron solutions because the retention capacity of the stomach may be reduced. The retention capacity is necessary for dissolving the shell of the iron tablet before the release of iron. A dose of 200 mg of elemental iron per day should result in the absorption of iron up to 50 mg/d. This supports a red cell production level of two to three times normal in an individual with a normally functioning marrow and appropriate erythropoietin (EPO) stimulus. However, as the hemoglobin level rises, EPO stimulation decreases, and the amount of iron absorbed is reduced. The goal of therapy in individuals with iron-deficiency anemia is not only to repair the anemia, but also to provide stores of at least 0.5–1 g of iron. Sustained treatment for a period of 6–12 months after correction of the anemia will be necessary to achieve this.

Of the complications of oral iron therapy, gastrointestinal distress is the most prominent and is seen in at least 15–20% of patients. Abdominal pain, nausea, vomiting, or constipation may lead to noncompliance. Although small doses of iron or iron preparations with delayed release may help somewhat, the gastrointestinal side effects are a major impediment to the effective treatment of a number of patients.

The response to iron therapy varies, depending on the EPO stimulus and the rate of absorption. Typically, the reticulocyte count should begin to increase within 4–7 days after initiation of therapy and peak at 1–1½ weeks. The absence of a response may be due to poor absorption, noncompliance (which is common), or a confounding diagnosis. A useful test in the clinic to determine the patient's ability to absorb iron is the *iron tolerance test*. Two iron tablets are given to the patient on an empty stomach, and the serum iron is measured serially over the subsequent 2–3 h. Normal absorption will result in an increase in the serum iron of at least 100 µg/dL. If iron deficiency persists despite adequate treatment, it may be necessary to switch to parenteral iron therapy.

PARENTERAL IRON THERAPY

Intravenous iron can be given to patients who are unable to tolerate oral iron; whose needs are relatively acute; or who need iron on an ongoing basis, usually due to persistent gastrointestinal or menstrual blood loss. Parenteral iron use has been increasing rapidly over the past several years with the recognition that recombinant EPO therapy induces a large demand for iron—a demand that frequently cannot be met through the physiologic release of iron from RE sources or oral iron absorption. The safety of parenteral iron has been a concern largely driven by the high adverse reaction rate to high-molecular-weight iron dextran. The newer iron complexes that are available, such as ferumoxytol (Feraheme), sodium ferric gluconate (Ferrlecit), iron sucrose (Venofer), low-molecular-weight (LMW) iron dextran (InFed), ferric derisomaltose (Monoferic), and ferric carboxymaltose (Injectafer), have much lower rates of adverse effects. Ferumoxytol delivers 510 mg of iron per infusion; ferric gluconate 125 mg per infusion; LMW iron dextran up to 1500 mg per infusion; ferric carboxymaltose 750 mg per infusion; ferric derisomaltose 1000 mg per infusion; and iron sucrose 200 mg per infusion.

Parenteral iron is used in two ways: one is to administer the total dose of iron required to correct the hemoglobin deficit and provide the patient with at least 500 mg of iron stores; the second is to give repeated small doses of parenteral iron over a protracted period. The latter approach is common in dialysis centers, where it is not unusual for 100 mg of elemental iron to be given weekly for 10 weeks to augment the response to recombinant EPO therapy. The amount of iron needed by an individual patient is calculated by the following formula:

$$\text{Body weight (kg)} \times 2.3 \times (15 - \text{patient's hemoglobin, g/dL}) \\ + 500 \text{ or } 1000 \text{ mg (for stores)}$$

In administering any intravenous iron preparation, anaphylaxis is a concern. Anaphylaxis is much rarer with the newer preparations. The factors that have correlated with an anaphylactic-like reaction include a history of multiple allergies or a prior allergic reaction to an iron preparation. Generalized symptoms appearing several days after the infusion of a large dose of iron can include arthralgias, skin rash, and low-grade fever. These may be dose-related, but they do not preclude the further use of parenteral iron in the patient. To date, patients with sensitivity to one iron preparation have been safely treated with other parenteral iron preparations. If a large dose of LMW iron dextran is to be given (>100 mg), the iron preparation should be diluted in 5% dextrose in water or 0.9% NaCl solution. The iron solution can then be infused over a 60- to 90-min period (for larger doses) or at a rate convenient for the attending nurse or physician. Although a test dose (25 mg) of parenteral LMW iron dextran is recommended, in reality, a slow infusion of a larger dose of parenteral iron solution will afford the same kind of early warning as a separately injected test dose. Early in the infusion of iron, if chest pain, wheezing, a fall in blood pressure, or other systemic symptoms occur, the infusion of iron should be stopped immediately.

OTHER HYPOPROLIFERATIVE ANEMIAS

In addition to mild to moderate iron-deficiency anemia, the hypoproliferative anemias can be divided into four categories: (1) chronic inflammation, (2) renal disease, (3) endocrine and nutritional deficiencies (hypometabolic states), and (4) marrow damage (Chap. 102). With chronic inflammation, renal disease, or hypometabolism, endogenous EPO production is inadequate for the degree of anemia observed. For the anemia of chronic inflammation, the erythroid marrow also responds inadequately to stimulation, due in part to defective *iron reutilization*. As a result of the lack of adequate EPO stimulation, an examination of the peripheral blood smear will disclose only an occasional polychromatophilic ("shift") reticulocyte. In cases of iron deficiency or marrow damage, appropriate elevations in endogenous EPO levels are typically found, and shift reticulocytes will be present on the blood smear.

■ ANEMIA OF ACUTE AND CHRONIC INFLAMMATION/INFECTION AI

AI, which encompasses inflammation, infection, tissue injury, and conditions (e.g., cancer) associated with the release of proinflammatory cytokines, is one of the most common forms of anemia seen clinically. It is the most important anemia in the differential diagnosis of iron deficiency because many of the features of the anemia are brought about by inadequate iron delivery to the marrow, despite the presence of normal or increased iron stores. This is reflected by a low serum iron, increased red cell protoporphyrin, a hypoproliferative marrow, transferrin saturation in the range of 15–20%, and a normal or increased serum ferritin. The serum ferritin values are often the most distinguishing features between true iron-deficiency anemia and the iron-restricted erythropoiesis associated with inflammation. Typically, serum ferritin values increase threefold over basal levels in the face of inflammation. These changes are due to the effects of inflammatory cytokines and hepcidin, the key iron regulatory hormone, acting at several levels of erythropoiesis (Fig. 97-4).

Interleukin 1 (IL-1) directly decreases EPO production in response to anemia. IL-1, acting through accessory cell release of interferon γ (IFN- γ), suppresses the response of the erythroid marrow to EPO—an effect that can be overcome by EPO administration *in vitro* and *in vivo*. In addition, tumor necrosis factor (TNF), acting through the release of IFN- β by marrow stromal cells, also suppresses the response to EPO. Hepcidin, made by the liver, is increased in inflammation via an IL-6-mediated pathway, and acts to suppress iron absorption and iron release from storage sites. The overall result is a chronic hypoproliferative anemia with classic changes in iron metabolism. The anemia is further compounded by a mild to moderate shortening in red cell survival.

With chronic inflammation, the primary disease will determine the severity and characteristics of the anemia. For example, many

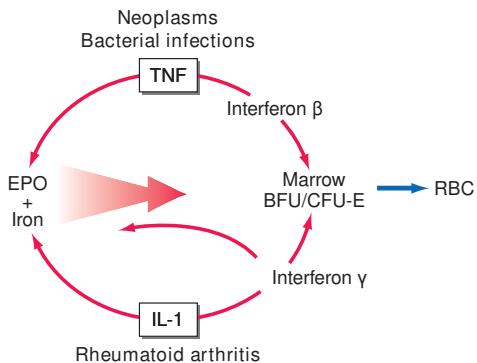


FIGURE 97-4 Suppression of erythropoiesis by inflammatory cytokines. Through the release of tumor necrosis factor (TNF) and interferon β (IFN- β), neoplasms and bacterial infections suppress erythropoietin (EPO) production and the proliferation of erythroid progenitors (erythroid burst-forming units and erythroid colony-forming units [BFU/CFU-E]). The mediators in patients with vasculitis and rheumatoid arthritis include interleukin 1 (IL-1) and IFN- γ . The red arrows indicate sites of inflammatory cytokine inhibitory effects. RBC, red blood cell.

patients with cancer also have anemia that is typically normocytic and normochromic. In contrast, patients with long-standing active rheumatoid arthritis or chronic infections such as tuberculosis will have a microcytic, hypochromic anemia. In both cases, the bone marrow is hypoproliferative, but the differences in red cell indices reflect differences in the availability of iron for hemoglobin synthesis. Occasionally, conditions associated with chronic inflammation are also associated with chronic blood loss. Under these circumstances, the measurement of soluble transferrin receptor protein may be necessary to rule out absolute iron deficiency. However, the administration of iron in this case will correct the iron-deficiency component of the anemia and leave the inflammatory component unaffected.

The anemia associated with acute infection or inflammation is typically mild but becomes more pronounced over time. Acute infection can produce a decrease in hemoglobin levels of 2–3 g/dL within 1 or 2 days; this is largely related to the hemolysis of red cells near the end of their natural life span. The fever and cytokines released exert a selective pressure against cells with more limited capacity to maintain the red cell membrane. In most individuals, the mild anemia is reasonably well tolerated, and symptoms, if present, are associated with the underlying disease. Occasionally, in patients with preexisting cardiac disease, moderate anemia (hemoglobin 10–11 g/dL) may be associated with angina, exercise intolerance, and shortness of breath. The erythropoietic profile that distinguishes the anemia of inflammation from the other causes of hypoproliferative anemias is shown in **Table 97-6**.

■ ANEMIA OF CHRONIC KIDNEY DISEASE CKD

Progressive CKD is usually associated with a moderate to severe hypoproliferative anemia; the level of the anemia correlates with the stage of CKD. Red cells are typically normocytic and normochromic, and reticulocytes are decreased. The anemia is primarily due to a failure of EPO production by the diseased kidney and a reduction in red cell survival. In certain forms of acute renal failure, the correlation

between the anemia and renal function is weaker. Patients with the hemolytic-uremic syndrome increase erythropoiesis in response to the hemolysis, despite renal failure. Polycystic kidney disease also shows a smaller degree of EPO deficiency for a given level of renal failure. By contrast, patients with diabetes or myeloma have more severe EPO deficiency for a given level of renal failure.

Assessment of iron status provides information to distinguish the anemia of CKD from the other forms of hypoproliferative anemia (Table 97-6) and to guide management. Patients with the anemia of CKD usually present with normal serum iron, TIBC, and ferritin levels. However, those maintained on chronic hemodialysis may develop iron deficiency from blood loss through the dialysis procedure. Iron must be replenished in these patients to ensure an adequate response to EPO therapy (see below).

■ ANEMIA IN HYPOMETABOLIC STATES

Patients who are starving, particularly for protein, and those with a variety of endocrine disorders that produce lower metabolic rates, may develop a mild to moderate hypoproliferative anemia. The release of EPO from the kidney is sensitive to the need for O_2 , not just O_2 levels. Thus, EPO production is triggered at lower levels of blood O_2 content in disease states (e.g., hypothyroidism and starvation) where metabolic activity, and thus O_2 demand, is decreased.

Endocrine Deficiency States The difference in the levels of hemoglobin between men and women is related to the effects of androgen and estrogen on erythropoiesis. Testosterone and anabolic steroids augment erythropoiesis; castration and estrogen administration to males decrease erythropoiesis. Patients who are hypothyroid or have deficits in pituitary hormones also may develop a mild anemia. Pathogenesis may be complicated by other nutritional deficiencies because iron and folic acid absorption can be affected by these disorders. Usually, correction of the hormone deficiency reverses the anemia.

Anemia may be more severe in Addison's disease, depending on the level of thyroid and androgen hormone dysfunction; however, anemia may be masked by decreases in plasma volume. Once such patients are given cortisol and volume replacement, the hemoglobin level may fall rapidly. Mild anemia complicating hyperparathyroidism may be due to decreased EPO production as a consequence of the renal effects of hypercalcemia or to impaired proliferation of erythroid progenitors.

Protein Starvation Decreased dietary intake of protein may lead to mild to moderate hypoproliferative anemia; this form of anemia may be prevalent in the elderly. The anemia can be more severe in patients with a greater degree of starvation. In marasmus, where patients are both protein- and calorie-deficient, the release of EPO is impaired in proportion to the reduction in metabolic rate; however, the degree of anemia may be masked by volume depletion and becomes apparent after refeeding. Deficiencies in other nutrients (iron, folate) may also complicate the clinical picture but may not be apparent at diagnosis. Changes in the erythrocyte indices on refeeding should prompt evaluation of iron, folate, and B_{12} status.

Anemia in Liver Disease A mild hypoproliferative anemia may develop in patients with chronic liver disease from nearly any cause. The peripheral blood smear may show spur cells and stomatocytes.

TABLE 97-6 Diagnosis of Hypoproliferative Anemias

TESTS	IRON DEFICIENCY	INFLAMMATION	RENAL DISEASE	HYPOMETABOLIC STATES
Anemia	Mild to severe	Mild	Mild to severe	Mild
MCV (fL)	60–90	80–90	90	90
Morphology	Normo-microcytic	Normocytic	Normocytic	Normocytic
SI ($\mu\text{g}/\text{dL}$)	<30	<50	Normal	Normal
TIBC ($\mu\text{g}/\text{dL}$)	>360	>300	Normal	Normal
Saturation (%)	<10	10–20	Normal	Normal
Serum ferritin ($\mu\text{g}/\text{L}$)	<15	30–200	115–150	Normal
Iron stores	0	2–4+	1–4+	Normal

Abbreviations: MCV, mean corpuscular volume; SI, serum iron; TIBC, total iron-binding capacity.

from the accumulation of excess cholesterol in the membrane from a deficiency of lecithin-cholesterol acyltransferase. Red cell survival is shortened, and the production of EPO is inadequate to compensate. In alcoholic liver disease, nutritional deficiencies are common and complicate the management. Folate deficiency from inadequate intake, as well as iron deficiency from blood loss and inadequate intake, can alter the red cell indices.

■ ANEMIA IN AGING

Anemia is common in people over age 65 years. It has been estimated to affect ~11% of community-living older adults and up to 40% of nursing home residents. In at least one-third of these anemic people, a cause for the anemia is not found. Patients with the unexplained anemia of aging do not have nutrient deficiency or renal dysfunction, and although older people can have an increase in systemic inflammatory cytokines (the inflammation of aging), the levels are not high enough to mimic the anemia of chronic inflammation. If hepcidin levels are elevated at all, they are minimally so.

Investigations into the cause(s) of this form of anemia have noted that EPO levels are generally in the normal range, that is, they are inappropriately low for the hemoglobin level. In general, in older people who maintain a normal hemoglobin level, EPO levels increase with age. This compensatory increase to maintain normal oxygen delivery seems to be due to a relative resistance to EPO stimulation; studies of red cell life span in older people have not noted a decrease in red cell survival. More data on the mechanism are needed.

The importance of this unexplained anemia of aging is that low hemoglobin levels are associated with increases in falls, hospitalizations, development of frailty, and mortality. It is not clear whether reversing the anemia would influence these increased risks. Anecdotal evidence suggests that this form of anemia is responsive to exogenous EPO.

TREATMENT

Hypoproliferative Anemias

Many patients with hypoproliferative anemias experience recovery of normal hemoglobin levels when the underlying disease is appropriately treated. For those in whom such reversals are not possible—such as patients with end-stage kidney disease, cancer, and chronic inflammatory diseases—symptomatic anemia requires treatment. The two major forms of treatment are transfusions and EPO.

TRANSFUSIONS

Thresholds for transfusion should be determined based on the patient's symptoms. In general, patients without serious underlying cardiovascular or pulmonary disease can tolerate hemoglobin levels above 7–8 g/dL and do not require intervention until the hemoglobin falls below that level. Patients with more physiologic compromise may need to have their hemoglobin levels kept above 11 g/dL. Usually, a unit of packed red cells increases the hemoglobin level by 1 g/dL. Transfusions are associated with certain infectious risks (Chap. 113), and chronic transfusions can produce iron overload. Importantly, the liberal use of blood has been associated with increased morbidity and mortality, particularly in the intensive care setting. Therefore, in the absence of documented tissue hypoxia, a conservative approach to the use of red cell transfusions is preferable.

ERYTHROPOIETIN

EPO is particularly useful in anemias in which endogenous EPO levels are inappropriately low, such as CKD or AI. Iron status must be evaluated and iron replaced to obtain optimal effects from EPO. In patients with CKD, the usual dose of EPO is 50–150 U/kg three times a week intravenously. Hemoglobin levels of 10–12 g/dL are usually reached within 4–6 weeks if iron levels are adequate; 90% of these patients respond. Once a target hemoglobin level is achieved, the EPO dose can be decreased. A decrease in hemoglobin level occurring in the face of EPO therapy usually signifies the development of an infection or iron depletion. Aluminum toxicity

and hyperparathyroidism can also compromise the response to EPO. When an infection intervenes, it is best to interrupt the EPO therapy and rely on transfusions to correct the anemia until the infection is adequately treated. The dose of EPO needed to correct chemotherapy-induced anemia in patients with cancer is higher, up to 300 U/kg three times a week, and only 60% of patients respond. Because of evidence that there is an increased risk of thromboembolic complications and tumor progression with EPO administration, the risks and benefits of using EPO in such patients must be weighed carefully, and the target hemoglobin should be that necessary to avoid transfusions.

Longer-acting preparations of EPO can reduce the frequency of injections. Darbepoetin alfa, a molecularly modified EPO with additional carbohydrate, has a half-life in the circulation that is three to four times longer than recombinant human EPO, permitting weekly or every other week dosing.

Orally bioavailable EPO mimetics such as roxadustat (usual dose 50 mg PO thrice weekly) that act to increase the biological half-life of active hypoxia-inducible factor (HIF) are demonstrating activity to increase hemoglobin levels in patients with chronic renal disease and other settings.

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98

Disorders of Hemoglobin

Martin H. Steinberg



Hemoglobinopathies affect the amino acid sequence of globin; thalassemia is a disorder of reduced globin biosynthesis. Together, these disorders of the hemoglobin molecule are our most common Mendelian genetic diseases. They are responsible for most cases of hemolytic anemia. Sickle cell disease and the hemoglobin E (HbE)-associated syndromes are the most prevalent hemoglobinopathies; β and α thalassemia are the most prevalent thalassemias. In addition to these common disorders of hemoglobin, rare globin mutations can cause hemoglobin instability, increased or decreased affinity of hemoglobin for oxygen (O_2), and oxidized hemoglobin reducing O_2 transport. O_2 transport by hemoglobin can also be reduced by exposure to carbon monoxide (CO) and some oxidizing agents (Table 98-1).

Phenotypic diversity among hemoglobin disorders is enormous. Mutations can be asymptomatic, for example in heterozygous carriers of sickle hemoglobin (HbS) and thalassemia, or cause intrauterine death as when all α -globin genes are deleted. Impressive gains in understanding the biological basis of hemoglobinopathies and thalassemia have led to novel therapeutics with the promise of improved patient outcomes.

TABLE 98-1 Disorders of Hemoglobin

- I. Hemoglobopathies—hemoglobin variants with amino acid sequence variants that alter the physical, chemical, or functional properties of hemoglobin
 - A. Common variants with unusual properties
 - 1. HbS—polymerization
 - 2. HbE—reduced biosynthesis
 - 3. HbC—hemoglobin-membrane interaction
 - B. Altered oxygen affinity
 - 1. High affinity—erythrocytosis
 - 2. Low affinity—cyanosis, anemia
 - C. Hemoglobins that oxidize readily
 - 1. Unstable hemoglobins—hemolytic anemia, jaundice
 - 2. M hemoglobins—methemoglobinemia, cyanosis
- II. Thalassemias—defective biosynthesis of globin chains
 - A. α Thalassemias
 - B. β Thalassemias
 - C. Complex thalassemias
- III. Hereditary persistence of fetal hemoglobin—persistence of higher than normal levels of HbF into adult life
 - A. Deletions within the *HBB* cluster—15–30% HbF in heterozygotes, pancellular
 - B. Point mutations in *HBG2/1* promoters—5–30% HbF in heterozygotes; panacellular or heterocellular
- IV. Acquired hemoglobopathies
 - A. Methemoglobin due to toxic exposures
 - B. Sulfhemoglobin due to toxic exposures
 - C. Carboxyhemoglobin
 - D. HbH in erythroleukemia
 - E. Elevated HbF in myelodysplasia

HEMOGLOBIN

Easy access to erythrocytes to study hemoglobin structure and function, reticulocytes to examine hemoglobin biosynthesis, and leukocyte DNA to define the mutations of hemoglobin and the availability of hematopoietic stem and progenitor cells from blood and bone marrow have placed hemoglobin disorders in the forefront of molecular medicine. A review of the biology of hemoglobin provides the background for understanding the pathophysiology of its many genetic and acquired disorders and approaches to their treatment.

■ DEVELOPMENTAL BIOLOGY

Successive waves of erythropoiesis beginning in the yolk sac, moving to the fetal liver and bone marrow, and culminating in the adult marrow direct the synthesis of different hemoglobin molecules that result from sequential activation and silencing of the globin genes (Fig. 98-1).

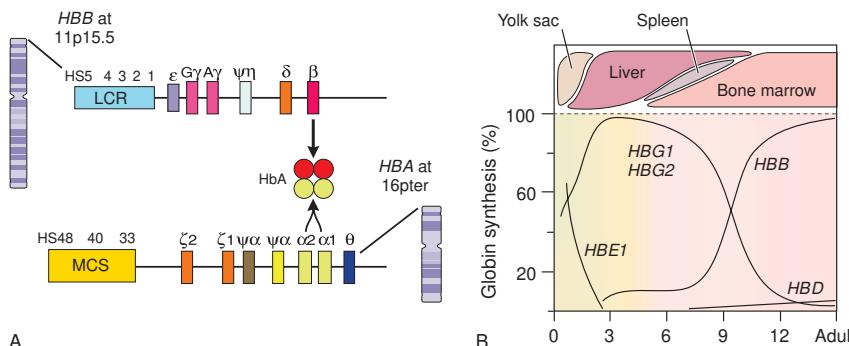


FIGURE 98-1 Globin gene clusters and their hemoglobin products during gestation. **A.** The order of globin genes in the β - and α -globin gene clusters along with their upstream enhancers, the locus control region (LCR) and multispecies conserved sequences (MCS). Normal hemoglobin tetramers contain two α -globin chains and two non- α -globin chains. In the example shown, this is adult HbA. **B.** Sites of erythropoiesis and globin synthesized from the yolk sac and the early embryo (months 1–3), the fetus (months 3–9), after delivery (months 9–12), and afterward (adult).

Hemoglobin is a tetramer of two pairs of unlike globin polypeptide chains, each chain containing a tetrapyrrole heme group. O_2 binds to heme as erythrocytes traverse the lungs and is released in the tissues. Heme is nestled within a protective pocket of each globin subunit.

■ GLOBIN GENE CLUSTERS

Globin is encoded in two nonallelic gene clusters. The β -globin gene cluster is on the short arm of chromosome 11 (11p15.4); the α -globin gene cluster is on chromosome 16 (16p13.3) (Fig. 98-1). The β -globin gene cluster contains an embryonic ϵ -globin gene (*HBE*), two nearly identical fetal γ -globin genes (*HBG2*, *HBG1*) a major adult β -globin gene (*HBB*), and a minor adult δ -globin gene (*HBD*). The α -globin gene cluster contains an embryonic ζ -globin gene (*HBZ*) and duplicated α -globin genes (*HBA2*, *HBA1*) with identical proteins. Embryonic hemoglobins include Gower I ($\zeta_1\epsilon$), Gower II ($\alpha_1\epsilon$), Portland I ($\zeta_2\gamma_2$), and Portland II ($\zeta_2\beta_2$). Fetal hemoglobin (HbF, $\alpha_2\gamma_2$) production begins at 6–8 weeks' gestation, peaks during mid-gestation, then falls to <1% of total hemoglobin during the first 6 months of extrauterine life. Adult hemoglobin A (HbA; $\alpha_2\beta_2$) production follows a pattern reciprocal to that of HbF. The hemoglobin composition of normal adults is >95% HbA, ~1% HbF, and 2–3% HbA₂ ($\alpha_2\delta_2$). In adults, HbF and HbA₂ have little functional significance because of their low concentrations, although they can be diagnostically important. Hemoglobin is also subject to posttranslational modifications, the most important being the nonenzymatic glycosylation of HbA forming the adduct HbA_{1c}, which is of diagnostic utility in the management of diabetes mellitus.

■ HEMOGLOBIN STRUCTURE

All globin polypeptides have similar but not identical primary structures. α -Globins contain 141 amino acids, and β -like globins have 146 amino acids. This primary structure dictates, according to the constraints of protein folding, the secondary structure of globin into α -helical sections joined by small nonhelical stretches. Each globin chain folds into a tertiary conformation known as the globin fold, whereby charged amino acid residues face the exterior of the molecules and uncharged residues face the hydrophobic interior. The iron-containing tetrapyrrole heme moiety is protected from oxidation and located between two of the helical segments; O_2 loading and unloading occur when heme iron is in its reduced ferrous form. Globin gene mutations affecting critical heme-binding amino acid residues allow iron to be oxidized, forming methemoglobin, which has high O_2 affinity and does not release O_2 in tissues. Dimers of α - and non- α -globin chains reversibly assemble into tetramers, forming a quaternary structure.

■ HEMOGLOBIN FUNCTION

Hemoglobin transports O_2 from lungs to tissues and carbon dioxide (CO_2) from tissues to lungs and is a nitrate reductase that releases nitric oxide (NO) from nitrite to promote vasodilation. Oxygen binding is defined by the sigmoidal shape of the hemoglobin- O_2 dissociation curve. P_{50} is a point on this curve that indicates the partial pressure of O_2 where hemoglobin is half saturated (Fig. 98-2). The P_{50} is influenced by the binding of 2,3-bisphosphoglycerate, a product of glycolysis, in the central cavity of hemoglobin, and by pH and temperature. Normal P_{50} is ~26 mmHg; low P_{50} indicates that hemoglobin has high O_2 affinity, decreasing O_2 delivery to tissues; high P_{50} indicates that hemoglobin has low O_2 affinity, releasing more O_2 to tissues. The conformation of hemoglobin fully saturated with O_2 is known as the R or relaxed state; desaturated hemoglobin is in the T or tense state. The transition between T and R states occurs when two or three O_2 molecules are bound. Cooperativity describes the progressively more rapid binding of O_2 once the first molecule

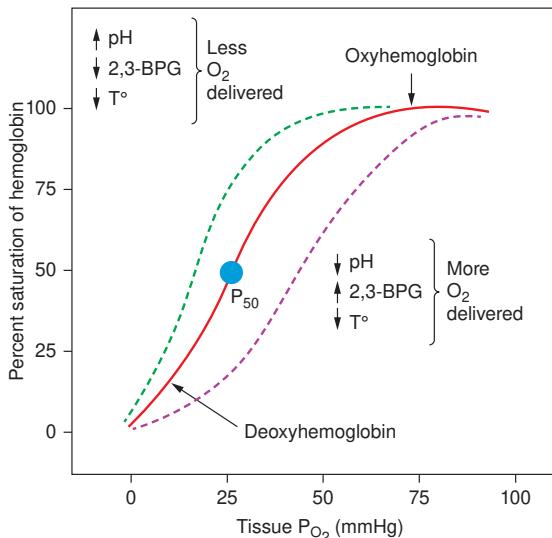


FIGURE 98-2 Hemoglobin-oxygen dissociation curve. The hemoglobin tetramer can bind up to four molecules of oxygen (O_2) in the iron-containing sites of the heme molecules. As O_2 is bound, 2,3-bisphosphoglycerate (2,3-BPG) and carbon dioxide (CO_2) are expelled. Salt bridges are broken, and each of the globin molecules changes its conformation to facilitate O_2 binding. O_2 release to the tissues is the reverse process, with salt bridges being formed and 2,3-BPG and CO_2 bound. Deoxyhemoglobin does not bind O_2 efficiently until the cell returns to conditions of higher pH, the most important modulator of O_2 affinity (Bohr effect). When acid is produced in the tissues, the dissociation curve shifts to the right, facilitating O_2 release and CO_2 binding. Alkalosis has the opposite effect, reducing O_2 delivery.

is bound. Hemoglobin variants that decrease P_{50} are characterized by isolated erythrocytosis as compensation for hypoxia; variants with increased P_{50} sometimes are accompanied by cyanosis and anemia as hemoglobin becomes unsaturated and O_2 delivery is enhanced. Mutations of residues critical for heme binding, R-T transitions, or tetramer stability cause hemoglobinopathies characterized by hemolytic anemia, methemoglobinemia, erythrocytosis and cyanosis.

GLOBIN GENE SWITCHING

The sequential activation and inactivation of globin genes during development shown in Fig. 98-1 is called “hemoglobin switching.” Transcription factors along with epigenetic elements such as DNA methyltransferases and demethylases, interact with enhancers “upstream” of the β -globin gene cluster that contact globin gene promoters, silencing the embryonic and fetal genes. Activation of fetal globin gene repressors during development allows expression of the adult genes. Developmental factors such as RNA-binding factors and microRNAs also impact hemoglobin switching.

β -Globin Gene Switching HbF reactivation by drugs and gene therapy is a prime therapeutic goal for treating the common disorders of hemoglobin, meriting a discussion of the controls of HbF gene silencing. An upstream super-enhancer called the β -globin locus control region (LCR) binds erythroid-specific and ubiquitous transcription factors. The LCR interacts directly with globin gene promoters; transcription factors that silence and activate genes also interact with elements of the globin genes. Competition among the β -like genes for the LCR and autonomous silencing of the embryonic and fetal globin genes depends on transcription factors. Silencing, first of HBE and then of $HBG2$ and $HBG1$, favors the interaction of the LCR with HBB . When $HBG2$ or $HBG1$ is upregulated by rare point mutations in their promoters, expression of the linked HBB is downregulated. Deletions of the HBB promoter remove competition for the LCR, increasing the expression of $HBG2$, $HBG1$, and HBD . The transcription factors BCL11A (2p16) and ZBTB7A (19p13) silence the HbF genes; BCL11A binds to the HbF gene promoters, repressing them and silencing transcription; ZBTB7A binds upstream of BCL11A with similar

repressive effects. This accounts for the bulk of the switch from HbF to HbA. Mutations in these binding sites abolish the normal silencing of the HbF genes, leading to one type of the benign condition called hereditary persistence of fetal hemoglobin (HPFH). Disruption of the *BCL11A* regulatory elements or the binding sites for BCL11A by gene editing is a prime therapeutic target for HbF induction.

α -Globin Gene Switching A less complex switch takes place in the α -globin gene cluster where a regulatory locus of four elements termed R1-R4 is present within introns of the gene *NPR3* that is upstream of *HBA2*. A developmental switch from embryonic ζ - to adult α -globin gene expression occurs at about 6 weeks’ gestation.

Modulation of HbF Level Variations in three quantitative trait loci (QTL), *BCL11A*, *MYB* (6q23), and a locus linked to the *HBB* cluster, account for a major portion of HbF variation among normal individuals and patients with sickle cell anemia and β thalassemia. BCL11A, a zinc finger protein that represses HbF genes, binds TGACCA motifs, the most important at position -115 in the promoter of each γ -globin gene. ZBTB7A binds 85 nucleotides upstream of these BCL11A binding sites; its binding also represses γ -globin gene transcription. When binding of either BCL11A or ZBTB7A is disrupted, silencing of *HBG2* and *HBG1* is abrogated. The unique impact of *BCL11A* variants on HbF in sickle cell anemia and β thalassemia is due to their large effect and the high frequency of the variant allele associated with increased HbF.

The *MYB* gene is essential for hematopoiesis and erythroid differentiation. *MYB* inhibits HbF expression directly by activation of *KLF1* and other repressors and indirectly through alteration of the kinetics of erythroid differentiation.

The third QTL is marked by a common variant 158 nucleotides upstream of the transcription start site of *HBG2* and could be a binding site for an uncharacterized HbF repressor. Haplotypes associated with the *HBB* cluster have been defined by single nucleotide polymorphisms (SNPs) among these genes. Sickle cell anemia patients with the Senegal and Arab-Indian HbS gene-associated haplotypes have higher HbF levels than patients with other haplotypes. These two haplotypes have the common-158 C-T variant in the *HBG2* promoter.

DIAGNOSIS OF HEMOGLOBIN DISORDERS

α -Globin gene mutations are expressed in the embryo and fetus and persist throughout life; HbF mutations are expressed in the fetus and in the first months of life, vanishing from notice afterward; δ -globin gene mutations are innocuous and usually not detected; β -globin gene mutations can become clinically apparent after the synthesis of HbF dwindles to stable adult levels.

With rare exceptions, all disorders of hemoglobin are autosomal recessive or co-dominant disorders; a family history of anemia, a common feature of most symptomatic hemoglobinopathies and thalassemias, is often present. In addition to pallor and jaundice, splenomegaly is often present. In sickle cell disease, acute painful vasoocclusive episodes are a diagnostic feature. A small number of laboratory tests can confirm the diagnosis starting with a complete blood count that includes a reticulocyte count with a careful review of a peripheral blood film. A sustained increase in reticulocyte count indicates the presence of hemolytic anemia. Hemoglobin fractionation by high-performance liquid chromatography (HPLC) or capillary electrophoresis, especially when, in addition to the index case, family members are available for study, is often sufficient to confirm a diagnosis at the level of hemoglobin phenotype. DNA sequencing of the globin genes should allow definitive diagnosis. DNA-based diagnosis, which is readily available from excellent reference laboratories, is a prerequisite for most instances of genetic counseling.

Sickle cell disease and β thalassemia have some features in common. They are caused by mutations in the β -globin gene; both are chronic hemolytic anemias sharing complications associated with hemolysis such as venous thrombosis, leg ulcers, and pulmonary hypertension; and they can be cured by hematopoietic stem cell transplantation. Key differences are that only HbS polymerizes and that ineffective

erythropoiesis is a prominent feature of β thalassemia and responsible for its severe anemia. Both diseases could be cured by inducing sufficiently high levels of HbF; in sickle cell disease, HbF prevents the polymerization of HbS; in β thalassemia, sufficient HbF compensates for the deficit of HbA.

SICKLE CELL DISEASE

Sickle cell disease is a clinical and hematologic phenotype caused by an assortment of genotypes (Table 98-2). Sickle cell anemia, defined as homozygosity for the sickle hemoglobin mutation ($\alpha_2\beta^S$; glutamic acid [E] 7 valine [V] GAG-GTG), is the most common of these genotypes, followed by HbSC disease or compound heterozygosity for HbS and HbC ($\alpha_2\beta^C$; E 7 lysine [K] GAG-AAG) genes. Many different thalassemia mutations contribute to the HbS- β thalassemias. The compound heterozygous genotypes are less common than HbS homozygotes; as a rule, their symptoms develop later in life and are less severe. HbS has also been described with many other variant hemoglobins. Few of these genotypes, other than HbSO^{Arab}, HbSE, and HbSD^{Punjab} are symptomatic.

■ ORIGIN, SPREAD, AND EPIDEMIOLOGY

HbS originated in Africa between 7000 and 22,000 years ago, reaching high frequencies because of the increased genetic fitness of heterozygotes under selective pressure from *Plasmodium falciparum*. The HbS gene became associated with five common β -globin gene haplotypes: Benin, Bantu, Senegal, Cameroon, and Arab-Indian. These haplotypes have a loose association with the severity of disease because each haplotype has a different average level of HbF. In some regions of Africa, India, and the Middle East, nearly half the population have sickle cell trait. Nigeria alone has ~150,000 newborns each year with sickle cell anemia, about one-third of the world's total newborns; most die before age 5. Coerced and free population movement have spread the HbS gene throughout the world. The HbS carrier, or sickle cell trait,

prevalence is 2–15% in emigrant populations; ~100,000 patients in the United States have sickle cell disease; their death in childhood is rare, with the median age of death in the fifth or sixth decade.

■ PATHOPHYSIOLOGY

Pathophysiologic features of sickle cell disease are summarized in Fig. 98-3. HbS is physiologically similar to HbA in most respects except it polymerizes when deoxygenated. Contacts between one of the β^S valine residues of deoxyHbS and specific amino acid residues of β - and α -globin culminate in fascicles of hemoglobin that injure the sickle erythrocyte. A delay occurs between the initiation of polymerization and the accumulation of sufficient polymer to damage the cell. It is unclear how much polymer is needed for cell injury, but it is clear that polymer leads directly and indirectly to the multiple abnormalities of the sickle erythrocyte that generate the pathophysiology of disease. Prominent among these abnormalities are HbS polymer penetration of the membrane causing vesiculation with membrane microparticle release; increased activity of the Gardos, K/CL cotransport, and P^{sickle} channels that dehydrate the cell, increasing mean corpuscular sickle hemoglobin concentration (MC(HbS)C), reducing cellular deformability, and increasing the polymerization potential of HbS; translocation of amino phospholipids such as phosphatidylserine to the outer leaflet of the membrane; and oxidation of erythrocyte contents. These and other abnormalities lead to the formation of irreversibly sickled cells (ISCs), which are sickle erythrocytes that are forever deformed because of permanent membrane damage regardless of whether HbS remains polymerized. Damaged sickle erythrocytes are responsible for initiating the vasoocclusive, hemolytic, and inflammatory features of the disease shown in Fig. 98-3.

■ DIAGNOSIS

Although sickle cell disease can appear in any ethnic group, most often it is present in people of African, Middle Eastern, Mediterranean, and

TABLE 98-2 Common Sickle Hemoglobinopathies

GENOTYPE	CLINICAL ABNORMALITIES	HEMOGLOBIN LEVEL, g/L (g/dL)/MCV, fL	HEMOGLOBIN FRACTIONS (%)
Sickle cell trait (HbAS)	8% of African Americans; hematuria, papillary necrosis, hyposthenuria, increased incidence of chronic kidney disease; 2–4 times increased VTE risk; ? stroke; splenic infarction at altitude; rhabdomyolysis	Normal	HbA: 60–70 HbS: 30–40 Percent HbS dependent on presence or absence of α thalassemia
Sickle cell anemia (HbSS)	Vasoocclusion related: pain, acute chest syndrome, osteonecrosis, splenic infarction Hemolysis related: stroke, pulmonary and systemic vasculopathy, nephropathy, leg ulceration/gallstones, priapism, leg ulcers	70–100 (7–10)/80–100	HbS: >75 HbF: 2–25 HbA ₂ : 3–4
HbS- β^0 thalassemia	Rate of complications similar to HbSS	80–100 (8–11)/60–85	HbS: >75 HbF: 2–15 HbA ₂ : 5–6
HbS- β^+ thalassemia	Rate of complications about half the rate of HbSS depending on percent HbA	100–140 (10–14)/70–80	HbS: 60–90 HbA: 5–40 HbF: 1–10 HbA ₂ : 5–6
Hemoglobin SC disease (HbSC)	Nearly asymptomatic to severe disease; about half the rate of complications as HbSS. Increased risk of retinopathy	100–140 (10–14)/70–100	HbS: 50 HbC: 50
HbSE	Resembles clinically HbS- β^+ thalassemia; symptoms delayed; often Asian/Indian ancestry	90–130 (9–13)/65–75	HbS: 65 HbE: 35 HbF: 1–5
HbSS- α thalassemia	Present in 30% of HbSS; phenocopies HbS- β^0 thalassemia; similar to HbSS but with fewer strokes and leg ulcers and less pulmonary vascular and renal disease	80–100 (8–11)/60–85	HbS: >75 HbF: 2–15 HbA ₂ : 4–5
HbS-HPFH	Most common genotype is due to large <i>HBB</i> deletions and is asymptomatic	110–140 (11–14)/70–80	HbS: 70 HbF: 20–30 HbA ₂ : 1–2

Note: Laboratory values are averages in untreated adults.

Abbreviation: VTE, venous thromboembolism.

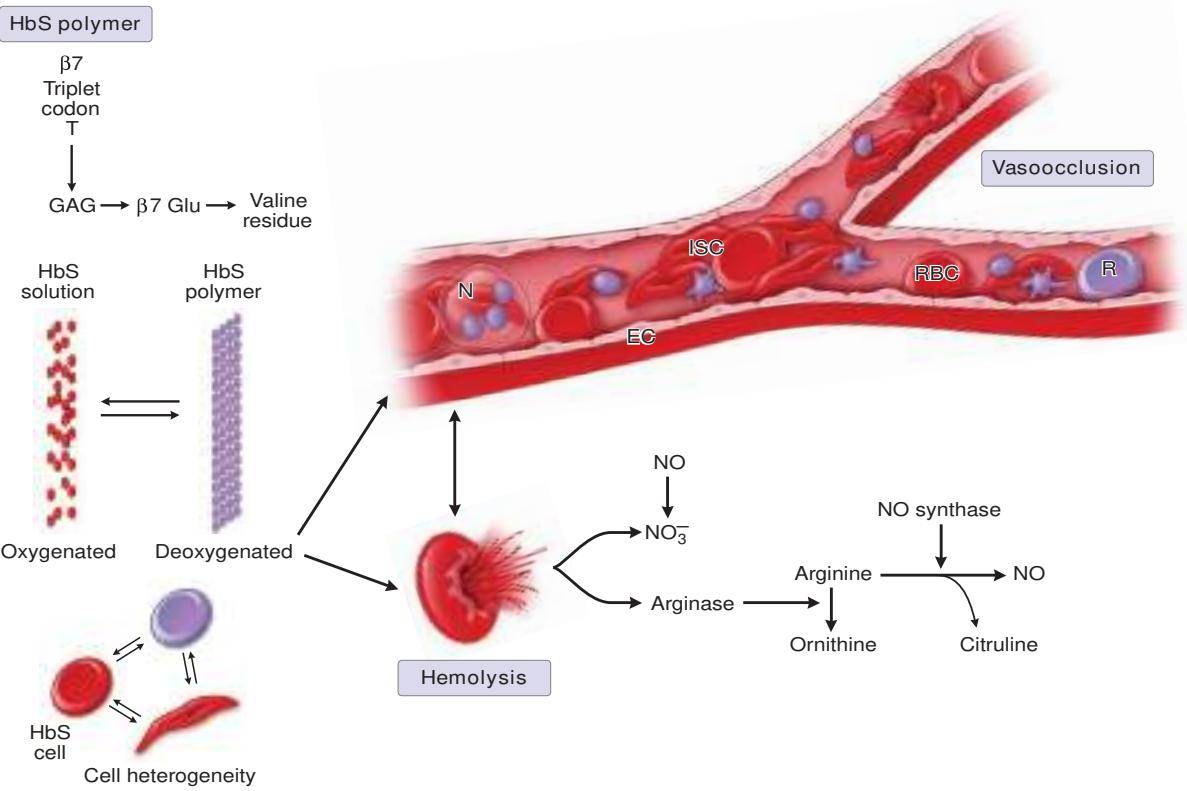


FIGURE 98-3 Pathophysiology of sickle cell disease. HbS is in solution when oxygenated but reversibly polymerizes when deoxygenated. Polymerization is dependent on the 30th power of hemoglobin concentration. In the sickle cell, this means that small changes in hemoglobin concentration or cell hydration can have large effects on polymerization. Polymerization begins seconds to minutes following deoxygenation. Erythrocyte deformation, or sickling, is initially reversible, but after an undetermined number of cell sickling events, the cell becomes irreversibly deformed. These are known as irreversibly sickled cells (ISCs). Their membrane is permanently damaged, although depending on their oxygen (O_2) content, HbS could be in solution. Sickled erythrocytes lead to the clinical and laboratory phenotypes of disease. Sickled cells interact with endothelial cells and other blood cells, occluding flow in small and sometimes large vessels and causing the many complications thought to be a result of vasoocclusion. Sickled cells also live <20 days (normal ~120 days) hemolyzing intra- and extravascularly. Intravascular hemolysis depletes haptoglobin and hemopexin while liberating heme, arginase, and other danger-associated molecular patterns (DAMPs) into the blood. This scavenges nitric oxide (NO), activates platelets and endothelium, reduces antioxidant activity, causes vasoconstriction, and is proinflammatory.

Indian descent. The chief presenting symptom is pain that might be an arthritis-like hand-foot syndrome in young children or the typical acute painful episode in older children and adults. In HbSC disease and HbS-β⁰ thalassemia, acute vasoocclusive episodes occur less often and complications develop later in life; rarely, patients with these genotypes are asymptomatic. The key elements of laboratory diagnosis are outlined in Table 98-2 showing typical hematologic findings and hemoglobin fractions. Figure 98-4 displays HPLC profiles and blood films in typical patients with sickle cell trait, sickle cell anemia, and HbSC disease. Clinical and basic laboratory diagnosis is sufficient for general management and counseling; genetic counseling and family planning usually require DNA-based diagnosis.

■ COMPLICATIONS

Complications of sickle cell disease can be grouped into those that likely are a consequence of sickle vasoocclusion and ones that appeared to be triggered by intravascular hemolysis. Although there is a relationship between these two limbs of pathophysiology, complications associated with vasoocclusion seem to respond best to induction of HbF. Some complications of disease are presented in Table 98-3. Early and effective treatment with hydroxyurea and the integration into management of new treatments discussed below should change this profile.

Acute Painful Episodes Characterized by unprovoked severe pain in extremities or torso that is often symmetrical and stereotypical

for each patient and usually requires treatment with strong opioids in the emergency department, acute painful episodes are the most common acute events in sickle cell disease. They are the chief cause of concern for patients, most of whom have them at some time in their life. Their frequency varies; most patients have one to two episodes a year; some rarely have them; others are hardly ever without them. Acute painful episodes last days to weeks. Complicating the diagnosis and management of the acute pain episode, pain in sickle cell disease can be chronic from complications such as osteonecrosis, osteoporosis, or leg ulcers; chronic and acute pain can overlap; and pain can also be induced by opioid treatment of pain. Diary studies have shown that most of the time patients have some degree of pain that does not reach the intensity of the acute episode. Most patients use oral opioid analgesics for control of this pain. Reliable patients can be given a reasonable supply of oral opioids on a monthly basis.

No diagnostic test can confirm or refute the presence of an acute pain episode; often a 1 to 2 g/dL decrease in hemoglobin level and a modest increase in the leukocyte count are noted during the painful episode. Drastic decreases in hemoglobin and platelet levels with more extreme leukocytosis can portend development of severe acute chest syndrome or multiorgan failure. Acute painful episodes have little to do with the presence of ISCs in the blood or the reticulocyte count. The most anemic patients seem to have the least pain. It is unusual for a cause of acute painful episodes to be identified. Physical examination is not often useful diagnostically. Some patients will have pain on

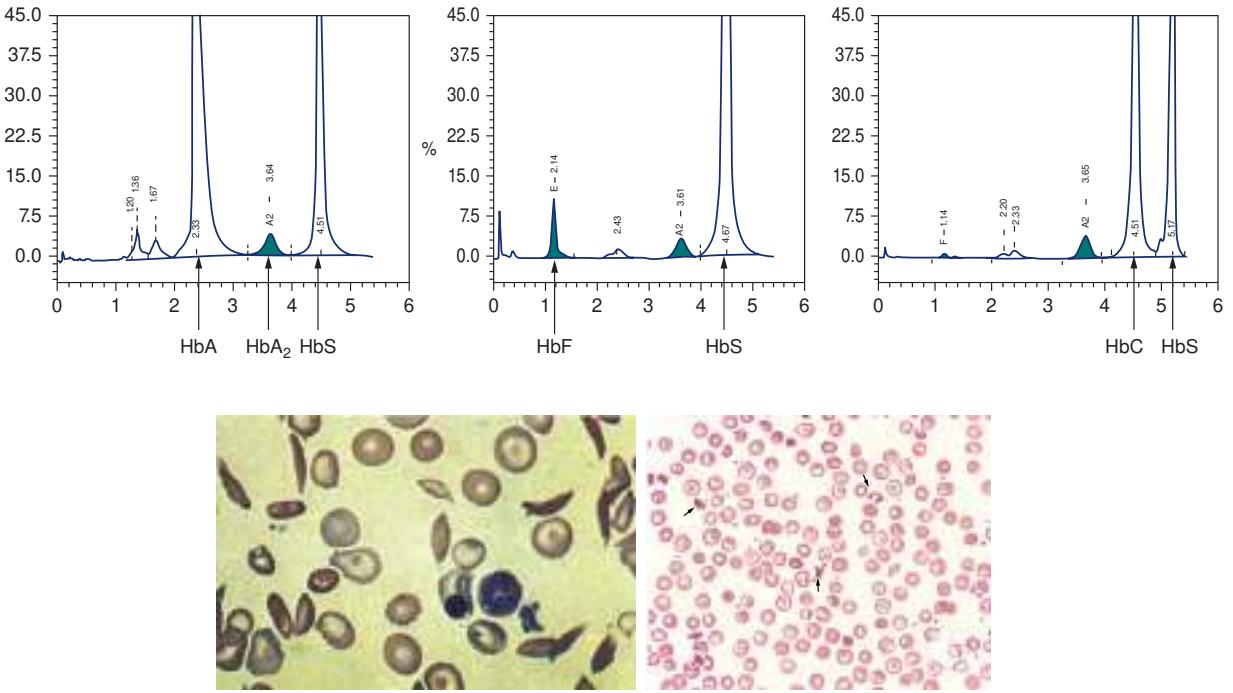


FIGURE 98-4 Diagnosis of sickle cell disease. *A*. From *left to right*, high-performance liquid chromatography separation in sickle cell trait, sickle cell anemia, and HbSC disease. Beneath each chromatogram, the individual protein peaks are identified. *B*. *Left*: Dense, elongated, and pointed cells are the irreversibly sickled cells characteristic of the sickle cell anemia and sickle cell- β^0 thalassemia. Target cells and nucleated red cells are also present. *Right*: Target cells, cells with squared ends of HbC crystals, cells folded like tacos, and contracted microspherocytes are typical of HbSC disease. (*Source: B [right]*: Reproduced with permission from American Society of Hematology.)

TABLE 98-3 Complications of Sickle Cell Disease

COMPLICATION	INCIDENCE, DIAGNOSIS, AND FEATURES	TREATMENT
Priapism	~30% of males; can be episodic and short duration (stuttering); severe episodes can cause impotence; associated with markers of hemolysis	Many unproven therapies including α -adrenergic agonists, stibesterol; consult urology for therapy, which is time-critical
Stroke and silent infarction	10–15% of all cases; infarction in early childhood into adulthood; hemorrhagic in adults; neurocognitive abnormalities in adults even without apparent stroke; associated with markers of hemolysis	Transcranial Doppler screening in children aged 2–16; transfusion for at-risk patients; hydroxyurea
Gallstones/surgery	~40% of patients; bilirubin levels and stones related to polymorphisms of <i>UGT1A</i> ; in surgery requiring general anesthesia, simple preoperative transfusion to a hemoglobin of 10 g/dL is recommended	If asymptomatic, usually let be; otherwise, laparoscopic cholecystectomy
Hepatic disease	>80% of patients have hepatomegaly; intrahepatic cholestasis can have bilirubin ~100 mg/dL; viral hepatitis, iron overload, RBC sequestration, extrahepatic cholestasis also contribute	Exchange transfusion for intrahepatic cholestasis; transplant for end-stage liver failure
Nephropathy	~30% of adults age >30 years; hyperfiltration in children, renal failure in adults; early albuminuria, later nephrotic-range proteinuria; associated with markers of hemolysis	Screen for microalbuminuria by age 10 years; avoid NSAIDs; use ACE inhibitors or receptor antagonists for albuminuria; erythropoietin for symptomatic anemia; dialysis or transplant for renal failure
Lung/pulmonary hypertension	Restrictive disease; asthma common; 5–10% have pulmonary hypertension by right heart catheterization; 30% have increased TRV that portends poor prognosis; associated with markers of hemolysis	Consult expert pulmonologist; screen yearly by echocardiography measurement of TRV
Retinopathy	30% in HbSC disease, 3% in HbSS; ^a develops in peripheral retina; vitreous hemorrhage and retinal detachment can cause blindness	Screen annually starting at age 10 years with fluorescein angiography; laser photocoagulation for proliferative disease
Acute anemic episodes	B19 parvovirus infection, folic acid deficiency, splenic sequestration, delayed hemolytic transfusion reaction with destruction of transfused and sometimes autologous red cells	RBC transfusion if symptomatic; splenectomy if more than one or two episodes of sequestration; anti-parvovirus IgM positive in acute infection, IgG in past infection
Multiorgan failure	Can accompany severe acute chest syndrome; often confused with sepsis and can coexist with sepsis; CNS, liver, muscle, lung, kidney affected	Exchange transfusion, ICU support
Pregnancy	Screening both partners for hemoglobin disorders with risk counseling is critical component of family planning.	All pregnancies are “high risk”; transfuse if sickle cell events increase, if previous miscarriage, multiple fetuses

^aSickle cell anemia (HbSS).

Abbreviations: ACE, angiotensin-converting enzyme; CNS, central nervous system; ICU, intensive care unit; NSAIDs, nonsteroidal anti-inflammatory drugs; TRV, tricuspid regurgitant jet velocity.

pressure over an affected area, perhaps accompanied by swelling; mild fever is common.

Some patients die suddenly shortly after admission for an acute painful episode. The cause of this sudden unexpected death is usually unknown; among the possibilities are arrhythmias and pulmonary embolism. Admitting patients to monitored beds or continuous pulse oximetry for the first 48–72 h of hospitalization might prevent some of these deaths and help identify acute chest syndrome that follows within 72 h in about a quarter of admissions for acute pain. After searching for possible precipitants such as infection or dehydration and treating these appropriately, the foundation of treatment is the proper dosing of opioid analgesics. By the time a patient presents at the emergency department or clinic requesting treatment, they have usually tried nonsteroidal anti-inflammatory drugs (NSAIDs) and oral opioids. In most patients, relief of pain requires the intravenous opioids morphine or hydromorphone. Many patients are opioid tolerant and require higher than usual doses for satisfactory relief. Dosing should not be on an “as-needed” schedule; patient-controlled analgesia or a frequent fixed dose of opioids with rescue doses for breakthrough pain are the preferred means of treatment, with frequent assessments to ensure pain relief without excessive sedation. Adjunctive treatment includes incentive spirometry to forestall pulmonary complications, maintaining hydration with half-normal saline with care not to overhydrate, prophylaxis for thromboembolism, and antihistamines and laxatives to counter expected side effects of opioids; unless hypoxia is present, supplemental O₂ is unnecessary. Ketorolac should not be used, and NSAIDs have little value in patients receiving intravenous opioids.

Acute Chest Syndrome This pneumonia-like illness is the second most frequent acute sickle cell–related event. It occurs in >50% of patients, often more than once. Acute chest syndrome can be mild, especially in children, in whom it can result from viral infection, or devastating, where multiple lobes of the lung are affected with severe hypoxia, multiorgan failure, and death. Chest pain, cough, fever, and hypoxia and a pulmonary infiltrate on chest x-ray are the major diagnostic criteria. The etiology includes in situ thrombosis, emboli, any type of infection, and postoperative hypoventilation. Management in adults is dictated by the severity of the episode. Patients who are hypoxic and febrile are often admitted directly to the intensive care unit. Antibiotics are almost always used in febrile patients even though a causative bacterium is not often cultured. Supplemental O₂ is given for an O₂ saturation <95%. Overhydration and excessive opioids can compound dyspnea and hypoxia. Hypoxic patients who are febrile with leukocytosis and have more than a trivial infiltrate on x-ray are transfused. In the more severely ill patient, exchange transfusion is the preferred modality. When hemoglobin level or symptoms indicate the need for transfusion of the severely ill patient and hours are needed to arrange red cell exchange, simple or top-up transfusion should be started first. Simple transfusions also suffice for less severely affected patients. Most patients survive acute chest syndrome, but in the most severe cases, often caused by embolization of necrotic bone marrow, death can be rapid even with prompt and proper treatment. Thrombocytopenia, leukocyte counts in excess of 20,000/dL, and rapidly developing acute anemia often portend severe acute chest syndrome with the possibility of acute respiratory distress syndrome and multiorgan failure. Many adults have chronic lung disease that could be a sequela of acute chest syndrome, and asthma is very common in patients with sickle cell disease.

Osteonecrosis This painful and sometimes crippling complication that most often affects hips bilaterally occurs in about half of all patients with sickle cell anemia and is also common in HbSC disease; shoulders are less often affected. Beginning with chronic pain that can become severe, loss of function is often the final stage, especially in the hips. MRI can detect the earliest stages, whereas x-ray is less sensitive. Physical therapy and NSAIDs provide some relief; unfortunately, oral opioids are sometimes required. Joint replacement can restore lost mobility and relieve pain, but the life span of prosthetic joints is finite

so surgery should be delayed as long as mobility is satisfactory and pain tolerable.

Leg Ulcers The incidence of leg ulcers is highly dependent on geography and hemoglobin genotype. They are far less common in HbSC disease and HbS-β⁰ thalassemia than in sickle cell anemia and HbS-β⁰ thalassemia. In temperate climates, 10–20% of patients are affected; tropical and subtropical areas have an incidence rate up to 75%; ulcers rarely occur in the Middle East. They can be small and superficial or deep and encompass most of the lower leg. Ulcers can be extraordinarily painful. Long-standing, recurrent large ulcers are difficult to treat. Wet-to-dry dressings and Unna boots are reasonable choices for initial treatment.

SICKLE CELL TRAIT CARRIERS, OR SIMPLE HETEROZYGOSITY FOR THE HbS GENE

Carriers of sickle cell trait outnumber patients with the disease by 25 to 1. Counseling and follow-up of carriers detected by cord blood screening are imperfect. Adolescents and adults can forget that they have sickle cell trait. Although usually a benign condition with a normal life expectancy, some features of this trait are shown in Table 98-2. Counseling sickle cell trait carriers about the small risks of complications and their likelihood of having offspring with sickle cell disease is essential. Counseling prior to participation in sports is also important because of the risk, albeit a very small one, of sudden death from heat-related exertional rhabdomyolysis. Optimal hydration before and during exercise can prevent most episodes of heat-related illness.

TREATMENT, SCREENING, COUNSELING, AND ANTENATAL DIAGNOSIS

Patients should, if possible, be referred to a sickle cell center for initial consultation, follow-up, and institution of therapy. Cooperation among primary care providers, hematologists, and other specialists can provide the best preventive care and management of complications. The frequency at which a patient is seen depends on their therapeutic regimen.

Remarkable changes in the treatment landscape have occurred with the promise of even greater benefits from new curative approaches based on gene therapy. The following discussion focuses on treatment to prevent the complications of disease.

Hydroxyurea Hydroxyurea is the standard of care for all patients with sickle cell anemia and HbS-β⁰ thalassemia. It is recommended for patients of all ages regardless of symptoms and should be started in the first year of life. The major mechanism of action of hydroxyurea is to induce high levels of HbF. Hydroxyurea increases HbF unevenly in the red cell population (heterocellularly), so some cells have greater protection from HbS polymerization than others. Although often employed in symptomatic patients with HbSC disease, its benefits in this genotype are understudied. In adults, where the average HbF is ~5%, the increase in HbF is often modest. Nevertheless, pain and acute chest syndrome are reduced by about half, hemoglobin concentration increases by ~1 g/dL, and after 17.5 years of follow-up, mortality was reduced by 49%. In contrast, all children respond robustly to hydroxyurea. When started at <1 year of age at a dose of ~27 mg/kg, HbF levels were 33.3 ± 9.1% and hemoglobin concentration was 10.1 ± 1.3 g/dL. Acute events were markedly reduced with little toxicity. Based on these and other studies in high- and low-resource countries, unless there is a contraindication, hydroxyurea is standard of care for all patients starting in the first year of life at a dose of ~20 mg/kg and titrated to the maximal tolerated dose based on neutrophil and platelet counts.

Voxelotor Voxelotor increases the affinity of the hemoglobin molecule for O₂ (decreases the P₅₀). Voxelotor, 1500 mg daily, was associated with a 1-g/dL increase in hemoglobin concentration in 59% of patients with a reduction in the biomarkers of hemolysis. Although vasoocclusive events were not significantly reduced in the initial report of efficacy, further analysis after a longer observation period suggested that patients achieving the highest hemoglobin had the fewest acute vasoocclusive events. Voxelotor increases hemoglobin-oxygen affinity in all

erythrocytes (pancellularly), and this should provide an increment in polymerization inhibition beyond hydroxyurea. Many questions remain about the long-term effects of voxelotor. Less hemolysis reduces the propensity for stroke, nephropathy, pulmonary hypertension, leg ulcers, and priapism. Will voxelotor be accompanied by these long-term benefits? Could the high O₂ affinity of a modified hemoglobin be harmful for some patients? The answers to these important questions require further study.

Crizanlizumab Downstream effects of HbS polymerization include adhesive interactions among endothelial cells, leukocytes, platelets, and erythrocytes. P-selectin is one molecule involved in these interactions; blocking selectins prevents sickle cell–endothelial adhesion. A P-selectin-blocking monoclonal antibody given intravenously every month reduced acute painful episodes by ~45%, a reduction similar to that seen with hydroxyurea. There were no effects on hemolysis.

-Glutamine The mechanism of action of this agent, presumed to be the reduction of oxidative stress in sickle erythrocytes, is unsettled. In a phase 3 clinical trial, compared with a placebo, -glutamine was associated with a 25% reduction in painful episodes and 33% reduction in hospitalization.

There is little consensus regarding how recently approved drugs should be integrated into treatment with hydroxyurea. The effects of voxelotor and crizanlizumab appear to be additive to those of hydroxyurea. Voxelotor can be added to hydroxyurea if the benefits of hydroxyurea alone are insufficient, as they are in most adults. If both hydroxyurea and voxelotor are taken at effective doses and acute vasoocclusive complications continue, crizanlizumab could then be added. The dropout rates in the crizanlizumab and -glutamine trials was ~35% so adherence to these therapeutics could be problematic.

Transfusion Transfusions are overutilized and underutilized. Major indications for transfusion include severe symptomatic anemia; treatment and prevention of stroke; increasing hemoglobin level to ~10 g/dL before surgery requiring general anesthesia; and acute chest syndrome with hypoxia or multiple lobe involvement. Sometimes transfusions are given during pregnancy when there is a history of complications or fetal loss. Transfusions should usually be avoided in acute pain episodes and for repair of stable chronic anemia. There is a preference for automated red cell exchange transfusion in acute stroke, severe acute chest syndrome, or multiorgan failure or when chronic transfusions are planned. Recent guidelines formulated by experts recommended extended red cell antigen profiling, if possible before the first transfusion, and antigen matching for Rh (C, E or C/c, E/e) and K antigens in addition to ABO/RhD. Complications of transfusion include hyperviscosity, alloimmunization (which occurred in 18.6% of patients transfused between 1979 and 1984 and 27.3% of patients transfused between 2001 and 2011), iron overload, delayed hemolytic transfusion reactions, and hyperhemolysis.

Stem Cell Transplantation Given the excellent results of human leukocyte antigen (HLA)-identical related donor transplants, which have an event-free survival of >95%, this option might be extended to all patients with a suitable donor. Unfortunately, only 15% of patients have a fully matched donor. New approaches to haploidentical transplants are improving event-free survival in these patients.

Preventive Measures and Screening Cord blood screening for sickle cell disease is done in many countries and all 50 states. Affected patients are then directed to clinics that can initiate early preventive care. In childhood, transcranial Doppler screening beginning at age 2 years and repeated annually until age 16 years, prophylactic penicillin (125 mg for children younger than 3 years; 250 mg for children 3 years and older) twice daily until age 5 years, and vaccination with pneumococcal vaccines are the main measures to prevent stroke and invasive pneumococcal infection. Folic acid, 1 mg daily, is given to prevent megaloblastic erythropoiesis; it is probably unnecessary in people with nutritious diets.

All women planning pregnancy should be screened for disorders of hemoglobin by blood counts, erythrocyte indices, and HPLC analysis

of hemoglobin. Individuals with HbS or β thalassemia trait should have their partners tested. Only then is it possible to know the risks of a fetus having sickle cell disease (Table 98-2). Antenatal diagnosis using chorionic villus sampling is widely available.

Emerging Treatments Gene therapy has curative potential and requires neither matched donors nor immunosuppression. Autologous hematopoietic CD34+ stem cells are mobilized and modified ex vivo to produce an antisickling globin. These cells are reinfused following myeloablative conditioning. Phase 1/2 clinical trials have used lentivirus transduction of CD34+ cells with an antisickling β-globin or have interfered with the HbF-suppressive effects of BCL11A using CRISPR/Cas, zinc finger nucleases, or shRNA. These approaches have resulted in HbF or antisickling hemoglobin levels of nearly 50%, reduced hemolysis, total hemoglobin levels of >11 g/dL, and resolution of acute vasoocclusive events. It is too early to know their long-term safety or cure rate.

THALASSEMIA

Thalassemia is caused by reduced accumulation of either α- or β-globin chains causing a relative excess of the unaffected chain. Unbalanced globin synthesis is the hallmark of thalassemia and the proximate cause of its pathophysiology; unpaired globin chains damage the developing erythroblast. Like the HbS mutation and many other red cell traits, thalassemia reached polymorphic levels in tropical and subtropical populations because heterozygotes are protected from *Plasmodium falciparum* infection. Estimates are that 1–5% of the world's population carries a thalassemia mutation; in some locales, most people have a thalassemia mutation. These mutations can affect any globin gene, but clinically, β and α thalassemia are the most important. With nearly 500 unique thalassemia-causing mutations (www.globin.bx.psu.edu) that can interact with each other and with hemoglobinopathies, thalassemia syndromes are remarkably diverse. Where resources permit and the mutation is known, genetic counseling can be provided and antenatal diagnosis is possible.

HbE (β²⁷ glu-lys) is a common variant whose biosynthesis is reduced because the site of the mutation alters its mRNA processing. Its reduced biosynthesis leads to a deficit of β^E-globin chains and features of β thalassemia. Hemoglobin Constant Spring is caused by a mutation of the termination codon of *HBA2* that leads to the synthesis of an elongated α-globin chain that is unstable and suboptimally synthesized. This variant therefore behaves as an α thalassemia variant.

a THALASSEMIA

■ EPIDEMIOLOGY

Once known as Mediterranean anemia, because of the concentration of cases in Italy, Greece, and other countries bordering the Mediterranean Sea, or as Cooley's anemia after the physician first describing cases, β thalassemia is common in most areas of the world where malaria was endemic. Effective programs of screening, counseling, and antenatal diagnosis have reduced the birth of new cases from the Mediterranean region. The bulk of new patients now are of Asian, Middle Eastern, and Indian origin. About 40,000 β thalassemia patients are born yearly. In the United States there are ~1000 cases of severe β thalassemia.

■ CLASSIFICATION

β⁰ Thalassemia mutations totally prevent the accumulation of any globin from the affected gene; β⁺ thalassemia mutations cause minor or extreme reductions in β-globin synthesis. β Thalassemia major and β thalassemia intermedia are now categorized as transfusion-dependent and non-transfusion-dependent based on the number and frequency of transfusions required to sustain a good quality of life.

Pathophysiology Single nucleotide changes are the most common β thalassemia mutations, but gene deletions also occur. A partial listing of the classes of mutations causing β thalassemia include mutations in the promoter elements affecting gene transcription causing mild and sometimes silent β⁺ thalassemia; mutations in the junctions between

exons and introns that affect mRNA processing causing β^0 and β^+ thalassemia; introduction of alternative splice sites into introns or exons usually causing β^+ thalassemia; 3' end-processing sequence mutations preventing RNA polyadenylation leading to mild or silent β^+ thalassemia; mutations preventing initiation of translation causing β^0 thalassemia; and introduction of stop codons that prematurely terminate translation (nonsense mutations) producing reading frameshifts and resulting in truncated globin mRNA and β^0 thalassemia.

In β thalassemia, the deficit in β -globin chain synthesis allows α -globin chains to accumulate in excess. Without a non- α -globin chain partner in dimer and tetramer formation, unpaired α -globin chains are unstable, cannot form a tetramer, and precipitate within the developing erythroblast, causing membrane lipid oxidation and damage. The predominant cause of anemia is intramedullary destruction of erythroid precursors, known as ineffective erythropoiesis. Reduced deformability and phosphatidyl serine exposure also cause extra- and intravascular hemolysis of those erythrocytes that gain entrance into the circulation. In poorly treated β thalassemia, severe anemia leads to bone marrow expansion; hepatosplenomegaly; iron accumulation in liver, heart, and endocrine organs; pulmonary hypertension; and thromboembolic disease.

Frightening pictures of children with severe β thalassemia permeate the literature. These examples of near-terminal disease should be relegated to history because treatment with transfusion and iron chelation can prevent their occurrence and hematopoietic stem cell transplantation can "cure" patients who have suitable donors.

■ DIAGNOSIS

Heterozygous β thalassemia, also known as β thalassemia trait and β thalassemia minor, has mild or no anemia but microcytic/hypochromic erythrocytes with minimal or no increase in reticulocyte count. After recognizing these hematologic abnormalities and excluding iron deficiency, finding an elevated level of HbA₂ and perhaps HbF by HPLC is sufficient to establish this diagnosis. The hematologic characteristics of this heterozygous carrier state are listed in Table 98-4. Sometimes, the spleen is enlarged. Before genetic counseling and antenatal diagnosis are considered after carrier identification by red cell indices and quantitation of HbA₂, the thalassemia-causing mutation should be identified. This is the key to preventing homozygotes or compound heterozygotes with transfusion-dependent thalassemia.

The more severe forms of β thalassemia are hemolytic anemias with hypochromia, microcytosis, reticulocytosis, marked anisocytosis, and

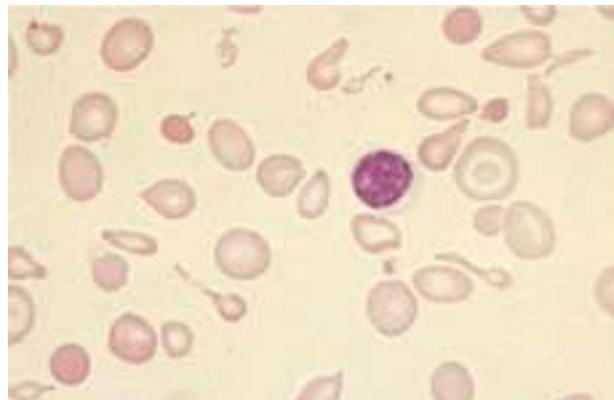


FIGURE 98-5 a Thalassemia intermedia. Target cells and marked variation in cell size and shape but with general hypochromia and microcytosis characterize the blood film. A lymphocyte is shown for size comparison.

poikilocytosis with variable numbers of circulating nucleated red cells (Fig. 98-5).

■ COMPLICATIONS

Complications of severe β thalassemia are many. They are a consequence of chronic hemolytic anemia, chronic transfusion, and iron loading. Increased iron absorption is especially common in non-transfusion-dependent thalassemia. Most complications, listed in Table 98-5, develop because of either inadequate blood transfusion and/or poor iron chelation and iron loading. Even when chelation is optimized, some complications attributable to iron toxicity will develop. Many complications have complex and multifactorial etiologies. Iron stores are estimated by serum ferritin levels; MRI is the most widespread means of noninvasively measuring iron accumulation in liver and heart.

■ MANAGEMENT, SCREENING, COUNSELING, AND ANTENATAL DIAGNOSIS

Heterozygote screening and counseling couples at risk for affected fetuses, with antenatal diagnosis, if needed, is an effective preventive approach. Severe thalassemia should be dealt with in specialized

TABLE 98-4 β Thalassemias

CLASSIFICATION	HEMOGLOBIN (g/dL)/MCV (fL)	HEMOGLOBIN FRACTIONS (%)	CLINICAL FEATURES
β Thalassemia trait	100–140 (10–14)/60–80	HbA: 94 HbF: 1–2 HbA ₂ : 4–6	Heterozygosity for β^+ or β^0 thalassemia mutations; "silent" carriers can have normal HbA ₂ and red cell indices.
Non-transfusion-dependent β thalassemia (thalassemia intermedia)	70–120 (7–12)/65–80	HbA: 60–90 HbF: 10–40 HbA ₂ : 4–6	Defined by infrequent or no transfusion requirement; caused by many different genotypes including homozygosity for "mild" β^+ mutations, combinations of β and α thalassemia, homozygous β thalassemia with high HbF producing capacity, and many others. Iron loading, thromboembolic disease, and pulmonary hypertension are major clinical events.
Transfusion-dependent β thalassemia (thalassemia major)	20–40 (2–4)/50–80	HbA: 0–5 HbF: 90–100 HbA ₂ : 2–5	Caused by many different genotypes including homozygosity and compound heterozygosity for β^0 and β^+ mutations, combinations of β and α thalassemia; transplantation curative; iron chelation required.
HbE- β thalassemia	50–80 (5–8)/60–70	HbE: 50–70 HbF: 30–50	Common in SE Asian populations; in some parts of the world, the most prevalent severe thalassemia; in HbE- β^0 thalassemia, only HbE and HbF are found; in HbE- β^+ thalassemia, HbA is present. Transfusion dependence depends in part on the thalassemia mutation.
$\delta\beta$ Thalassemia and hemoglobin Lepore	110–120 (11–12)/65–75	HbA: 70 HbF: 7–13 HbA ₂ : 2	Rare; deletions removing the δ - and β -globin genes cause $\delta\beta$ thalassemia; Lepore hemoglobins are fusion globin chains; values are for heterozygotes; homozygotes have 100% HbF with hemoglobin 10–11 g/dL.
Gene deletion HPPH	120–140 (12–14)/75–85	HbA: 70 HbF: 15–30 HbA ₂ : 2	Rare; large deletions removing the δ - and β -globin genes; values are for heterozygotes; homozygotes, who are asymptomatic, have 100% HbF without anemia.

Note: Laboratory results are averages in adults.

TABLE 98-5 Complications of β Thalassemia

COMPLICATION	INCIDENCE, DIAGNOSIS, AND FEATURES
Growth retardation	Most often a feature of delayed or inadequate transfusions but can occur in well-transfused children.
Delayed puberty; secondary amenorrhea	50% and 25%, respectively.
Splenomegaly	Can trap 1–40% of red blood cell volume; increases plasma volume, worsening heart failure. Splenectomy indicated when transfusion requirement to maintain ideal hemoglobin increases. Prophylactic penicillin after splenectomy.
Heart	Due to chronic anemia, heightened sensitivity to iron toxicity, thromboembolic pulmonary hypertension, other causes. Progresses through stages to congestive failure and arrhythmias. Assessed by T2* on MRI. The available chelating agents might have differential effects on different measures of cardiac function and can be used in combination.
Leg ulcers	Common in thalassemia intermedia.
Hepatic disease	Fibrosis progressing to cirrhosis is related to hepatic iron concentration that can be monitored by MRI. Hepatitis also plays a role.
Lung disease/pulmonary hypertension	Fibrosis, chronic thromboembolic disease, restrictive pathophysiology, intravascular hemolysis, and reduced nitric oxide bioavailability
Thromboembolism	Multifactorial etiology including platelet activation, red cell–endothelial interactions, thrombocytosis; endothelial activation; splenectomy.
Endocrinopathies	Diabetes, hypothyroidism, hypoparathyroidism, adrenal insufficiency; hypogonadism; hypothalamic-pituitary axis might be especially sensitive to iron.
Bone disease	Caused by bone marrow expansion, severe iron loading, hypogonadism; osteoporosis in ~50% of patients, even those well treated. Extramedullary hematopoietic masses are a feature of thalassemia intermedia.
Infections	Transfusion associated; linked to iron overload (<i>Yersinia</i>); malaria.

centers where these and other services are available and managed by a team led by a hematologist experienced with this disease with help from endocrinologists, cardiologists, transfusion medicine specialists, and social services.

Transfusion and Iron Chelation Transfusion every 2–4 weeks with a goal pretransfusion hemoglobin concentration of 9–10.5 g/dL, coupled with oral iron chelation to prevent the accumulation of excess toxic iron that accompanies transfusion, has prevented the development of cardiomyopathy and endocrinopathies while extending life to at least 50 years. When to begin transfusions, whether partial exchange transfusion is preferable to simple transfusion, and the choice of blood product require consultation with experts. To be effective, transfusions and iron chelation must be started early, be uninterrupted, and continue lifelong. Older patients who did not have the advantage of effective chelation are more likely to develop multiple disease-related morbidities such as osteoporosis, endocrinopathies, liver disease, and renal failure. Two orally effective chelating agents, deferasirox and deferiprone, and one intravenous chelator, deferoxamine, are available.

Hematopoietic Stem Cell Transplantation There is consensus that patients with available donors should be offered transplantation because of the difficulty of lifelong transfusion and chelation and its imperfect efficacy. Quality of life in successfully transplanted patients exceeds that in patients treated with transfusion and chelation. Transplantation from matched sibling donors is curative in >80% of all cases. Unfortunately, only a third of patients have matched donors. The best results are in the youngest patients who have been effectively chelated and received fewer transfusions. Graft failure, graft rejection,

graft-versus-host disease, and a mortality of 5–20% depending on risk factors are the major drawbacks of this procedure. Results of haploidentical and unrelated donor transplants are improving but lag those of matched sibling donors.

Improving Ineffective Erythropoiesis Luspatercept, a fusion protein containing the extracellular domain of human activin type IIB receptor and the Fc domain of human IgG, was recently approved for treatment of transfusion-dependent thalassemia. By binding transforming growth factor β superfamily ligands and reducing Smad2/3 signaling, luspatercept enhances late-stage erythropoiesis. Given subcutaneously, 1 mg/kg every 3 weeks, it was associated with a 33% reduction in transfusion requirements.

Gene Therapy Lentiviral mediated gene therapy using autologous CD34+ hematopoietic stem cells has been approved in Europe for some patients with transfusion-dependent thalassemia who lack a matched donor. In a clinical trial with a median follow-up of 26 months, where patients received autologous CD34+ cells transduced with a lentiviral vector containing a modified HbA, transfusions were reduced or eliminated and hemoglobin levels stabilized between 8.2 and 13.7 g/dL. However, the results were dependent on the β thalassemia mutation, and although transfusion independence was achieved, some features of disease such as ineffective erythropoiesis were not eliminated. The initial results of CRISPR/Cas editing to downregulate *BCL11A* in β thalassemia have eliminated the need for transfusion and normalized hemoglobin levels (see Sickle Cell Disease).

THALASSEMIA

In some respects the obverse of β thalassemia, clinically consequential α thalassemia is less common than severe β thalassemia. α Thalassemia is most often found in Asian populations and is usually caused by deletion of α -globin genes rather than point mutations.

EPIDEMIOLOGY

Carriers of the most common α thalassemia chromosomes (Table 98-6) are found in 5–80% of people from tropical and subtropical regions of Africa, the Middle East, India, Southern China, and Melanesia. About 30% of African Americans carry the common $- \alpha^{\text{3.7}}$ chromosome that contains a single functional α -globin gene. HbH disease, the chief clinically important α thalassemia, is most prevalent in Southern China and Southeast Asia. Estimates are that in Thailand ~3500 patients with severe α thalassemia are born yearly. Pregnancies affected by hemoglobin (Hb) Bart's hydrops fetalis occur mainly in Southern China and Southeastern Asia.

CLASSIFICATION

Each normal chromosome 16 contains two α -globin genes; normal diploid individuals have four α -globin genes. A classification of inherited α thalassemia, as summarized in Table 98-6, is based on the number of functional α -globin genes. If one or two α -globin genes are missing or poorly expressed, these people have α thalassemia trait. Their hematologic abnormalities are almost always trivial. HbH disease is usually caused by deletion or malfunction of three α -globin genes. Hb Bart's hydrops fetalis fetuses have no normally functioning α -globin genes. Hundreds of different sized deletions and rarer point mutations affect the production of α globin and the magnitude of imbalanced globin synthesis. Because of this mutational complexity, many different variations of the common α thalassemia syndromes are found.

PATHOPHYSIOLOGY

Reduced accumulation of α -globin leaves non- α -globins unpaired and unable to participate in the formation of functional hemoglobin tetramers. In the fetus, absent or reduced synthesis of α -globin allows unpaired γ -globin chains, which are usually part of the HbF tetramer, to form γ_4 or Hb Bart's; in adults, when γ -globin synthesis is mostly silenced, unpaired β -globin chains, lacking a suitable partner to form HbA, tetramerize as β_4 or HbH. Both Hb Bart's and HbH have very high O₂ affinity and do not unload O₂ in tissues; HbH is also unstable. Severe anemia in Hb Bart's hydrops fetalis is a result of absent normal

TABLE 98-6 α Thalassemias

CLASSIFICATION	α -GLOBIN GENE ARRANGEMENT	HEMOGLOBIN LEVEL, g/L (g/dL)/MCV (fL)	CLINICAL FEATURES
α Thalassemia trait	- α/α - $\alpha/-\alpha$ - $/-\alpha$ $\alpha^{\text{pt}}/\alpha/\alpha$	120–150 (12–15)/65–80	The chromosome with one deleted α gene (- α) is called α^+ thalassemia (α thalassemia-2); the chromosome with both deleted α genes is α^0 thalassemia (α thalassemia-1); non-gene deletion α thalassemias (α^{pt}) often have a more severe phenotype.
Hemoglobin H disease	- $/-\alpha$ $\alpha^{\text{pt}}/\alpha/-$ $\alpha^{\text{pt}}/\alpha/\alpha^{\text{pt}}$	50–120 (5–12)/60–70	Mild to moderate anemia depending on genotype; non-gene deletion forms of α thalassemia can produce severe HbH disease.
Hb Bart's hydrops fetalis	--/--		Fatal in utero or at birth with rare survivors. Hydrops can also result from combinations of gene deletion and non-gene deletion α thalassemia.
α Thalassemia/intellectual disability syndromes (ATR-16) (ATR-X)	- $/\alpha\alpha$ or --/ α in ATR-16 $\alpha\alpha/\alpha\alpha$ in ATR-X		ATR-16: Large deletions and rearrangements in chr16p. ATR-X: No α -globin gene deletion or mutation, ATRX mutations, X-linked.
α Thalassemia with myelodysplasia (ATMDS)	$\alpha\alpha/\alpha\alpha$		Mutations in ATRX; striking male predominance. Hematologic findings of HbH disease.

Note: Laboratory values are averages in adults. $\alpha\alpha$ denotes the chromosome with two intact α -globin genes; - α chromosome with one α -globin gene deleted; -- chromosome with both α -globin genes deleted; α^{pt} represents non-gene deletion α thalassemia caused by point mutations. The - α chromosome, referred to as α^+ or α thalassemia-2, most often has a deletion of 3.7 kb of DNA (- α^{37}) or 4.2 kb of DNA (- α^{42}) that leaves but a single α -globin gene intact. The chromosome where both α -globin genes are deleted (--) is called α^0 thalassemia or α thalassemia-1. These chromosomes are caused by different-sized deletions that are usually named after their regions of highest frequency such as -SEA, -MED, -Fl, and -THAI.

hemoglobin and ineffective erythropoiesis; in HbH disease, unstable HbH leads to oxidative membrane damage with extravascular hemolysis in the spleen and ineffective erythropoiesis.

■ DIAGNOSIS

Microcytosis/hypochromia with nearly normal hemoglobin concentrations, in the absence of iron deficiency and the increased level of HbA₂, that is diagnostic of β thalassemia, is sufficient for a presumptive diagnosis of α thalassemia trait. When genetic counseling is needed and antenatal diagnosis contemplated, the molecular basis of the presumed α thalassemia is required. HbH disease, which is usually due to compound heterozygosity for one chromosome with both α -globin genes deleted and one chromosome with only a single α -globin gene, is defined by the hematologic findings shown in Table 98-6 along with varying levels of reticulocytosis. At birth, when hemoglobin is separated by HPLC, 20–30% Hb Bart's is present; in adults, traces to 40% HbH are present along with residual Hb Bart's in some cases. HbH inclusions can be induced in some red cells after incubation and staining with brilliant cresyl blue. Hemoglobin composition in Hb Bart's hydrops fetalis is predominantly Hb Bart's with some Hb Portland if the deletion removing α -globin genes preserves the ζ -globin gene.

■ COMPLICATIONS

HbH disease is very heterogeneous because of the different combinations of genotypes that can cause this phenotype. Generally, when non-gene deletion mutants, such as Hb Constant Spring, contribute to the genotype, the disease is more severe. In the most common --/-- genotype, mean hemoglobin in adults is ~11 g/dL Hepatosplenomegaly, jaundice, thalassemic bone changes in the face, and growth impairment are seen 20–50% of cases, depending on the underlying genotype. Iron loading occurs but is not the severe problem; it is in β thalassemia. Pregnancy in these patients should be considered high risk and managed accordingly. Mothers of infants with Hb Bart's hydrops fetalis have a history of stillbirth and develop preeclampsia, polyhydramnios, and antepartum hemorrhage and have difficult labor and delivery. Intrauterine transfusion of the fetus is possible.

■ MANAGEMENT, SCREENING, COUNSELING, AND ANTENATAL DIAGNOSIS

When planning families, couples from regions where α thalassemia is common who have red cell indices that suggest the possibility of carrying an α thalassemia gene should have genetic counseling based

on DNA analysis of their globin genes. Iron should be avoided in non-iron-deficient individuals with α thalassemia trait and microcytosis. Transfusions are not usually needed in HbH disease. Nevertheless, depending on the genotype of disease, transfusions might be necessary especially when anemia becomes more severe, for example, with acute anemic episodes or pregnancy. Iron stores should be checked periodically by measuring serum ferritin or MRI; chelation does not appear to be needed.

Hb Bart's hydrops fetalis is best prevented by screening couples at risk and antenatal diagnosis. Intrauterine therapy and perinatal intensive care have permitted survival of some infants with Hb Bart's hydrops fetalis. As growth retardation affects ~40% and neurodevelopmental delay is present in 20% of survivors, prevention is the best approach.

OTHER HEMOGLOBINOPATHIES OF CLINICAL IMPORTANCE TABLE 98-7

Thirteen-hundred mutations affecting hemoglobin structure have been described (www.globin.bx.psu.edu). Most are clinically silent. HbC and HbE are common. HbC is found in people of African descent and HbE in South China and Southeast Asia. Heterozygotes for HbC and HbE are clinically well. Even individuals homozygous for these mutations, where the variant hemoglobin comprises >90% of the hemolysate, are clinically well with very mild anemia and microcytosis. The major importance of these variants is the interaction of HbC with HbS and HbE with β thalassemia, as outlined in Tables 98-2 and 98-4. A definitive diagnosis for all rare variants depends on DNA analysis.

Unexpected low O₂ saturation by pulse oximetry (SpO₂) with normal O₂ saturation of arterial blood is occasionally seen in rare hemoglobin variants with clinical phenotypes. Asymptomatic patients with unexpectedly low SpO₂ should not be subjected to unneeded cardiopulmonary investigations in search of the cause of their "hypoxemia" until the existence of a hemoglobin variant is excluded.

■ M HEMOGLOBINS

M (met) hemoglobins are characterized by oxidation of the heme-iron from its ferrous (Fe⁺⁺) to ferric (Fe⁺⁺⁺) form. The major clinical feature of these disorders is cyanosis that is asymptomatic. Nine M hemoglobin variants have been described. In seven, the mutation involves histidine residues that interact with heme. Asymptomatic slate gray/brownish pseudocyanosis is the main clinical finding. Spectrophotometric recording of the visible spectrum of the hemolysate is

TABLE 98-7 HbC, HbE, and Rare Hemoglobinopathies

CLASSIFICATION	CLINICAL ABNORMALITIES	HEMOGLOBIN LEVEL, g/L (g/dL)/MCV, fL	HEMOGLOBIN FRACTIONS (%)
HbC trait	2% of African Americans; target cells; no disease	Normal	HbC: 30–40 HbA ₂ : 2–3
HbC disease	Target cells; HbC crystals; mild reticulocytosis; splenomegaly	100–130 (10–13)/60–70	HbC: >95 HbF: 2–4 HbA ₂ : 2–3
HbE trait	50% incidence in some Asian populations; a few target cells; clinically normal	120–140 (12–14)/80–90	HbE: 27–31 ^b HbF: 1 HbA ₂ : 3
HbE disease	No hemolysis; 20–80% target cells; no splenomegaly	100–120 (10–12)/65–75	HbE: 85–95 HbF: 3–7 HbA ₂ : 3
High O ₂ affinity hemoglobins	Isolated erythrocytosis; often familial; no splenomegaly; no <i>JAK2</i> ^{W617F} mutation	150–200 (15–20)	Variants in α- and β-globin genes; patients are heterozygotes: ~25–50% variant
Low O ₂ affinity hemoglobins	Asymptomatic mild anemia; cyanosis	100–140 (10–14)	~50% variant
Unstable hemoglobins	Pigmenturia; hemolysis; reticulocytosis; splenomegaly	90–140 (9–14)/70–90	20–35% variant; rare hyperunstable variants can be undetectable and have the phenotype of thalassemia
M hemoglobins	Some have mild hemolysis; few symptoms	100–140 (10–14)/80–90	20–50% variant depending on gene affected

Note: Laboratory values are averages in adults. As noted for HbAS, the amount of HbC and HbE in heterozygotes depends on the number of α-globin genes.

diagnostic. To distinguish M hemoglobins from methemoglobinemia due to drugs or cytochrome b5 reductase (*CYB5R3*) deficiency, potassium cyanide (KCN) can be added to the hemolysate; methemoglobin-containing blood will turn red, but KCN has no effect on M hemoglobin. Treatment is not needed.

■ UNSTABLE HEMOGLOBINS

Sometimes referred to as congenital Heinz body hemolytic anemias, some mutations result in a hemoglobin tetramer that is unstable and precipitates intracellularly. Such variants are rare and often a result of a new mutation that affects the tertiary or quaternary structure of the molecule. The most common class of mutations introduce a proline residue in the α helix or a polar amino acid into the interior of the molecule. Heinz bodies are intraerythrocytic precipitates that are detectable as dark globular aggregates after staining with a dye such as brilliant cresyl blue. Three unstable hemoglobins are the most common of these rare variants. Hemoglobin Köln (β⁹⁹ val-met) has been found in multiple families. Hb Hasharon (α⁴⁷ asp-his) is found in Ashkenazi Jews, and Hb Zurich (β⁶³ his-arg) is susceptible to oxidant drug-induced hemolysis. Unstable variants present with nonspherocytic hemolytic anemia, but presentation is highly variable. The associated disease is usually mild and does not require transfusion. Heating blood to 50°C or incubation with isopropanol precipitates unstable hemoglobins but must be done with careful controls. Some variants can be detected by HPLC.

■ HEMOGLOBINS WITH HIGH OXYGEN AFFINITY AND LOW OXYGEN AFFINITY

Rare mutations in areas involved in the R-T transition, at critical interfaces between globin chains of the tetramer that reduce the affinity for 2,3-bisphosphoglycerate, or present in the heme pocket account for most of these variants. High O₂ affinity hemoglobins outnumber low O₂ affinity variants by two to one. Isolated erythrocytosis in the absence of splenomegaly suggests the presence of a high O₂ affinity hemoglobin. High O₂ affinity hemoglobin variants shift the hemoglobin-O₂ dissociation curve leftward, causing a low P₅₀ and thereby stimulating erythropoiesis. Many of these variants are due to new mutations. The clinical course is benign, and phlebotomy because of erythrocytosis is usually not required. Early diagnosis is important to forestall unnecessary diagnostic procedures and therapeutics such as cardiac catheterization to exclude congenital heart disease or treatment for polycythemia vera. Low O₂ affinity variants often present with cyanosis. Their hemoglobin-O₂ dissociation curve is right-shifted with

high P₅₀. HPLC might reveal the presence of a hemoglobin variant. Treatment is often not necessary.

■ ACQUIRED DISORDERS OF HEMOGLOBIN

CO binds hemoglobin with high affinity forming carboxyhemoglobin. Carboxyhemoglobin levels can be accurately measured by co-oximetry of arterial blood. Standard pulse oximeters cannot accurately make this measurement. Some newly developed pulse oximeters are able to measure both carboxyhemoglobin and methemoglobin. Bound CO inhibits the transport of O₂; the hemoglobin-O₂ binding curve is left-shifted. Acute and chronic CO intoxication, caused by occupational exposure and other sources of incomplete combustion of hydrocarbons, presents with headache, altered mental status, and other constitutional symptoms. High-flow O₂ via facemask is the preferred treatment; criteria have been developed to guide the use of hyperbaric O₂.

Acquired methemoglobinemia and methemoglobinemia due to deficiency of *CYB5R3* are more common than the M hemoglobins. *CYB5R3* is required for the reduction of methemoglobin by NADH. Affected individuals with “toxic” methemoglobinemia can be cyanotic and symptomatic. As in carboxyhemoglobinemia, O₂ transport is reduced and reflected by the left-shift in the hemoglobin-O₂ binding curve. *CYB5R3* deficiency usually affects only erythrocytes (type I), causing a mild disorder; when all cells are affected (type II), a severe disease results. Intravenous methylene blue is the preferred treatment in symptomatic patients with acquired methemoglobinemia and 40–60% methemoglobin. The usual dose is 1–2 mg/kg. Alternative treatment with ascorbic acid is preferable in people who are glucose-6-phosphate dehydrogenase deficient. Methylene blue interferes with co-oximetry, reducing the value of co-oximetry for monitoring treatment.

Many drugs and chemicals can induce methemoglobin in the absence of *CYB5R3* deficiency. Dapsone and topical anesthetics such as benzocaine are the most common offending agents.

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does not have the ability to degrade cobalamin, daily requirements are also about 1–3 µg. Body stores are of the order of 2–3 mg, sufficient for 3–4 years if supplies are completely cut off.

■ ABSORPTION

Two mechanisms exist for cobalamin absorption. One is passive, occurring equally through buccal, duodenal, and ileal mucosa; it is rapid but extremely inefficient, with <1% of an oral dose being absorbed by this process. The normal physiologic mechanism is active; it occurs through the ileum and is efficient for small (a few micrograms) oral doses of cobalamin, and it is mediated by gastric intrinsic factor (IF). Dietary cobalamin is released from protein complexes by enzymes in the stomach, duodenum, and jejunum; it combines rapidly with a salivary glycoprotein that belongs to the family of cobalamin-binding proteins known as haptocorrins (HCs). In the intestine, the HC is digested by pancreatic trypsin and the cobalamin is transferred to IF.

IF (gene at chromosome 11q13) is produced in the gastric parietal cells of the fundus and body of the stomach, and its secretion parallels that of hydrochloric acid. Normally, a vast excess of IF is available. The IF-cobalamin complex passes to the ileum, where IF attaches to a specific receptor (cubilin) on the microvillus membrane of the enterocytes. Cubilin also is present in yolk sac and renal proximal tubular epithelium. Cubilin appears to traffic by means of amnionless (AMN), an endocytic receptor protein that directs sublocalization and endocytosis of cubilin with its ligand IF-cobalamin complex. The cobalamin-IF complex enters the ileal cell, where IF is destroyed. After a delay of about 6 h, the cobalamin appears in portal blood attached to transcobalamin (TC) II.

Between 0.5 and 5 µg of cobalamin enter the bile each day. This binds to IF, and a major portion of biliary cobalamin normally is reabsorbed together with cobalamin derived from sloughed intestinal cells. Because of the appreciable amount of cobalamin undergoing enterohepatic circulation, cobalamin deficiency develops more rapidly in individuals who malabsorb cobalamin than it does in vegans, in whom reabsorption of biliary cobalamin is intact.

■ TRANSPORT

Two main cobalamin transport proteins exist in human plasma; they both bind cobalamin—one molecule for one molecule. One HC, also known as TC I, is closely related to other cobalamin-binding HCs in milk, gastric juice, bile, saliva, and other fluids. The gene *TCNL* is at chromosome 11q11-q12.3. These HCs differ from each other only in the carbohydrate moiety of the molecule. TC I is derived primarily from the specific granules in neutrophils. Normally, it is about two-thirds saturated with cobalamin, which it binds tightly. TC I does not enhance cobalamin entry into tissues. Glycoprotein receptors on liver cells are involved in the removal of TC I from plasma, and TC I may play a role in the transport of cobalamin analogues (which it binds more effectively than IF) to the liver for excretion in bile.

The other major cobalamin transport protein in plasma is transcobalamin, also known as TC II. The gene is on chromosome 22q11-q13.1. As for IF and HC, there are nine exons. The three proteins are likely to have a common ancestral origin. TC II is synthesized by liver and by other tissues, including macrophages, ileum, and vascular endothelium. It normally carries only 20–60 ng of cobalamin per liter of plasma and readily gives up cobalamin to marrow, placenta, and other tissues, which it enters by receptor-mediated endocytosis involving the TC II receptor and megalin (encoded by the *LRP-2* gene). The TC II cobalamin is internalized by endocytosis via clathrin-coated pits; the complex is degraded, but the receptor probably is recycled to the cell membrane as is the case for transferrin. Export of “free” cobalamin is via the ATP-binding cassette drug transporter alias multidrug resistance protein 1.

FOLATE

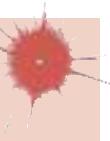
■ DIETARY FOLATE

Folic (pteroylglutamic) acid is a yellow, crystalline, water-soluble substance. It is the parent compound of a large family of natural folate compounds, which differ from it in three respects: (1) they are partly or

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Megaloblastic Anemias

A. Victor Hoffbrand



The megaloblastic anemias are a group of disorders characterized by the presence of distinctive morphologic appearances of the developing red cells in the bone marrow. The marrow is usually hypercellular, and the anemia is based on ineffective erythropoiesis. The cause is usually a deficiency of either cobalamin (vitamin B₁₂) or folate, but megaloblastic anemia may occur because of genetic or acquired abnormalities that affect the metabolism of these vitamins or because of defects in DNA synthesis not related to cobalamin or folate (Table 99-1).

COBALAMIN

Cobalamin (vitamin B₁₂) exists in a number of different chemical forms. All have a cobalt atom at the center of a corrin ring. In nature, the vitamin is mainly in the 2-deoxyadenosyl (ado) form, which is located in mitochondria. It is the cofactor for the enzyme L-methylmalonyl coenzyme A (CoA) mutase. The other major natural cobalamin is methylcobalamin, the form in human plasma and in cell cytoplasm. It is the cofactor for methionine synthase. Minor amounts of hydroxocobalamin are also present to which methyl- and adocobalamin are converted rapidly by exposure to light.

■ DIETARY SOURCES AND REQUIREMENTS

Cobalamin is synthesized solely by microorganisms. Ruminants obtain cobalamin from the foregut, but the only source for humans is food of animal origin, for example, meat, fish, and dairy products. Vegetables, fruits, and other foods of nonanimal origin are free from cobalamin unless they are contaminated by bacteria. A normal Western diet contains 5–30 µg of cobalamin daily. Adult daily losses (mainly in the urine and feces) are 1–3 µg (0.1% of body stores), and because the body

TABLE 99-1 Causes of Megaloblastic Anemia

Cobalamin deficiency or abnormalities of cobalamin metabolism (see Tables 99-3, 99-4)

Folate deficiency or abnormalities of folate metabolism (see Table 99-5)

Therapy with antifolate drugs (e.g., methotrexate)

Independent of either cobalamin or folate deficiency and refractory to cobalamin and folate therapy:

Some cases of acute myeloid leukemia, myelodysplasia

Therapy with drugs interfering with synthesis of DNA (e.g., cytosine arabinoside, hydroxyurea, 6-mercaptopurine, azidothymidine [AZT])

Orotic aciduria (responds to uridine)

Thiamine-responsive

TABLE 99-2 Biochemical Reactions of Folate Coenzymes

REACTION	COENZYME FORM OF FOLATE INVOLVED	SINGLE CARBON UNIT TRANSFERRED	IMPORTANCE
<i>Formate activation</i>	THF	-CHO	Generation of 10-formyl-THF
<i>Purine synthesis</i>			
Formation of glycaminamide ribonucleotide	5,10-Methylene-THF	-CHO	Formation of purines needed for DNA, RNA synthesis, but reactions probably not rate-limiting
Formylation of aminoimidazole carboxamide ribonucleotide (AICAR)	10-Formyl (CHO)THF		
<i>Pyrimidine synthesis</i>			
Methylation of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (dTTP)	5,10-Methylene-THF	-CH ₃	Rate limiting in DNA synthesis Oxidizes THF to DHF Some breakdown of folate at the C-9-N-10 bond
<i>Amino acid interconversion</i>			
Serine-glycine interconversion	THF	=CH ₂	Entry of single carbon units into active pool
Homocysteine to methionine	5-Methyl(M)THF	-CH ₃	Demethylation of 5-MTHF to THF; also requires cobalamin, flavine adenine dinucleotide, ATP, and adenosylmethionine
Forminoglutamic acid to glutamic acid in histidine catabolism	THF	-HN-CH=	

Abbreviations: DHF, dihydrofolate; THF, tetrahydrofolate.

completely reduced to dihydrofolate (DHF) or tetrahydrofolate (THF) derivatives, (2) they usually contain a single carbon unit (Table 99-2), and (3) 70–90% of natural folates are folate-polyglutamates.

Most foods contain some folate. The highest concentrations are found in liver, yeast, spinach, other greens, and nuts (>100 µg/100 g). The total folate content of an average Western diet is 250 µg daily, but the amount varies widely according to the type of food eaten and the method of cooking. Folate is easily destroyed by heating, particularly in large volumes of water. Total-body folate in the adult is 10 mg, with the liver containing the largest store. Daily adult requirements are 100 µg, and so stores are sufficient for only 3–4 months in normal adults, and severe folate deficiency may develop rapidly.

■ ABSORPTION

Folates are absorbed rapidly from the upper small intestine. The absorption of folate polyglutamates is less efficient than that of monoglutamates; on average, 50% of food folate is absorbed. Polyglutamate forms are hydrolyzed to the monoglutamate derivatives either in the lumen of the intestine or within the mucosa. All dietary folates are converted to 5-methyl-THF (5-MTHF) within the small intestinal mucosa before entering portal plasma. The monoglutamates are actively transported across the enterocyte by a proton-coupled folate transporter (PCFT, SCL46A1). This is situated at the apical brush border and is most active at pH 5.5, which is about the pH of the duodenal and jejunal surface. Genetic mutations of this protein underlie hereditary malabsorption of folate (see below). Pteroylglutamic acid at doses >400 µg is absorbed largely unchanged and converted to natural folates in the liver. Lower doses are converted to 5-MTHF during absorption through the intestine.

About 60–90 µg of folate enter the bile each day and are excreted into the small intestine. Loss of this folate, together with the folate of sloughed intestinal cells, accelerates the speed with which folate deficiency develops in malabsorption conditions.

■ TRANSPORT

Folate is transported in plasma; about one-third is loosely bound to albumin, and two-thirds are unbound. In all body fluids (plasma, cerebrospinal fluid, milk, bile), folate is largely, if not entirely, 5-MTHF in the monoglutamate form. Three types of folate-binding protein are involved. A reduced folate transporter (RFC, SLC19A1) is the major route of delivery of plasma folate (5-MTHF) to cells. Two folate receptors, FR2 and FR3 embedded in the cell membrane by a glycosyl phosphatidylinositol anchor, transport folate into the cell via receptor-mediated endocytosis. The third protein, proton-coupled folate

transporter (PCFT), transports folate at low pH from the vesicle to the cell cytoplasm. The reduced folate transporter also mediates uptake of methotrexate by cells.

■ BIOCHEMICAL FUNCTIONS

Folates (as the intracellular polyglutamate derivatives) act as coenzymes in the transfer of single-carbon units (Fig. 99-1 and Table 99-2). Two of these reactions are involved in purine synthesis and one in pyrimidine synthesis necessary for DNA and RNA replication. Folate is also a coenzyme for methionine synthesis, in which methylcobalamin is also involved and in which THF is regenerated. THF is the acceptor of single carbon units newly entering the active pool via conversion of serine to glycine. Methionine, the other product of the methionine synthase reaction, is the precursor for S-adenosylmethionine (SAM), the universal methyl donor involved in >100 methyltransferase reactions (Fig. 99-1).

During thymidylate synthesis, 5,10-methylene-THF is oxidized to DHF. The enzyme DHF reductase converts this to THF. The drugs methotrexate, pyrimethamine, and (mainly in bacteria) trimethoprim inhibit DHF reductase and so prevent formation of active THF coenzymes from DHF. A small fraction of the folate coenzyme is not recycled during thymidylate synthesis but is degraded at the C9-N10 bond.

BIOCHEMICAL BASIS OF MEGALOBLASTIC ANEMIA

The common feature of all megaloblastic anemias is a defect in DNA synthesis that affects rapidly dividing cells in the bone marrow. All conditions that give rise to megaloblastic changes have in common a disparity in the rate of synthesis or availability of the four immediate precursors of DNA: the deoxyribonucleoside triphosphates (dNTPs)—dA(adenine)TP and dG(guanine)TP (purines), dT(thymine)TP, and dC(cytosine)TP (pyrimidines). In deficiencies of either folate or cobalamin, there is failure to convert deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTTP), the precursor of dTTP (Fig. 99-1). This is the case because folate is needed as the coenzyme 5,10-methylene-THF polyglutamate for conversion of dUMP to dTMP; the availability of 5,10-methylene-THF is reduced in either cobalamin or folate deficiency. DNA replication from multiple origins along the chromosome is slower than normal during mitosis, and there is failure of joining up the incomplete replicons with resulting single-stranded DNA breaks. An alternative theory for megaloblastic anemia in cobalamin or folate deficiency is misincorporation of uracil into DNA because of the accumulation of deoxyuridine triphosphate (dUTP) at

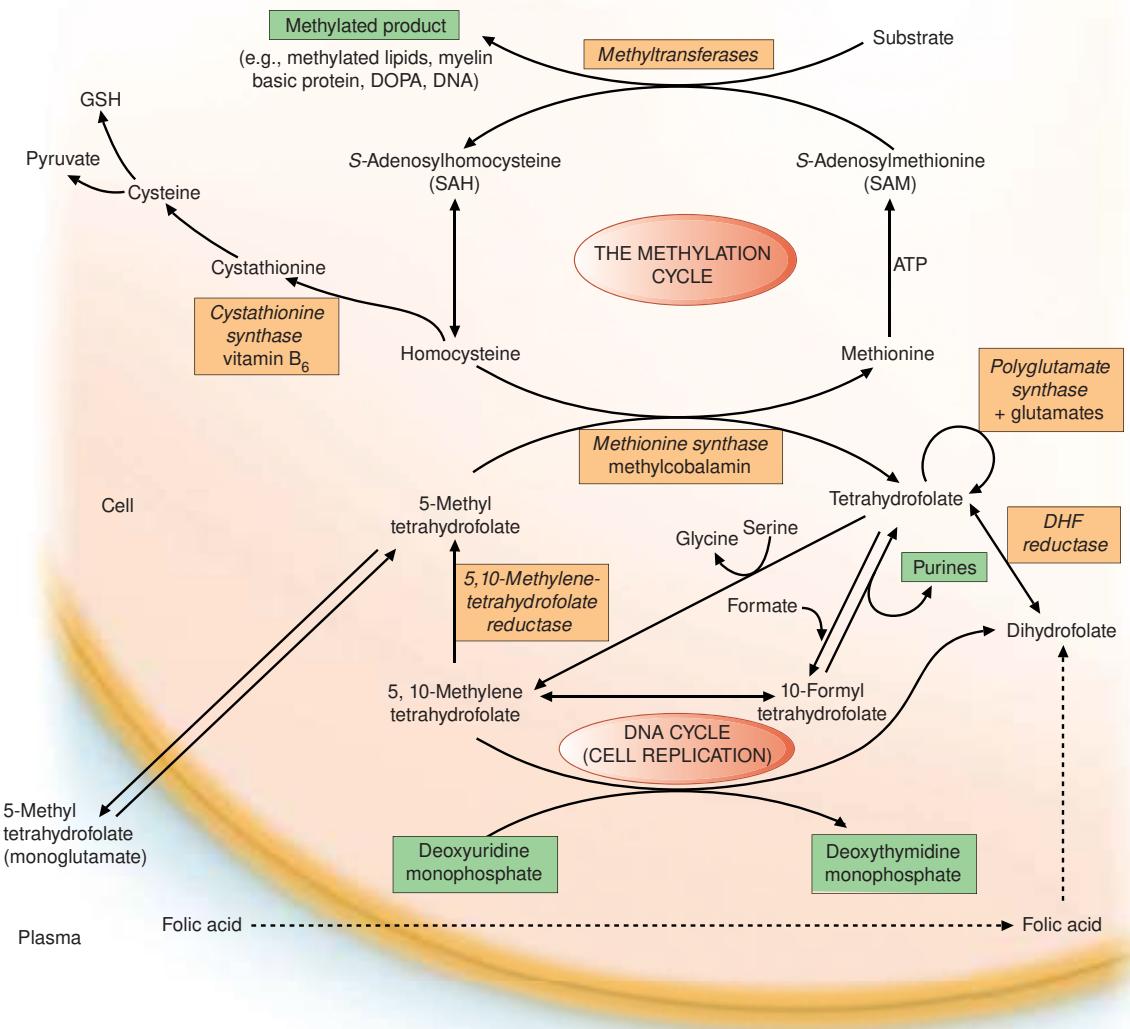


FIGURE 99-1 The role of folates in DNA synthesis and in formation of S-adenosylmethionine (SAM), which is involved in numerous methylation reactions. DHF, dihydrofolate; GSH, glutathione. (Reproduced with permission from AV Hoffbrand et al [eds]: Postgraduate Haematology, 5th ed. Oxford, UK, Blackwell Publishing, 2005.)

the DNA replication fork as a consequence of the block in conversion of dUMP to dTMP.

■ COBALAMIN FOLATE RELATIONS

Folate is required for many reactions in mammalian tissues. Only two reactions in the body are known to require cobalamin. Methylmalonyl-CoA isomerization requires adocobalamin, and the methylation of homocysteine to methionine requires both methylcobalamin and 5-MTHF (Fig. 99-1). This reaction is the first step in the pathway by which 5-MTHF, which enters bone marrow and other cells from plasma, is converted into all the intracellular folate coenzymes. The coenzymes are all polyglutamated (the larger size aiding retention in the cell), but the enzyme folate polyglutamate synthase can use only THF, not MTHF, as substrate. In cobalamin deficiency, MTHF accumulates in plasma, and intracellular folate concentrations fall due to failure of formation of THF, the substrate on which folate polyglutamates are built. This has been termed *THF starvation*, or the *methylfolate trap*.

This theory explains the abnormalities of folate metabolism that occur in cobalamin deficiency (high serum folate, low cell folate, positive purine precursor aminoimidazole carboxamide ribonucleotide [AICAR] excretion; Table 99-2) and also why the anemia of cobalamin deficiency responds to folic acid in large doses.

CLINICAL FEATURES

Many symptomless patients are detected through the finding of a raised mean corpuscular volume (MCV) on a routine blood count. The main clinical features in more severe cases are those of anemia. Anorexia is usually marked, and there may be weight loss, diarrhea, or constipation. Glossitis, angular cheilosis, a mild fever in more severely anemic patients, jaundice (unconjugated), and reversible melanin skin hyperpigmentation also may occur with a deficiency of either folate or cobalamin. Thrombocytopenia sometimes leads to bruising, and this may be aggravated by vitamin C deficiency or alcohol in malnourished patients. The anemia and low leukocyte count may predispose to infections, particularly of the respiratory and urinary tracts. Cobalamin deficiency has also been associated in a few studies with impaired bactericidal function of phagocytes and with osteoporosis.

Neurologic Manifestations Vitamin B₁₂ is needed for the myelination of the central nervous system. Its deficiency may cause a bilateral peripheral neuropathy or degeneration (demyelination) of the cervical and thoracic posterior and lateral (pyramidal) tracts of the spinal cord and, less frequently, of the cranial nerves and of the white matter of the brain. Optic atrophy and cerebral symptoms including dementia, depression, psychotic symptoms, and cognitive impairment may be

prominent. There may also be anosmia and loss of taste. MRI may show the "spongy" degeneration of the cord.

The patient, more frequently male, typically presents with paresthesias, muscle weakness, or difficulty in walking but sometimes may present with dementia, psychotic disturbances, or visual impairment. There is usually loss of proprioception and vibration sensation with positive Romberg and Lhermitte signs. Gait may be ataxic with spasticity (hyperreflexia). Autonomic nervous dysfunction can result in postural hypotension, impotence, and incontinence.

Long-term nutritional cobalamin deficiency in infancy leads to poor brain development and impaired intellectual development. In infancy, there may be feeding difficulties, lethargy, and coma. Convulsions and myoclonus have been described. An important clinical problem is the nonanemic patient with neurologic or psychiatric abnormalities and a low or borderline serum cobalamin level. In such patients, it is necessary to try to establish whether there is significant cobalamin deficiency, for example, by careful examination of the blood film, tests for pernicious anemia (PA) by serum gastrin level and for antibodies to IF or parietal cells, along with serum methylmalonic acid (MMA) measurement if available. A trial of cobalamin therapy for at least 3 months will usually also be needed to determine whether the symptoms improve.

The biochemical basis for cobalamin neuropathy remains obscure. Its occurrence in the absence of methylmalonic aciduria in TC II deficiency suggests that the neuropathy is related to the defect in homocysteine-methionine conversion. Accumulation of S-adenosylhomocysteine in the brain, resulting in inhibition of transmethylation reactions, has been suggested. Folate deficiency has been suggested to cause organic nervous disease, but this is uncertain, although methotrexate injected into the cerebrospinal fluid may cause brain or spinal cord damage.

Psychiatric disturbance as discussed above is common in both folate and cobalamin deficiencies. This, like the neuropathy, has been attributed to a failure of the synthesis of SAM, which is needed in methylation of biogenic amines (e.g., dopamine) as well as that of proteins, phospholipids, and neurotransmitters in the brain (Fig. 99-1). Associations between lower serum folate or cobalamin levels and higher homocysteine levels and the development of decreased cognitive function and dementia in Alzheimer's disease have been reported. A meta-analysis of randomized, placebo-controlled trials of homocysteine-lowering B-vitamin supplementation of individuals with and without cognitive impairment, however, showed that supplementation with vitamin B₁₂, vitamin B₆, and folic acid alone or in combination did not improve cognitive function. Some studies done in China suggest some cognitive improvement with supplements of both vitamins. It is unknown whether prolonged treatment with these B vitamins can reduce the risk of dementia in later life.

■ GENERAL TISSUE EFFECTS OF COBALAMIN AND FOLATE DEFICIENCIES

Epithelial Surfaces After the marrow, the next most frequently affected tissues are the epithelial cell surfaces of the mouth (with glossitis), stomach, and small intestine and the respiratory, urinary, and female genital tracts. The cells show macrocytosis, with increased numbers of multinucleate and dying cells. The deficiencies may cause cervical smear abnormalities.

Complications of Pregnancy The gonads are also affected, and infertility is common in both men and women with severe deficiency of either vitamin. Maternal folate deficiency has been implicated as a cause of prematurity, and both folate deficiency and cobalamin deficiency have been implicated in recurrent fetal loss and neural tube defects, as discussed below.

Neural Tube Defects Folic acid supplements at the time of conception and in the first 12 weeks of pregnancy reduce by 70% the incidence of neural tube defects (NTDs) (anencephaly, meningomyelocele, encephalocele, and spina bifida) in the fetus. Most of this protective effect can be achieved by taking folic acid, 0.4 mg daily, at the time of conception.

The incidence of cleft palate and harelip also can be reduced by prophylactic folic acid. There is no clear simple relationship between maternal folate status and these fetal abnormalities, although overall, the lower the maternal folate, the greater is the risk to the fetus. NTDs also can be caused by antifolate and antiepileptic drugs.

An underlying maternal folate metabolic abnormality has also been postulated. One abnormality has been identified: reduced activity of the enzyme 5,10-methylene-THF reductase (MTHFR) (Fig. 99-1) caused by a common C677T polymorphism in the *MTHFR* gene. In one study, the prevalence of this polymorphism was found to be higher than in controls in the parents of NTD fetuses and in the fetuses themselves: homozygosity for the TT mutation was found in 13% of cases compared with 5% of control subjects. The polymorphism codes for a thermolabile form of MTHFR. The homozygous state results in a lower mean serum and red cell folate level compared with control subjects, as well as significantly higher serum homocysteine levels. Tests for mutations in other enzymes possibly associated with NTDs, for example, methionine synthase and serine-glycine hydroxymethylase, have been negative. Serum vitamin B₁₂ levels are also lower in the sera of mothers of NTD infants than in controls. In addition, maternal TC II receptor polymorphisms are associated with increased risk of NTD births. However, no studies show that dietary fortification with vitamin B₁₂ reduces the incidence of NTDs.

Cardiovascular Disease Children with severe homocystinuria (blood levels $\geq 100 \mu\text{mol/L}$) due to deficiency of one of three enzymes (methionine synthase, MTHFR, or cystathione synthase; Fig. 99-1) have vascular disease, for example, ischemic heart disease, cerebrovascular disease, or pulmonary embolus, as teenagers or in young adulthood. Lesser degrees of raised serum homocysteine and low levels of serum folate and homozygous inherited mutations of *MTHFR* have been found to be associated with cerebrovascular, peripheral vascular, and coronary heart disease and with deep vein thrombosis. Prospective randomized trials of lowering homocysteine levels with supplements of folic acid, vitamin B₁₂, and vitamin B₆ against placebo over a 5-year period in patients with vascular disease or diabetes have not, however, shown a reduction of first event fatal or nonfatal myocardial infarction, nor have these supplements reduced the risk of recurrent cardiovascular disease after an acute myocardial infarct. Meta-analysis showed an 18% reduction in strokes. The benefit for stroke prevention has been confirmed by a large (>20,000 subjects) randomized prospective study in hypertensive subjects in China. This showed a significant reduction in the first incidence of stroke in subjects receiving enalapril and folic acid compared to enalapril alone. The effect was especially marked in the subjects commencing the prospective trial with the lowest serum folate levels. Venous thrombosis has been reported to be more frequent in folate-deficient or vitamin B₁₂-deficient subjects than in controls and to occur at unusual sites such as cerebral venous sinuses. This tendency was ascribed to raised plasma homocysteine levels in folate or vitamin B₁₂ deficiency.

Malignancy Prophylactic folic acid in pregnancy has been found in some but not all studies to reduce the subsequent incidence of acute lymphoblastic leukemia (ALL) in childhood. A significant negative association has also been found with the *MTHFR* C677T polymorphism and leukemias with mixed lineage leukemia (MLL) translocations, but a positive association was found with hyperdiploidy in infants with ALL or acute myeloid leukemia or with childhood ALL. A second polymorphism in the *MTHFR* gene, A1298C, is also strongly associated with hyperdiploid leukemia. Various positive and negative associations are noted between polymorphisms in folate-dependent enzymes and the incidence of adult ALL. The C677T polymorphism is thought to lead to increased thymidine pools and "better quality" of DNA synthesis by shunting one-carbon groups toward thymidine and purine synthesis. This may explain its reported association with a lower risk for colorectal cancer. Most but not all studies suggest that prophylactic folic acid also protects against colon adenomas. Other tumors that have been associated with folate polymorphisms or status include follicular lymphoma, breast cancer, and gastric cancer. A meta-analysis of 50,000 individuals given folic acid (0.5–40 mg daily) or placebo in cardiovascular or colon

adenoma prevention trials found that folic acid supplementation did not significantly increase or decrease the overall incidence of cancer or of any site-specific cancer during a weighted average scheduled treatment duration of 5.7 years. Because folic acid may “feed” tumors, it probably should be avoided in those with established tumors unless there is severe megaloblastic anemia due to folate deficiency.

HEMATOLOGIC FINDINGS

■ PERIPHERAL BLOOD

Oval macrocytes, usually with considerable anisocytosis and poikilocytosis, are the main feature (Fig. 99-2A). The MCV is usually >100 fL unless a cause of microcytosis (e.g., iron deficiency or thalassemia trait) is present. Some of the neutrophils are hypersegmented (more than five nuclear lobes). There may be leukopenia due to a reduction in granulocytes and lymphocytes, but this is usually $>1.5 \times 10^9/L$. The platelet count may be moderately reduced, rarely to $<40 \times 10^9/L$. The severity of all these changes parallels the degree of anemia. In a nonanemic patient, the presence of a few macrocytes and hypersegmented neutrophils in the peripheral blood may be the only indication of the underlying disorder.

■ BONE MARROW

In a severely anemic patient, the marrow is hypercellular with an accumulation of primitive cells due to selective death by apoptosis of more mature forms. The erythroblast nucleus maintains a primitive appearance despite maturation and hemoglobinization of the cytoplasm. The cells are larger than normoblasts, and an increased number of cells with eccentric lobulated nuclei or nuclear fragments may be present (Fig. 99-2B). Giant and abnormally shaped metamyelocytes and enlarged hyperpolyploid megakaryocytes are characteristic. In severe cases, the accumulation of primitive cells may mimic acute myeloid leukemia, whereas in less anemic patients, the changes in the marrow may be difficult to recognize. The terms *intermediate*, *mild*, and *early* have been used. The term *megaloblastoid* does not mean mildly megaloblastic. It is used to describe cells with both immature-appearing nuclei and defective hemoglobinization and is usually seen in myelodysplasia.

■ CHROMOSOMES

Bone marrow cells, transformed lymphocytes, and other proliferating cells in the body show a variety of changes, including random breaks, reduced contraction, spreading of the centromere, and exaggeration of

secondary chromosomal constrictions and overprominent satellites. Similar abnormalities may be produced by antimetabolite drugs (e.g., cytosine arabinoside, hydroxyurea, and methotrexate) that interfere with either DNA replication or folate metabolism and that also cause megaloblastic appearances.

■ INEFFECTIVE HEMATOPOIESIS

Unconjugated bilirubin accumulates in plasma due to the death of nucleated red cells in the marrow (ineffective erythropoiesis). Other evidence for this includes raised urine urobilinogen, reduced haptoglobins and positive urine hemosiderin, and a raised serum lactate dehydrogenase. A weakly positive direct antiglobulin test due to complement can lead to a false diagnosis of autoimmune hemolytic anemia.

CAUSES OF COBALAMIN DEFICIENCY

Cobalamin deficiency is usually due to malabsorption. The only other cause is inadequate dietary intake.

■ INADEQUATE DIETARY INTAKE

Adults Dietary cobalamin deficiency arises in vegans who omit meat, fish, eggs, cheese, and other animal products from their diet. The largest group in the world consists of Hindus, and it is likely that many millions of Indians are at risk of deficiency of cobalamin on a nutritional basis. Subnormal serum cobalamin levels are found in up to 50% of randomly selected, young, adult Indian vegans, but the deficiency usually does not progress to megaloblastic anemia since the diet of most vegans is not totally lacking in cobalamin and the enterohepatic circulation of cobalamin is intact. Dietary cobalamin deficiency may also arise rarely in nonvegetarian individuals who exist on grossly inadequate diets because of poverty or psychiatric disturbance.

Infants Cobalamin deficiency has been described in infants born to severely cobalamin-deficient mothers. These infants develop megaloblastic anemia at about 3–6 months of age, presumably because they are born with low stores of cobalamin and because they are fed breast milk with low cobalamin content. The babies have also shown growth retardation, impaired psychomotor development, and other neurologic sequelae. MRI shows delayed myelination and atrophy.

■ GASTRIC CAUSES OF COBALAMIN MALABSORPTION

See Tables 99-3 and 99-4.

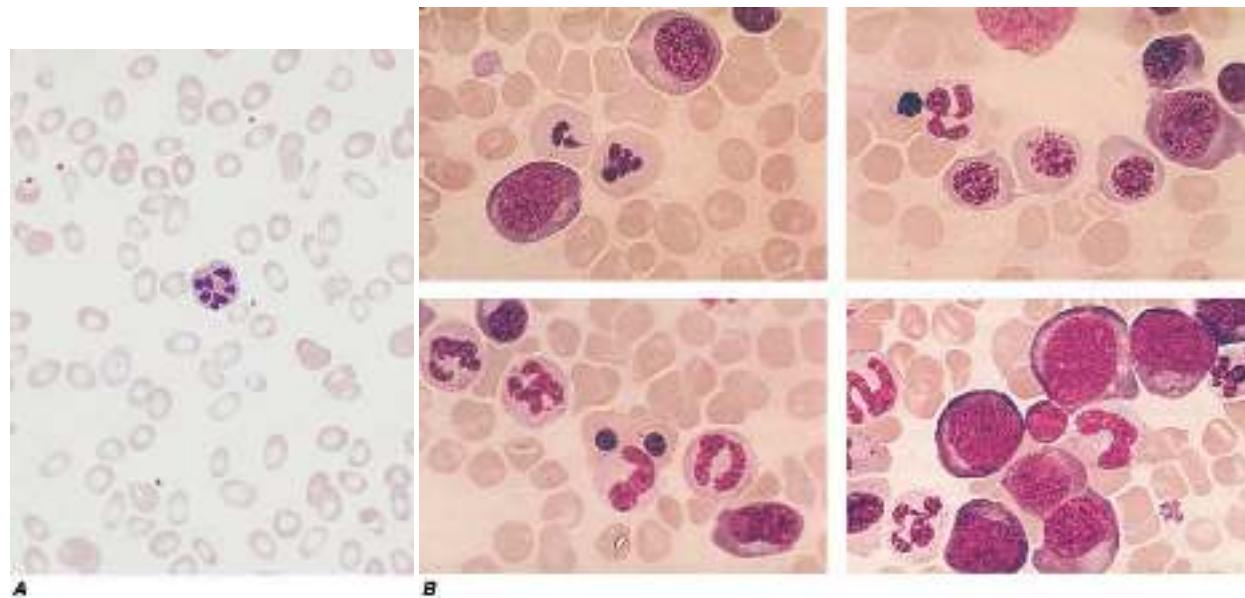


FIGURE 99-2. A. The peripheral blood in severe megaloblastic anemia. B. The bone marrow in severe megaloblastic anemia. (Reprinted from AV Hoffbrand et al [eds]: Postgraduate Haematology, 5th ed. Oxford, UK, Blackwell Publishing, 2005; with permission.)

TABLE 99-3 Causes of Cobalamin Deficiency Sufficiently Severe to Cause Megaloblastic Anemia

NUTRITIONAL	VEGANS
Malabsorption	Pernicious anemia
Gastric causes	Congenital absence of intrinsic factor or functional abnormality Total or partial gastrectomy
Intestinal causes	Intestinal stagnant loop syndrome; jejunal diverticulosis, ileocolic fistula, anatomic blind loop, intestinal stricture, etc. Ileal resection and Crohn's disease Selective malabsorption with proteinuria Tropical sprue Transcobalamin II deficiency Fish tapeworm

Formerly, the pathogenesis of B_{12} malabsorption was distinguishable based on the results of a Schilling test in which a radioactive form of B_{12} was administered orally and its appearance in the urine was a sign of absorption. Radioactive B_{12} is no longer available, and Schilling tests are no longer performed. Other approaches to the differential diagnosis of B_{12} malabsorption are now employed.

Pernicious Anemia PA may be defined as a severe lack of IF due to gastric atrophy. It is a common disease in northern Europeans but occurs in all countries and ethnic groups. It is more frequent in people of African than Asian ancestry. The overall incidence is about 120 per 100,000 population in the United Kingdom (UK). The ratio of incidence in men and women among whites is 1:1.6, and the median age of onset is 70–80 years, with only 10% of patients being <40 years of age. However, in some ethnic groups, notably blacks and Latin Americans, the age at onset of PA is generally lower. The disease occurs more commonly than by chance in close relatives and in persons with other organ-specific autoimmune diseases, for example, thyroid diseases, vitiligo, hypoparathyroidism, type 1 diabetes, and Addison's disease. It is also associated with hypogammaglobulinemia, premature graying or blue eyes, and persons of blood group A. An association with human leukocyte antigen (HLA) 3 has been reported in some but not all series and, in those with endocrine disease, with HLA-B8, - B_{12} , and -BW15. Life expectancy is normal in women once regular treatment has begun. Men had a slightly subnormal life expectancy as a result of a higher incidence of carcinoma of the stomach than in control subjects, but

TABLE 99-4 Malabsorption of Cobalamin May Occur in the Following Conditions but Is Not Usually Sufficiently Severe and Prolonged to Cause Megaloblastic Anemia

Gastric causes	Simple atrophic gastritis (food cobalamin malabsorption) Zollinger-Ellison syndrome Gastric bypass or bariatric surgery Use of proton pump inhibitors
Intestinal causes	Gluten-induced enteropathy Severe pancreatitis HIV infection Radiotherapy Graft-versus-host disease
	Deficiencies of cobalamin, folate, protein, ?riboflavin, ?nicotinic acid
	Therapy with colchicine, para-aminosalicylate, neomycin, slow-release potassium chloride, anticonvulsant drugs, metformin, ^a cytotoxic drugs
	Alcohol

^aIt is now thought that metformin lowers serum vitamin B_{12} level by lowering the level of transcobalamin I.

current data on their life expectancy are unavailable. Gastric output of hydrochloric acid, pepsin, and IF is severely reduced. The serum gastrin level is raised, and serum pepsinogen I levels are low.

Gastric Biopsy A single endoscopic examination is recommended if PA is diagnosed. Gastric biopsy usually shows atrophy of all layers of the body and fundus, with loss of glandular elements, an absence of parietal and chief cells and replacement by mucous cells, a mixed inflammatory cell infiltrate, and perhaps intestinal metaplasia. The infiltrate of plasma cells and lymphocytes contains an excess of CD4 cells. These are directed against gastric H/K-ATPase. The antral mucosa is usually well preserved. *Helicobacter pylori* infection occurs infrequently in PA, but it has been suggested that *H. pylori* gastritis occurs at an early phase of atrophic gastritis and presents in younger patients as iron-deficiency anemia but in older patients as PA. *H. pylori* is suggested to stimulate an autoimmune process directed against parietal cells, with the *H. pylori* infection then being gradually replaced, in some individuals, by an autoimmune process.

Serum Antibodies Two types of IF immunoglobulin G antibody may be found in the sera of patients with PA. The “blocking,” or type I, antibody prevents the combination of IF and cobalamin, whereas the “binding,” or type II, antibody prevents attachment of IF to ileal mucosa. Type I occurs in the sera of 55% of patients, and type II in 35%. IF antibodies cross the placenta and may cause temporary IF deficiency in a newborn infant. Patients with PA also show cell-mediated immunity to IF. Type I antibody has been detected rarely in the sera of patients without PA but with thyrotoxicosis, myxedema, Hashimoto's disease, or diabetes mellitus and in relatives of PA patients. IF antibodies also have been detected in gastric juice in 80% of PA patients. These gastric antibodies may reduce absorption of dietary cobalamin by combining with small amounts of remaining IF.

Parietal cell antibody is present in the sera of almost 90% of adult patients with PA but is frequently present in other subjects. Thus, it occurs in as many as 16% of randomly selected female subjects age >60 years. The parietal cell antibody is directed against the α and β subunits of the gastric proton pump (H^+, K^+ -ATPase).

JUVENILE PERNICIOUS ANEMIA

This usually occurs in older children and resembles PA of adults. Gastric atrophy, achlorhydria, and serum IF antibodies are all present, although parietal cell antibodies are usually absent. About one-half of these patients show an associated endocrinopathy such as autoimmune thyroiditis, Addison's disease, or hypoparathyroidism; in some, mucocutaneous candidiasis occurs.

CONGENITAL INTRINSIC FACTOR DEFICIENCY OR FUNCTIONAL ABNORMALITY

An affected child usually presents with megaloblastic anemia in the first to third year of life; a few have presented as late as the second decade. The child usually has no demonstrable IF but has a normal gastric mucosa and normal secretion of acid. The inheritance is autosomal recessive. Parietal cell and IF antibodies are absent. Variants have been described in which the child is born with IF that can be detected immunologically but is unstable or functionally inactive, unable to bind cobalamin or to facilitate its uptake by ileal receptors.

GASTRECTOMY

After total gastrectomy, cobalamin deficiency is inevitable, and prophylactic cobalamin therapy should be commenced immediately after the operation. After partial gastrectomy, 10–15% of patients also develop this deficiency. The exact incidence and time of onset are most influenced by the size of the resection and the preexisting size of cobalamin body stores.

FOOD COBALAMIN MALABSORPTION

Failure of release of cobalamin from binding proteins in food is believed to be responsible for this condition, which is more common in the elderly. It is associated with low serum cobalamin levels, with or without raised serum levels of MMA and homocysteine. Typically,

these patients have normal cobalamin absorption, as measured with crystalline cobalamin, but show malabsorption when a modified test using food-bound cobalamin is used. It is usually due to mild forms of atrophic gastritis or therapy with proton pump inhibitors. Bariatric surgery is likely to be an increasing cause of this form of B_{12} malabsorption and deficiency. The frequency of progression to severe cobalamin deficiency and the reasons for this progression are not clear.

■ INTESTINAL CAUSES OF COBALAMIN MALABSORPTION

Intestinal Stagnant Loop Syndrome Malabsorption of cobalamin occurs in a variety of intestinal lesions in which there is colonization of the upper small intestine by fecal organisms. This may occur in patients with jejunal diverticulosis, enteroanastomosis, or an intestinal stricture or fistula or with an anatomic blind loop due to Crohn's disease, tuberculosis, or an operative procedure.

Ileal Resection Removal of ≥ 1.2 m of terminal ileum causes malabsorption of cobalamin. In some patients after ileal resection, particularly if the ileocecal valve is incompetent, colonic bacteria may contribute further to the onset of cobalamin deficiency.

Selective Malabsorption of Cobalamin with Proteinuria (Imerslund's Syndrome; Imerslund-Gräsbeck Syndrome; Congenital Cobalamin Malabsorption; Autosomal Recessive Megaloblastic Anemia; MGA1) This autosomal recessive disease is the most common cause of megaloblastic anemia due to cobalamin deficiency in infancy in Western countries. More than 200 cases have been reported with familial clusters in Finland, Norway, the Middle East, and North Africa. The patients secrete normal amounts of IF and gastric acid but are unable to absorb cobalamin. In Finland, impaired synthesis, processing, or ligand binding of cubilin due to inherited mutations is found. In Norway, mutation of the gene for *AMN* has been reported. Other tests of intestinal absorption are normal. Over 90% of these patients show nonspecific proteinuria, but renal function is otherwise normal, and renal biopsy has not shown any consistent renal defect. A few have shown aminoaciduria and congenital renal abnormalities, such as duplication of the renal pelvis.

Tropical Sprue Nearly all patients with acute and subacute tropical sprue show malabsorption of cobalamin; this may persist as the principal abnormality in the chronic form of the disease, when the patient may present with megaloblastic anemia or neuropathy due to cobalamin deficiency. Absorption of cobalamin usually improves after antibiotic therapy and, in the early stages, folic acid therapy.

Fish Tapeworm Infestation The fish tapeworm (*Diphyllobothrium latum*) lives in the small intestine of humans and accumulates cobalamin from food, rendering the cobalamin unavailable for absorption. Individuals acquire the worm by eating raw or partly cooked fish. Infestation is common around the lakes of Scandinavia, Germany, Japan, North America, and Russia. Megaloblastic anemia or cobalamin neuropathy occurs only in those with a heavy infestation.

Gluten-Induced Enteropathy Malabsorption of cobalamin occurs in 30% of untreated patients (presumably those in whom the disease extends to the ileum). Cobalamin deficiency is not severe in these patients and is corrected with a gluten-free diet.

Severe Chronic Pancreatitis In this condition, lack of trypsin is thought to cause dietary cobalamin attached to gastric non-IF (R) binder to be unavailable for absorption. It also has been proposed that in pancreatitis, the concentration of calcium ions in the ileum falls below the level needed to maintain normal cobalamin absorption.

HIV Infection Serum cobalamin levels tend to fall in patients with HIV infection and are subnormal in 10–35% of those with AIDS. Malabsorption of cobalamin not corrected by IF has been shown in some, but not all, patients with subnormal serum cobalamin levels. Cobalamin deficiency sufficiently severe to cause megaloblastic anemia or neuropathy is rare.

Zollinger-Ellison Syndrome Malabsorption of cobalamin has been reported in the Zollinger-Ellison syndrome. It is thought that there is a failure to release cobalamin from R-binding protein due to inactivation of pancreatic trypsin by high acidity, as well as interference with IF binding of cobalamin.

Radiotherapy Both total-body irradiation and local radiotherapy to the ileum (e.g., as a complication of radiotherapy for carcinoma of the cervix) may cause malabsorption of cobalamin.

Graft-versus-Host Disease This commonly affects the small intestine. Malabsorption of cobalamin due to abnormal gut flora, as well as damage to ileal mucosa, is common.

Drugs The drugs that have been reported to cause malabsorption of cobalamin are listed in Table 99-4. However, megaloblastic anemia due to these drugs is rare. It has been suggested that metformin lowers serum B_{12} by lowering TC I level rather than causing malabsorption of B_{12} .

■ ABNORMALITIES OF COBALAMIN METABOLISM

Congenital Transcobalamin II Deficiency or Abnormality Infants with TC II deficiency usually present with megaloblastic anemia within a few weeks of birth. Serum cobalamin and folate levels are normal, but the anemia responds to massive (e.g., 1 mg three times weekly) injections of cobalamin. Some cases show neurologic complications. The protein may be present but functionally inert. Genetic abnormalities found include mutations of an intraexonic cryptic splice site, extensive deletion, single nucleotide deletion, nonsense mutation, and an RNA editing defect. Malabsorption of cobalamin occurs in all cases, and serum immunoglobulins are usually reduced. Failure to institute adequate cobalamin therapy or treatment with folic acid may lead to neurologic damage.

Congenital Methylmalonic Acidemia and Aciduria Infants with this abnormality are ill from birth with vomiting, failure to thrive, severe metabolic acidosis, ketosis, and mental retardation. Anemia, if present, is normocytic and normoblastic. The condition may be due to a functional defect in either mitochondrial methylmalonyl-CoA mutase or its cofactor adocobalamin. Mutations in the methylmalonyl-CoA mutase are not responsive or are only poorly responsive to treatment with cobalamin. A proportion of infants with failure of adocobalamin synthesis respond to cobalamin in large doses. Some children have combined methylmalonic aciduria and homocystinuria due to defective formation of both cobalamin coenzymes. This usually presents in the first year of life with feeding difficulties, developmental delay, microcephaly, seizures, hypotonia, and megaloblastic anemia.

Acquired Abnormality of Cobalamin Metabolism: Nitrous Oxide Inhalation Nitrous oxide (N_2O) irreversibly oxidizes methylcobalamin to an inactive precursor; this inactivates methionine synthase. Megaloblastic anemia has occurred in patients undergoing prolonged N_2O anesthesia (e.g., in intensive care units). A neuropathy resembling cobalamin neuropathy has been described in dentists and anesthetists who are exposed repeatedly to N_2O . Methylmalonic aciduria does not occur as adocobalamin is not inactivated by N_2O .

CAUSES OF FOLATE DEFICIENCY (Table 99-5)

■ NUTRITIONAL

Dietary folate deficiency is common. Indeed, in most patients with folate deficiency, a nutritional element is present. Certain individuals are particularly prone to have diets containing inadequate amounts of folate (Table 99-5). In the United States and other countries where fortification of the diet with folic acid has been adopted, the prevalence of folate deficiency has dropped dramatically and is now almost restricted to high-risk groups with increased folate needs. Nutritional folate deficiency occurs in kwashiorkor and scurvy and in infants with repeated infections or those who are fed solely on goats' milk, which has a low folate content.

TABLE 99-5 Causes of Folate Deficiency**Dietary^a**

Particularly in: old age, infancy, poverty, alcoholism, chronic invalids, and the psychiatrically disturbed; may be associated with scurvy or kwashiorkor

Malabsorption**Major causes of deficiency**

Tropical sprue, gluten-induced enteropathy in children and adults, and in association with dermatitis herpetiformis, specific malabsorption of folate, intestinal megaloblastosis caused by severe cobalamin or folate deficiency

Minor causes of deficiency

Extensive jejunal resection, Crohn's disease, partial gastrectomy, congestive heart failure, Whipple's disease, scleroderma, amyloid, diabetic enteropathy, systemic bacterial infection, lymphoma, sulfasalazine (Salazopyrin)

Excess utilization or loss**Physiologic**

Pregnancy and lactation, prematurity

Pathologic

Hematologic diseases: chronic hemolytic anemias, sickle cell anemia, thalassemia major, myelofibrosis

Malignant diseases: carcinoma, lymphoma, leukemia, myeloma

Inflammatory diseases: tuberculosis, Crohn's disease, psoriasis, exfoliative dermatitis, malaria

Metabolic disease: homocystinuria

Excess urinary loss: congestive heart failure, active liver disease

Hemodialysis, peritoneal dialysis

Antifolate drugs^b

Anticonvulsant drugs (phenytoin, primidone, barbiturates), sulfasalazine

Nitrofurantoin, tetracycline, antituberculosis (less well documented)

Mixed causes

Liver diseases, alcoholism, intensive care units

^aIn severely folate-deficient patients with causes other than those listed under Dietary, poor dietary intake is often present. ^bDrugs inhibiting dihydrofolate reductase are discussed in the text.

■ MALABSORPTION

Malabsorption of dietary folate occurs in tropical sprue and in gluten-induced enteropathy. In the rare congenital recessive syndrome of selective malabsorption of folate due to mutation of the PCFT, there is an associated defect of folate transport into the cerebrospinal fluid, and these patients show megaloblastic anemia, which responds to physiologic doses of folic acid given parenterally but not orally. They also show mental retardation, convulsions, and other central nervous system abnormalities. Minor degrees of malabsorption may also occur after jejunal resection or partial gastrectomy, in Crohn's disease, and in systemic infections, but in these conditions, if severe deficiency occurs, it is usually largely due to poor nutrition. Malabsorption of folate has been described in patients receiving sulfasalazine (Salazopyrin), cholestyramine, and triamterene.

■ EXCESS UTILIZATION OR LOSS

Pregnancy Folate requirements are increased by 200–300 µg to 400 µg daily in a normal pregnancy, partly because of transfer of the vitamin to the fetus but mainly because of increased folate catabolism due to cleavage of folate coenzymes in rapidly proliferating tissues. Megaloblastic anemia due to this deficiency is prevented by prophylactic folic acid therapy. It occurred in 0.5% of pregnancies in the UK and other Western countries before prophylaxis with folic acid, but the incidence is much higher in countries where the general nutritional status is poor.

Prematurity A newborn infant, whether full term or premature, has higher serum and red cell folate concentrations than does an adult. However, a newborn infant's demand for folate has been estimated to

be up to 10 times that of adults on a weight basis, and the neonatal folate level falls rapidly to the lowest values at about 6 weeks of age. The falls are steepest and are liable to reach subnormal levels in premature babies, a number of whom develop megaloblastic anemia responsive to folic acid at about 4–6 weeks of age. This occurs particularly in the smallest babies (<1500 g birth weight) and those who have feeding difficulties or infections or have undergone multiple exchange transfusions. In these babies, prophylactic folic acid should be given.

Hematologic Disorders Folate deficiency frequently occurs in chronic hemolytic anemia, particularly in sickle cell disease, autoimmune hemolytic anemia, and congenital spherocytosis. In these and other conditions of increased cell turnover (e.g., myelofibrosis, malignancies), folate deficiency arises because it is not completely reutilized after performing coenzyme functions.

Inflammatory Conditions Chronic inflammatory diseases such as tuberculosis, rheumatoid arthritis, Crohn's disease, psoriasis, exfoliative dermatitis, bacterial endocarditis, and chronic bacterial infections cause deficiency by reducing the appetite and increasing the demand for folate. Systemic infections also may cause malabsorption of folate. Severe deficiency is virtually confined to the patients with the most active disease and the poorest diet.

Homocystinuria This is a rare metabolic defect in the conversion of homocysteine to cystathione. Folate deficiency occurring in most of these patients may be due to excessive utilization because of compensatory increased conversion of homocysteine to methionine.

Long-Term Dialysis Because folate is only loosely bound to plasma proteins, it is easily removed from plasma by dialysis. In patients with anorexia, vomiting, infections, and hemolysis, folate stores are particularly likely to become depleted. Routine folate prophylaxis is now given.

Congestive Heart Failure and Liver Disease Excess urinary folate losses of >100 µg per day may occur in some of these patients. The explanation appears to be release of folate from damaged liver cells.

■ ANTIFOLATE DRUGS

A large number of epileptics who are receiving long-term therapy with phenytoin or primidone, with or without barbiturates, develop low serum and red cell folate levels. The exact mechanism is unclear. Alcohol may also be a folate antagonist, as patients who are drinking spirits may develop megaloblastic anemia that will respond to normal quantities of dietary folate or to physiologic doses of folic acid only if alcohol is withdrawn. Macrocytosis of red cells is associated with chronic alcohol intake even when folate levels are normal. Inadequate folate intake is the major factor in the development of deficiency in spirit-drinking alcoholics. Beer is relatively folate-rich in some countries, depending on the technique used for brewing.

The drugs that inhibit DHF reductase include methotrexate, pyrimethamine, and trimethoprim. Methotrexate has the most powerful action against the human enzyme, whereas trimethoprim is most active against the bacterial enzyme and is likely to cause megaloblastic anemia only when used in conjunction with sulfamethoxazole in patients with preexisting folate or cobalamin deficiency. The activity of pyrimethamine is intermediate. The antidote to these drugs is folinic acid (5-formyl-THF).

■ CONGENITAL ABNORMALITIES OF FOLATE METABOLISM

Some infants with congenital defects of folate enzymes (e.g., cyclohydrolase or methionine synthase) have had megaloblastic anemia.

DIAGNOSIS OF COBALAMIN AND FOLATE DEFICIENCIES

The diagnosis of cobalamin or folate deficiency has traditionally depended on the recognition of the relevant abnormalities in the peripheral blood and analysis of the blood levels of the vitamins.

Serum Cobalamin This is measured by an automated enzyme-linked immunosorbent assay (ELISA) or competitive-binding luminescence assay (CBLA). Normal serum levels range from 118–148 pmol/L (160–200 ng/L) to 738 pmol/L (1000 ng/L). In patients with megaloblastic anemia due to cobalamin deficiency, the level is usually <74 pmol/L (100 ng/L). In general, the more severe the deficiency, the lower is the serum cobalamin level. In patients with spinal cord damage due to the deficiency, levels are very low even in the absence of anemia. Values between 74 and 148 pmol/L (100 and 200 ng/L) are regarded as borderline. They may occur, for instance, in pregnancy, in patients with megaloblastic anemia due to folate deficiency. They may also be due to heterozygous, homozygous, or compound heterozygous mutations of the gene *TCN1* that codes for HC (TC I). There is then no clinical or hematologic abnormality. The serum cobalamin level is sufficiently robust, cost-effective, and most convenient to rule out cobalamin deficiency in the vast majority of patients suspected of having this problem. However, problems have arisen with commercial CBLA assays involving IF in PA patients with intrinsic antibodies in serum. These antibodies may cause false normal serum vitamin B_{12} levels in up to 50% of cases tested. Where clinical indications of PA are strong, a normal serum vitamin B_{12} does not rule out the diagnosis. Serum MMA levels will be elevated in untreated PA (see below).

Folate deficiency, TC I (HC) deficiency, oral contraceptives, and multiple myeloma have all been associated with low serum B_{12} levels that do not indicate B_{12} deficiency. On the other hand, high serum B_{12} levels are usually due to raised serum TC I levels and can be due to the presence of liver, renal, or myeloproliferative diseases or to cancer of the breast, colon, or liver.

Serum Methylmalonate and Homocysteine In patients with cobalamin deficiency sufficient to cause anemia or neuropathy, the serum MMA level is raised. Sensitive methods for measuring MMA and homocysteine in serum have been introduced and recommended for the early diagnosis of cobalamin deficiency, even in the absence of hematologic abnormalities or subnormal levels of serum cobalamin. Serum MMA levels fluctuate, however, in patients with renal failure. Mildly elevated serum MMA and/or homocysteine levels occur in up to 30% of apparently healthy volunteers, with serum cobalamin levels up to 258 pmol/L (350 ng/L) and normal serum folate levels; 15% of elderly subjects, even with cobalamin levels >258 pmol/L (>350 ng/L), have this pattern of raised metabolite levels. These findings bring into question the exact cutoff points for normal MMA and homocysteine levels. It is also unclear at present whether these mildly raised metabolite levels have clinical consequences.

Serum homocysteine is raised in both early cobalamin and folate deficiency but may be raised in other conditions, for example, chronic renal disease, alcoholism, smoking, pyridoxine deficiency, hypothyroidism, and therapy with steroids, cyclosporine, and other drugs. Levels are also higher in serum than in plasma, in men than in premenopausal women, in women taking hormone replacement therapy or in oral contraceptive users, and in elderly persons and patients with several inborn errors of metabolism affecting enzymes in transsulfuration pathways of homocysteine metabolism. Thus, homocysteine levels must be carefully interpreted for diagnosis of cobalamin or folate deficiency.

Tests for the Cause of Cobalamin Deficiency Only vegans, strict vegetarians, or people living on a totally inadequate diet will become vitamin B_{12} deficient because of inadequate intake. Studies of cobalamin absorption once were widely used, but difficulty in obtaining radioactive cobalamin and ensuring that IF preparations are free of viruses has made these tests obsolete. Tests to diagnose PA include serum gastrin, which is raised; serum pepsinogen I, which is low in PA (90–92%) but also in other conditions; and gastric endoscopy. Tests for IF and parietal cell antibodies are also used, as well as tests for individual intestinal diseases.

Patients with atrophic gastritis may also have sufficient occult gastrointestinal blood loss to have iron deficiency as well as vitamin B_{12}

deficiency. Iron deficiency may blunt the development of macrocytosis when iron deficiency and B_{12} deficiency coexist. Iron deficiency is much more common than B_{12} deficiency, and in people older than age 60 years, B_{12} deficiency may accompany iron deficiency in 15–20% of cases. Thus, patients diagnosed with iron-deficiency anemia should have B_{12} levels assessed, and those diagnosed with B_{12} deficiency should have their iron status assessed.

■ FOLATE DEFICIENCY

Serum Folate This is also measured by an ELISA technique. In most laboratories, the normal range is from 11 nmol/L (2 μ g/L) to 82 nmol/L (15 μ g/L). The serum folate level is low in all folate-deficient patients. It also reflects recent diet. Because of this, serum folate may be low before there is hematologic or biochemical evidence of deficiency. Serum folate rises in severe cobalamin deficiency because of the block in conversion of MTHF to THF inside cells; raised levels have also been reported in the intestinal stagnant loop syndrome due to absorption of bacterially synthesized folate.

Red Cell Folate The red cell folate assay is a valuable test of body folate stores. It is less affected than the serum assay by recent diet and traces of hemolysis. In normal adults, concentrations range from 880 to 3520 μ mol/L (160–640 μ g/L) of packed red cells. Subnormal levels occur in patients with megaloblastic anemia due to folate deficiency but also in nearly two-thirds of patients with severe cobalamin deficiency. False-normal results may occur if a folate-deficient patient has received a recent blood transfusion or if a patient has a raised reticulocyte count. Serum homocysteine assay is discussed earlier.

Tests for the Cause of Folate Deficiency The diet history is important. Tests for transglutaminase antibodies are performed to confirm or exclude celiac disease. If positive, duodenal biopsy is needed. An underlying disease causing increased folate breakdown should also be excluded.

TREATMENT

Cobalamin and Folate Deficiency

It is usually possible to establish which of the two deficiencies, folate or cobalamin, is the cause of the anemia and to treat only with the appropriate vitamin. In patients who enter the hospital severely ill, however, it may be necessary to treat with both vitamins in large doses once blood samples have been taken for cobalamin and folate assays and a bone marrow biopsy has been performed (if deemed necessary). Transfusion is usually unnecessary and inadvisable. If it is essential, packed red cells should be given slowly, one or two units only, with the usual treatment for heart failure if present. Potassium supplements have been recommended to obviate the danger of the hypokalemia but are not necessary. Occasionally, an excessive rise in platelets occurs after 1–2 weeks of therapy. Antiplatelet therapy, for example, aspirin, should be considered if the platelet count rises to $>800 \times 10^9/L$.

COBALAMIN DEFICIENCY

It is usually necessary to treat patients who have developed cobalamin deficiency with lifelong regular cobalamin injections. In the UK, the form used is hydroxocobalamin; in the United States, cyanocobalamin. In a few instances, the underlying cause of cobalamin deficiency can be permanently corrected, for example, fish tapeworm, tropical sprue, or an intestinal stagnant loop that is amenable to surgery. The indications for starting cobalamin therapy are a well-documented megaloblastic anemia or other hematologic abnormalities and neuropathy due to the deficiency. Patients with borderline serum cobalamin levels but no hematologic or other abnormality may be followed to make sure that the cobalamin deficiency does not progress (see below). If malabsorption of cobalamin or rises in serum MMA levels have been demonstrated, however, these patients also should be given regular maintenance cobalamin

therapy. Cobalamin should be given routinely to all patients who have had a total gastrectomy or ileal resection. Patients who have undergone gastric reduction for control of obesity or who are receiving long-term treatment with proton pump inhibitors should be screened and, if necessary, given cobalamin replacement.

Replenishment of body stores should be complete with six 1000- μ g IM injections of hydroxocobalamin given at 3- to 7-day intervals. More frequent doses are usually used in patients with cobalamin neuropathy, but there is no evidence that they produce a better response. Allergic reactions are rare and may require desensitization or antihistamine or glucocorticoid cover. For maintenance therapy, 1000 μ g hydroxocobalamin IM once every 3 months is satisfactory. Because of the poorer retention of cyanocobalamin, protocols generally use higher and more frequent doses, for example, 1000 μ g IM, monthly, for maintenance treatment.

Because a small fraction of cobalamin can be absorbed passively through mucous membranes even when there is complete failure of physiologic IF-dependent absorption, large daily oral doses (1000–2000 μ g) of cyanocobalamin are used in PA for replacement (especially in Canada and Sweden) and maintenance of normal cobalamin status in, for example, food malabsorption of cobalamin. Sublingual therapy has also been proposed for those in whom injections are difficult because of a bleeding tendency and who may not tolerate oral therapy. If oral therapy is used, it is important to monitor compliance, particularly with elderly, forgetful patients. This author prefers parenteral therapy for initial treatment, particularly in severe anemia or if a neuropathy is present, and for maintenance in PA. Oral B_{12} therapy even with low doses of 50 μ g daily may have a larger role in treating food malabsorption of B_{12} .

For treatment of patients with subnormal serum vitamin B_{12} levels with a normal MCV and no hypersegmentation of neutrophils, a negative IF antibody test in the absence of tests of B_{12} absorption is problematic. Some (perhaps 15%) cases may be due to TC I (HC) deficiency. Homocysteine and/or MMA measurements may help, but in the absence of these tests and with otherwise normal gastrointestinal function, repeat serum B_{12} assay after 6–12 months may help one decide whether to start cobalamin therapy.

Vitamin B_{12} injections are used in a wide variety of diseases, often neurologic, despite normal serum B_{12} and folate levels and a normal blood count and in the absence of randomized, double-blind, controlled trials. These conditions include multiple sclerosis and chronic fatigue syndrome/myalgic encephalomyelitis (ME). It seems probable that any benefit is due to the placebo effect of a usually painless, pink injection. In ME, oral B_{12} therapy, despite providing equally large amounts of B_{12} , has not been beneficial, supporting the view of the effect of the injections being placebo only.

FOLATE DEFICIENCY

Oral doses of 5–15 mg of folic acid daily are satisfactory, as sufficient folate is absorbed from these extremely large doses even in patients with severe malabsorption. The length of time therapy must be continued depends on the underlying disease. It is customary to continue therapy for about 4 months, when all folate-deficient red cells will have been eliminated and replaced by new folate-replete populations.

Before large doses of folic acid are given, cobalamin deficiency must be excluded and, if present, corrected; otherwise, cobalamin neuropathy may develop despite a response of the anemia of cobalamin deficiency to folate therapy. Studies in the United States, however, suggest that there is no increase in the proportion of individuals with low serum cobalamin levels and no anemia since food fortification with folic acid, but it is unknown if there has been a change in incidence of cobalamin neuropathy.

Long-term folic acid therapy is required when the underlying cause of the deficiency cannot be corrected and the deficiency is likely to recur, for example, in chronic dialysis or hemolytic anemias. It may also be necessary in gluten-induced enteropathy that does not respond to a gluten-free diet. Where mild but chronic folate deficiency occurs, it is preferable to encourage improvement

in the diet after correcting the deficiency with a short course of folic acid. In any patient receiving long-term folic acid therapy, it is important to measure the serum cobalamin level at regular (e.g., once-yearly) intervals to exclude the coincidental development of cobalamin deficiency.

Folinic Acid (5-Formyl-THF) This is a stable form of fully reduced folate. It is given orally or parenterally to overcome the toxic effects of methotrexate or other DHF reductase inhibitors, for example, trimethoprim or cotrimoxazole.

PROPHYLACTIC FOLIC ACID

Prophylactic folic acid is used in chronic dialysis patients and in parenteral feeds. Prophylactic folic acid has been used to reduce homocysteine levels to prevent cardiovascular disease and for cognitive function in the elderly, but there are no firm data to show any benefit.

Pregnancy In over 70 countries (but none in Europe), food is fortified with folic acid (in grain or flour) to reduce the risk of NTDs. Nevertheless, folic acid, 400 μ g daily, should be given as a supplement before and throughout pregnancy to prevent megaloblastic anemia and reduce the incidence of NTDs, even in countries with fortification of the diet. The levels of fortification provide up to 400 μ g daily on average in Chile, but in most countries, it is nearer to 200 μ g, so periconceptual folic acid is still needed. Most if not all the folic acid used in fortification and eaten over three meals a day will be converted during absorption to methyltetrahydrofolate. This compound will not correct the anemia in B_{12} deficiency. Studies in early pregnancy show significant lack of compliance with the folic acid supplements, emphasizing the benefit of food fortification. Supplemental folic acid reduces the incidence of birth defects in babies born to diabetic mothers. In women who have had a previous fetus with an NTD, a dose of 5 mg daily is recommended when pregnancy is contemplated and throughout the subsequent pregnancy.

Infancy and Childhood The incidence of folate deficiency is so high in the smallest premature babies during the first 6 weeks of life that folic acid (e.g., 1 mg daily) should be given routinely to those weighing <1500 g at birth and to larger premature babies who require exchange transfusions or develop feeding difficulties, infections, or vomiting and diarrhea.

The World Health Organization currently recommends routine supplementation with iron and folic acid in children in countries where iron deficiency is common and child mortality, largely due to infectious diseases, is high. However, some studies suggest that in areas where malaria rates are high, this approach may increase the incidence of severe illness and death. Even where malaria is rare, there appears to be no survival benefit.

MEGALOBLASTIC ANEMIA NOT DUE TO COBALAMIN OR FOLATE DEFICIENCY OR ALTERED METABOLISM

This may occur with many antimetabolic drugs (e.g., hydroxyurea, cytosine arabinoside, 6-mercaptopurine) that inhibit DNA replication. Antiviral nucleoside analogues used in treatment of HIV infection may also cause macrocytosis and megaloblastic marrow changes. In the rare disease orotic aciduria, two consecutive enzymes in purine synthesis are defective. The condition responds to therapy with uridine, which bypasses the block. In thiamine-responsive megaloblastic anemia, there is a genetic defect in the high-affinity thiamine transport (*SLC19A2*) gene. This causes defective RNA ribose synthesis through impaired activity of transketolase, a thiamine-dependent enzyme in the pentose cycle. This leads to reduced nucleic acid production. It may be associated with diabetes mellitus and deafness and the presence of many ringed sideroblasts in the marrow. The explanation is unclear for megaloblastic changes in the marrow in some patients with acute myeloid leukemia and myelodysplasia.

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Hemolytic Anemias

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DEFINITIONS

A finite life span is a distinct characteristic of red cells. Hence, a logical, time-honored classification of anemias is in three groups: (1) decreased production of red cells, (2) increased destruction of red cells, and (3) acute blood loss. Decreased production is covered in [Chaps. 97, 98, and 102](#); acute blood loss in [Chap. 101](#); increased destruction is covered in this chapter.

All patients who are anemic as a result of either increased destruction of red cells or acute blood loss have one important element in common: the anemia results from overconsumption of red cells from the peripheral blood, whereas the supply of cells from the bone marrow is normal (indeed, it is usually increased). However, with blood loss, as in acute hemorrhage, the red cells are physically lost from the body itself; this is fundamentally different from destruction of red cells *within* the body, as in hemolytic anemias (HAs).

With respect to primary etiology, HAs may be *inherited* or *acquired*; from a clinical point of view, they may be more *acute* or more *chronic*, and they may vary from mild to very severe; the site of hemolysis may be predominantly *intravascular* or *extravascular*. With respect to mechanisms, HAs may be due to *intracorporeal* causes or to *extracorporeal* causes ([Table 100-1](#)). But before reviewing the individual types

TABLE 100-1 Classification of Hemolytic Anemias^a

	INTRACORPOERAL DEFECTS	EXTRACORPOERAL FACTORS
Inherited	Hemoglobinopathies Enzymopathies Membrane-cytoskeletal defects	Familial (atypical) hemolytic-uremic syndrome
Acquired	Paroxysmal nocturnal hemoglobinuria (PNH)	Mechanical destruction (microangiopathic) Toxic agents Drugs Infectious Autoimmune

^aHeredity causes correlate with intracorporeal defects because these defects are due to inherited mutations; the one exception is PNH because the defect is due to an acquired somatic mutation. Conversely, acquired causes correlate with extracorporeal factors because mostly these factors are exogenous; the one exception is familial hemolytic-uremic syndrome (HUS; often referred to as atypical HUS) because here an inherited abnormality permits complement activation triggered by exogenous factors, to become excessive, with bouts of production of membrane attack complex capable of destroying normal red cells. Interestingly, in both PNH and aHUS hemolysis is complement-mediated.

of HA, it is appropriate to consider what general features they have in common, in terms of clinical aspects and pathophysiology.

GENERAL CLINICAL AND LABORATORY FEATURES

The clinical presentation of a patient with anemia is greatly influenced in the first place by whether the onset is abrupt or gradual and HAs are no exception. A patient with autoimmune HA or with favism may be a medical emergency, whereas a patient with mild hereditary spherocytosis (HS) or with cold agglutinin disease (CAD) may be diagnosed after years. This is due in large measure to the remarkable ability of the body to adapt to anemia when it is slowly progressing ([Chap. 63](#)).

What differentiates HAs from other anemias is that the patient has signs and symptoms arising directly from hemolysis ([Table 100-2](#)). At the clinical level, the main sign is *jaundice*; in addition, the patient may report discoloration of the urine. In many cases of HA, the spleen is enlarged because it is a preferential site of hemolysis; and in some cases, the liver may be enlarged as well. In all severe congenital forms of HA, there may also be skeletal changes due to overactivity of the bone marrow: they are never as severe as in thalassemia major because there is less ineffective erythropoiesis, or none at all.

The laboratory features of HA are related to (i) hemolysis per se, and (ii) the erythropoietic response of the bone marrow. In most cases hemolysis is largely extravascular, and it produces an increase in unconjugated bilirubin and aspartate aminotransferase (AST) in the serum; urobilinogen will be increased in both urine and stool. If hemolysis is mainly intravascular, the telltale sign is hemoglobinuria (often associated with hemosiderinuria); in the serum there is free hemoglobin, lactate dehydrogenase (LDH) is increased, and haptoglobin is reduced. In contrast, the serum bilirubin level may be normal

TABLE 100-2 Features Common to Most Patients with a Hemolytic Disorder

General examination	Jaundice, pallor
Other physical findings	Spleen may be enlarged; bossing of skull in severe congenital cases
Hemoglobin level	From normal to severely reduced
MCV, MCH	Usually increased
Reticulocytes	Usually increased
Bilirubin	Almost always increased (mostly unconjugated)
LDH	Increased (up to 10x normal with intravascular hemolysis)
Haptoglobin	Reduced to absent if hemolysis is at least in part intravascular

Abbreviations: LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

or only mildly elevated. The main sign of the erythropoietic response by the bone marrow is an increase in reticulocytes (a test all too often neglected in the initial workup of a patient with anemia). Usually the increase will be reflected in both the percentage of reticulocytes (the more commonly quoted figure) and in the absolute reticulocyte count (the more definitive parameter). The increased number of reticulocytes is associated with an increased mean corpuscular volume (MCV) in the blood count. On the blood smear, this is reflected in the presence of macrocytes; there is also polychromasia, and sometimes one sees nucleated red cells. In most cases, a bone marrow aspirate is not necessary in the diagnostic workup; if it is done, it will show erythroid hyperplasia. In practice, once an HA is suspected, specific tests will usually be required for a definitive diagnosis of a specific type of HA.

■ GENERAL PATHOPHYSIOLOGY

The mature red cell is the product of a developmental pathway that brings the phenomenon of differentiation to an extreme. An orderly sequence of events produces synchronous changes, whereby the gradual accumulation of a huge amount of hemoglobin in the cytoplasm (to a final level of 340 g/L, i.e., about 5 mM) goes hand in hand with the gradual loss of cellular organelles and of biosynthetic abilities. In the end, the erythroid cell undergoes a process that has features of apoptosis, including nuclear pyknosis and eventually extrusion of the nucleus. However, the final result is more altruistic than suicidal; the cytoplasmic body, instead of disintegrating, is now able to provide oxygen to all cells in the human organism for some remaining 120 days of the red cell life span.

As a result of this unique process of differentiation and maturation, intermediary metabolism is drastically curtailed in mature red cells (Fig. 100-1); for instance, cytochrome-mediated oxidative phosphorylation has been lost with the loss of mitochondria (through a process of physiologic autophagy); therefore, there is no backup to anaerobic glycolysis, which in the red cell is the only provider of adenosine triphosphate (ATP). Also, the capacity of making protein has been lost with the loss of ribosomes. This places the cell's limited metabolic apparatus at risk, because if any protein component deteriorates, it cannot be replaced, as it would be in most other cells; and in fact, the activity of most enzymes gradually decreases as red cells age. At the same time, during their long time in circulation, various red cell components inevitably accumulate damage and become physically denser. The anion exchanger known as band 3 is the most abundant protein in the red cell membrane (Fig. 100-2 and Table 100-3), with about 1.2 million molecules per red cell. As red cells age and become denser, probability is increased that a region of the band 3 molecule becomes exposed on the cell surface and contributes to creating an antigenic site recognizable by low-avidity naturally occurring anti-band 3 IgG antibodies. This process might be enhanced by the clustering of band 3 molecules favored by the antibody itself and by the binding of hemichromes arising from hemoglobin degradation. Senescent red cells thus become opsonized, and this is the signal for phagocytosis by macrophages in the spleen, in the liver, and elsewhere. This process may become accelerated in various ways in HA.

Another consequence of the relative simplicity of red cells is that they have a limited range of ways to manifest distress under hardship; in essence, any sort of metabolic failure will eventually lead either to structural damage to the membrane or to failure of the cation pump. In either case, the life span of the red cell is reduced, which is the definition of a *hemolytic disorder*. If the rate of red cell destruction exceeds the capacity of the bone marrow to produce more red cells, the hemolytic disorder will manifest as HA.

Thus, the essential pathophysiologic process common to all HAs is an increased red cell turnover; in many HAs, this is due at least in part to an acceleration of the senescence process described above. The gold standard for proving that the life span of red cells is reduced (compared to the normal value of about 120 days) is a *red cell survival study*, which can be carried out by labeling the red cells with ^{51}Cr and measuring the fall in radioactivity over several days or weeks (this classic test can now be replaced by a methodology using the nonradioactive isotope ^{15}N). If the hemolytic event is transient, it does not usually cause any long-term

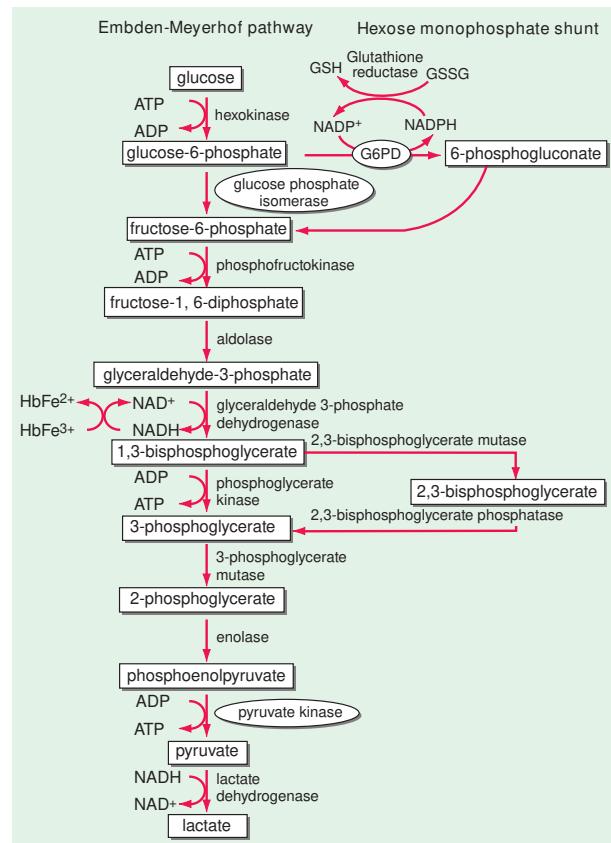


FIGURE 100-1 Red blood cell (RBC) metabolism. The Embden-Meyerhof pathway (glycolysis) generates ATP required for cation transport and for membrane maintenance. The generation of NADH maintains hemoglobin iron in a reduced state. The hexose monophosphate shunt generates NADPH that is used to reduce glutathione, which protects the red cell against oxidant stress; the 6-phosphogluconate, after decarboxylation, can be recycled via pentose sugars to glycolysis. Regulation of the 2,3-bisphosphoglycerate level is a critical determinant of oxygen affinity of hemoglobin. Enzyme deficiency states in order of prevalence: glucose-6-phosphate dehydrogenase (G6PD) > pyruvate kinase > glucose-6-phosphate isomerase > rare deficiencies of other enzymes in the pathway. The more common enzyme deficiencies are encircled.

consequences, except for an increased requirement for erythropoietic factors, particularly folic acid. However, if hemolysis is recurrent or persistent, the increased bilirubin production favors the formation of gallstones. If a considerable proportion of hemolysis takes place in the spleen, as is often the case, splenomegaly may become increasingly a feature, and hypersplenism may develop, with consequent neutropenia and/or thrombocytopenia.

The increased red cell turnover has important consequences. In normal subjects, the iron from effete red cells is very efficiently recycled by the body; however, with chronic intravascular hemolysis, the persistent hemoglobinuria will cause considerable iron loss, needing replacement. With chronic extravascular hemolysis, the opposite problem, iron overload, is more common, especially if the patient needs frequent blood transfusions. Even without blood transfusion, when erythropoiesis is massively increased, the release of erythroferrone from erythroid cells suppresses hepcidin, causing increased iron absorption. In the long run, in the absence of iron-chelation therapy, iron overload will cause secondary hemochromatosis; this will cause damage particularly to the liver, eventually leading to cirrhosis; and to the heart muscle, eventually causing heart failure.

Compensated Hemolysis versus Hemolytic Anemia Red cell destruction is a potent stimulus for erythropoiesis, which is mediated

by erythropoietin (EPO) produced by the kidney. This mechanism is so effective that in many cases the increased output of red cells from the bone marrow can fully balance an increased destruction of red cells. In such cases, we say that hemolysis is *compensated*. The pathophysiology of compensated hemolysis is similar to what we have just described, except there is no anemia. This notion is important from the diagnostic point of view, because a patient with a hemolytic condition, even an inherited one, may present without anemia; and it is also important from the point of view of management because compensated hemolysis may become “decompensated,” i.e., anemia may suddenly appear in certain circumstances, for instance in pregnancy, folate deficiency, or renal failure interfering with adequate EPO production. Another general feature of chronic HAs is seen when any intercurrent condition, such as an acute infection, depresses erythropoiesis. When this happens, in view of the increased rate of red cell turnover, the effect will be predictably much more marked than in a person who does not have hemolysis. The most dramatic example is infection by parvovirus B19, which may cause a rather precipitous fall in hemoglobin—an occurrence sometimes referred to as *aplastic crisis*.

■ INHERITED HEMOLYTIC ANEMIAS

The red cell has three essential components: (1) hemoglobin, (2) the membrane-cytoskeleton complex, and (3) the metabolic machinery necessary to keep hemoglobin and the membrane-cytoskeleton complex in working order. Diseases caused by inherited abnormalities of hemoglobin, or hemoglobinopathies, are covered in *Chap. 98*. Here we will deal with diseases of the other two components.

Hemolytic Anemias due to Abnormalities of the Membrane-Cytoskeleton Complex The detailed architecture of the red cell membrane is complex, but its basic design is relatively simple (*Fig. 100-2*). The lipid bilayer incorporates phospholipids and cholesterol, and it is spanned by a number of proteins that have their hydrophobic transmembrane domain(s) embedded in the membrane; most of these

proteins also extend to both the outside (extracellular domains) and the inside of the cell (cytoplasmic domains). Other proteins are tethered to the membrane through a glycosylphosphatidylinositol (GPI) anchor; these have only an extracellular domain. Membrane proteins include energy-dependent ion transporters, ion channels, receptors for complement components, and receptors for other ligands. The most abundant red cell membrane proteins are glycophorins and the so-called band 3, an anion transporter that is an integral membrane protein. The extracellular domains of many of these proteins are heavily glycosylated, and they carry antigenic determinants that correspond to blood groups. Underneath the membrane, and tangential to it, is a network of other proteins that make up the cytoskeleton. The main cytoskeletal protein is the spectrin tetramer, consisting of a head-to-head association of two α -spectrin- β -spectrin heterodimers. The cytoskeleton is linked to the membrane through the *ankyrin complex* (that includes also band 4.2) and the *junctional complex* (that includes adducin and band 4.1) (*Fig. 100-2*). These multiprotein complexes make membrane and cytoskeleton intimately connected to each other, thus supporting membrane stability and at the same time providing the erythrocyte with the important property of deformability.

The membrane-cytoskeleton complex has essentially three functions: It is an envelope for the red cell cytoplasm; it maintains the normal red cell shape; it provides cross-membrane transport of electrolytes and of metabolites such as glucose and amino acids. In the membrane-cytoskeleton complex, the individual components are so intimately associated with each other that an abnormality of almost any of them will be disturbing or disruptive, causing mechanical instability of the membrane and/or reduced red cell deformability, ultimately causing hemolysis. These abnormalities are almost invariably inherited mutations; thus diseases of the membrane-cytoskeleton complex belong to the category of inherited HAs. Before the red cells lyse, they often exhibit more or less specific changes that alter the normal biconcave disk shape. Thus, the majority of the diseases in this group have been known for over a century as hereditary spherocytosis (HS) and hereditary elliptocytosis (HE). More recently a third morphologic entity, whereby on a blood smear the round-shaped central pallor of a red cell is replaced by a linear-shaped central pale area, has earned the name *stomatocytosis*; because this abnormal shape is related to abnormalities of channel molecules, the underlying disorders are also referred to as *channelopathies*. From an understanding of the molecular basis of these disorders, it has emerged (*Table 100-3*) that, although these disorders are predominantly monogenic, no one-to-one correlation exists between a certain gene and a certain disorder. Rather, what has been regarded as a single disorder (e.g., HS) can arise through mutation of one of several genes; conversely, what have been regarded as different disorders can arise through different mutations of the very same gene (*Fig. 100-3*).

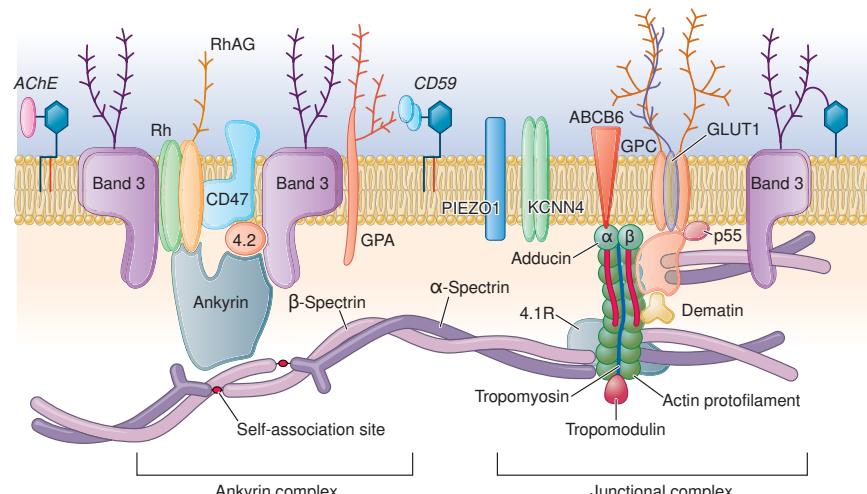


FIGURE 100-2 The red cell membrane and cytoskeleton. Within the membrane lipid bilayer several integral membrane proteins are shown: band 3 (anion exchanger 1 [AE1]) is the most abundant. *PIEZ01* is a mechanoreceptor, *KCNN4*, a Ca^{2+} activated K^+ channel, and *ABCB6* is an ion channel: they are important in the regulation of the red cell volume. Other proteins, e.g., acetylcholinesterase (AChE) and the two complement-regulatory proteins CD59 and CD55, are tethered to the membrane through the glycosylphosphatidylinositol (GPI) anchor; in these cases the entire polypeptide chain is extracellular. Many of the membrane proteins bear polypeptide and/or carbohydrate red cell antigens. Underneath the membrane, the α - β spectrin dimers, that associate head-to-head into tetramers, together with actin and other proteins, form most of the cytoskeleton. The *ankyrin complex*, that also involves the band 4.2 protein, and the *junctional complex*, that involves the band 4.1 protein and dematin, connect the membrane to the cytoskeleton. The ankyrin complex provides mainly radial (also called vertical) connections; the junctional complex provides mainly tangential (also called horizontal) connections: pathogenic changes in the former can cause spherocytosis, whereas pathogenic changes in the latter can cause elliptocytosis; pathogenic changes in spectrin can cause either. Branched lines symbolize carbohydrate moiety of proteins. The various molecules are obviously not drawn to the same scale. Additional explanations are found in the text. (Reproduced with permission from N Young et al: *Clinical Hematology*. Philadelphia, Elsevier, 2006.)

HEREDITARY SPHEROCYTOSIS This is most common among this group of HAs, with an estimated prevalence of 1:2000–1:5000 in populations of European ancestry. Its identification is credited to Minkowsky and Chauffard, who, at the end of the nineteenth century, reported families who had spherocytes in their peripheral blood (*Fig. 100-4A*). In vitro studies revealed that the red cells were abnormally susceptible to lysis in hypotonic media; indeed, the presence of

TABLE 100-3 Inherited Diseases of the Red Cell Membrane-Cytoskeleton Complex

GENE	CHROMOSOMAL LOCATION	PROTEIN PRODUCED	DISEASE(S) WITH CERTAIN MUTATIONS (INHERITANCE)	COMMENTS
SPTA1	1q22-q23	α -Spectrin	HS (recessive) HE (dominant)	Rare Mutations of this gene account for about 65% of HE. More severe forms may be due to coexistence of an otherwise silent mutant allele.
SPTB	14q23-q24.1	β -Spectrin	HS (dominant) HE (dominant)	Rare Mutations of this gene account for about 30% of HE, including some severe forms.
ANK1	8p11.2	Ankyrin	HS (dominant)	May account for majority of HS.
SLC4A1	17q21	Band 3; also known as AE (anion exchanger) or AE1	HS (dominant) Southeast Asia ovalocytosis (dominant) Stomatocytosis (cryohydrocytosis)	Mutations of this gene may account for about 25% of HS. Polymorphic mutation (deletion of nine amino acids); in heterozygotes clinically asymptomatic and protective against <i>Plasmodium falciparum</i> . Certain specific missense mutations shift protein function from anion exchanger to cation conductance.
EPB41	1p33-p34.2	Band 4.1	HE (dominant)	Mutations of this gene account for about 5% of HE, mostly with prominent morphology but little/no hemolysis in heterozygotes; severe hemolysis in homozygotes.
EPB42	15q15-q21	Band 4.2	HS (recessive)	Mutations of this gene account for about 3% of HS.
RHAG	6p21.1-p11	Rhesus-associated glycoprotein	Chronic nonspherocytic hemolytic anemia (recessive)	Very rare; associated with total loss of all Rh antigens. One specific mutation in this gene entails loss of stomatin from the cell membrane, causing overhydrated stomatocytosis.
PIEZ01	16q23-q24	PIEZ01 (mechanosensitive ion channel component 1 channel)	Dehydrated hereditary stomatocytosis (dominant)	Also known as xerocytosis with pseudohyperkalemia. Patients may present with perinatal edema.
KCNN4	19q13.31	KCNN4 Intermediate conductance calcium-activated potassium channel protein 4 (Gardos channel)	Dehydrated hereditary stomatocytosis (dominant)	Clinical presentation similar to that of <i>PIEZ01</i> mutants.
ABCB6	2q35-q36	ATP-binding cassette subfamily B member 6	Familial pseudohyperkalemia (dominant)	Increased potassium leakage upon storage in blood bank condition: this can cause hyperkalemia in the recipient. ABCB6 mutation is present in 0.3% of blood donors.
SLC2A1	1p34.2	GLUT1 glucose transporter	Overhydrated hereditary stomatocytosis	Associated with serious neurological manifestations.

Note: *PIEZ01*, *KCNN4*, *ABCB6*, and *GLUT1* are channel molecules; conditions associated with mutations in the respective genes are appropriately named channelopathies.

Abbreviations: HE, hereditary elliptocytosis; HS, hereditary spherocytosis.

osmotic fragility became the main diagnostic test for HS. Today we know that HS, thus defined, is genetically heterogeneous; i.e., it can arise from a variety of mutations in one of several genes (Table 100-3). It has been also recognized that the inheritance of HS is not always autosomal dominant (with the patient being heterozygous); indeed, some of the most severe forms are instead autosomal recessive (with the patient being homozygous).

Clinical Presentation and Diagnosis The spectrum of clinical severity of HS is broad. Severe cases may present in infancy with severe anemia, whereas mild cases may present in young adults or even later in life. The main clinical findings are jaundice, an enlarged spleen, and often gallstones; indeed, it may be the finding of gallstones in a young person that triggers diagnostic investigations.

The variability in clinical manifestations that is observed among patients with HS is largely due to the different underlying molecular lesions (Table 100-3). Not only are mutations of several genes involved, even different mutations of the same gene can give very different clinical manifestations. In milder cases, hemolysis is often compensated (see above), but changes in clinical expression may be seen even in the same patient because intercurrent conditions (e.g., pregnancy, infection) may cause decompensation. The anemia is usually normocytic with the characteristic morphology that gives the disease its name. An increased mean corpuscular hemoglobin concentration (MCHC >34) and increased red cell distribution width (RDW >14%) associated with normal or slightly decreased MCV on an ordinary blood count report should raise the suspicion of HS. The spleen plays a key role in HS

through a dual mechanism. On one hand, like in many other HAs, the spleen itself is a major site of destruction; on the other hand, because the red cells in HS are less deformable, transit through the splenic circulation makes them more prone to vesiculate, thus accelerating their demise.

When there is a family history, it is usually easy to make a diagnosis based on features of HA and typical red cell morphology. However, family history may be negative for at least two reasons. First, the patient may have a de novo mutation, i.e., a mutation that has taken place in a germ cell of one of the patient's parents or early after zygote formation. Second, the patient may have a recessive form of HS (Table 100-3). In such cases, more extensive laboratory investigations are required, including osmotic fragility, the acid glycerol lysis test, the eosin-5'-maleimide (EMA)-binding test, and SDS-gel electrophoresis of membrane proteins; these tests are usually carried out in laboratories with special expertise in this area. Sometimes a definitive diagnosis can be obtained only by molecular studies demonstrating a mutation in one of the genes underlying HS (Table 100-3).

TREATMENT

Heredity Spherocytosis

We do not have a causal treatment for HS; i.e., no way has yet been found to correct the basic defect in the membrane-cytoskeleton structure. Given the special role of the spleen in HS (see above),

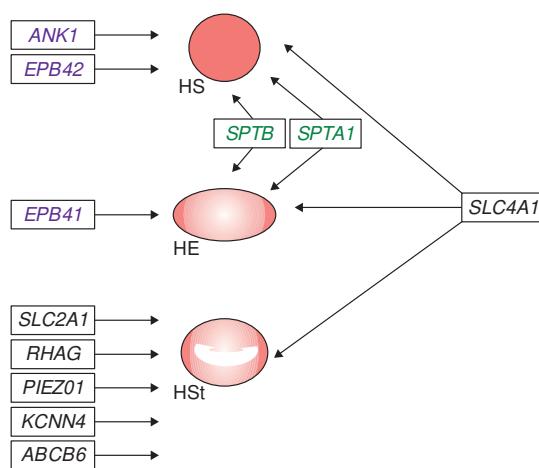


FIGURE 100-3 Hereditary spherocytosis (HS), hereditary elliptocytosis (HE), and hereditary stomatocytosis (HSt) are three morphologically distinct forms of congenital hemolytic anemia. It has emerged that each one can arise from mutation of one of several genes and that different mutations of the same gene can give one or another form. (See also Table 100-3.) Genes encoding membrane proteins are in black; genes encoding cytoskeleton proteins are in green; genes encoding proteins in the junctional and ankyrin complexes are in purple.

splenectomy is often beneficial. Current recommendations are to proceed with splenectomy at the age of 4–6 years in severe cases, to delay splenectomy until puberty in moderate cases, and to avoid splenectomy in mild cases. Partial splenectomy can be considered in certain cases; and it is helpful to know about the outcome of splenectomy in the patient's affected relatives. Before splenectomy, vaccination against encapsulated bacteria (*Neisseria meningitidis* and *Streptococcus pneumoniae*) is imperative; penicillin prophylaxis after splenectomy is controversial. Along with splenectomy, cholecystectomy should not be carried out automatically; but it should be carried out, usually by the laparoscopic approach, whenever it is clinically indicated.

HEREDITARY ELLIPTOCYTOSIS HE is at least as heterogeneous as HS, both from the genetic point of view (Table 100-3, Fig. 100-3) and from the clinical point of view. The global incidence of HE is 1:2000–4000 subjects. Again, it is the shape of the red cells (Fig. 100-4B) that gives the name to the condition, but there is no direct correlation between the elliptocytic morphology and clinical severity. In fact, some mild or even asymptomatic cases may have nearly 100% elliptocytes (or ovalocytes). Indeed, the diagnosis of HE is generally incidental, because hemolysis may be compensated and there may be no anemia, although this may become evident in the course of infection. One particular in-frame deletion of nine amino acids in the *SLC4A1* gene encoding band 3 underlies the so-called Southeast Asia ovalocytosis (SAO): it is not a disease, but rather a polymorphism with a frequency of up to 5–7% in certain populations (e.g., Papua New Guinea, Indonesia, Malaysia, Philippines), presumably as a result of malaria selection; it is asymptomatic in heterozygotes and probably lethal in homozygotes. The cases of HE with the most severe HA are those with biallelic mutations of one of the genes involved (see Fig. 100-3), and these are said to have pyropoikilocytosis (HPP): here the instability of the cytoskeleton protein network may result from decreased tetramerization of spectrin dimers. The red cell volume is decreased (MCV: 50–60 fL), and all kinds of bizarre poikilocytes are seen on the blood smear (Fig. 100-4C). HPP patients have splenomegaly and often benefit from splenectomy.

Channelopathies These rare conditions (see Fig. 100-3) are characterized by abnormalities in red cell ion content and alteration of erythrocyte volume. Cation leak can cause hyperkalemia; in some cases, this leak is accelerated in the cold (the resulting spuriously high serum K⁺ is then referred to as pseudo-hyperkalemia). The less rare form,

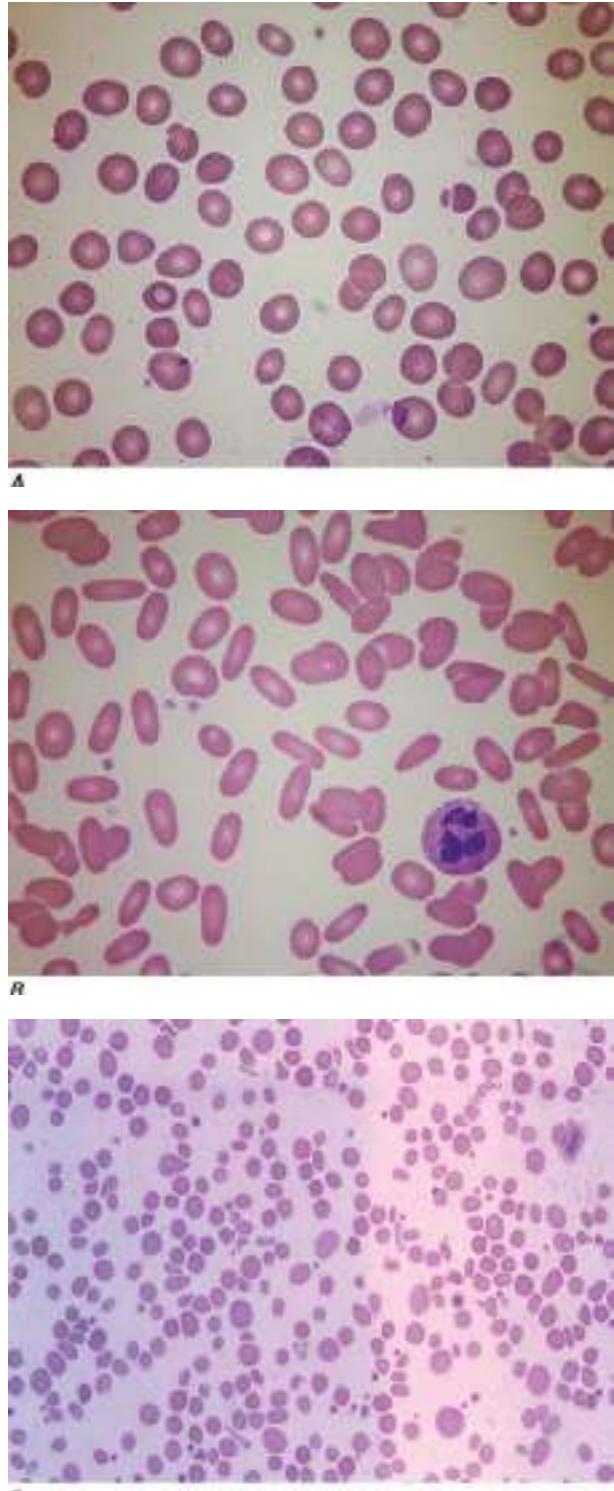


FIGURE 100-4 Peripheral blood smear from patients with membrane-cytoskeleton abnormalities. *A*. Hereditary spherocytosis. *B*. Hereditary elliptocytosis, heterozygote. *C*. Pyropoikilocytosis, with both alleles of the α -spectrin gene mutated.

dehydrated stomatocytosis (DHS; also referred to as xerocytosis) is a (usually compensated) macrocytic hemolytic disorder, with increased MCHC (generally higher than 36 g/dL) associated with mild jaundice. Mutations in either *PIEZ01*, encoding an ion channel activated by

pressure (mechanoreceptor), or in *KCCN4*, encoding the Ca^{2+} activated K^+ channel (Gardos channel) have been recognized to cause DHS (see Table 100-3).

Another form is overhydrated stomatocytosis (OHS); this too is macrocytic ($\text{MCV} > 110 \text{ fL}$), but the MCHC is low ($< 30 \text{ g/dL}$). The underlying mutation is in the Rhesus gene *RHAG*, which encodes an ammonia channel. Yet other patients with stomatocytosis (Table 100-3) have mutations in *SLC4A1* (encoding band 3) and *SLC2A1* (encoding the glucose transporter GLUT1). Mutations of the latter are responsible for *cryohydrocytosis*, a channeopathy in which the red cells swell and burst when they are cooled. In vivo hemolysis can vary from relatively mild to quite severe. Familial hyperkalemia has been recently linked to mutations in *ABCB6*, resulting in abnormal cation leak with extracellular release of a large amount of K^+ (hyperkalemia). Mutations in *ABCB6* have been identified in almost 0.3% of blood donors. However, splenectomy is contraindicated in stomatocytosis due to the significant proportion of severe thromboembolic complications observed in splenectomized DHS patients.

A specialized technique to measure erythrocyte deformability through laser diffraction analysis is *ektacytometry*; this has been used extensively in order to investigate membrane-cytoskeleton abnormalities. For diagnostic purposes, systematic sequencing of a panel of genes in patients' DNA is a powerful approach already in use and destined to be used increasingly.

Enzyme Abnormalities When an important defect in a component of the membrane-cytoskeleton complex is present, hemolysis is a direct consequence of the fact that the very structure of the red cell is compromised. Instead, when one of the enzymes is defective, the consequences will depend on the precise role of that enzyme in the metabolic machinery of the red cell. This machinery has two main

functions: (1) to provide energy in the form of ATP, and (2) to prevent oxidative damage to hemoglobin and to other proteins by providing sufficient reductive potential; the key molecule for this is NADPH.

ABNORMALITIES OF THE GLYCOLYTIC PATHWAY Because red cells, in the course of their differentiation, have sacrificed not only their nucleus and their ribosomes but also their mitochondria, they rely exclusively on the anaerobic portion of the glycolytic pathway for producing ATP, most of which is required by the red cell for cation transport against a concentration gradient across the membrane. If this fails due to a defect of any of the enzymes of the glycolytic pathway (Table 100-4), the result will be hemolytic disease.

Pyruvate Kinase Deficiency Abnormalities of the glycolytic pathway are all inherited and all rare. Among them, deficiency of pyruvate kinase (PK) is the least rare, with an estimated prevalence in most populations of 1:10,000. However, recently, a polymorphic PK mutation (E277K) was found in some African populations with heterozygote frequencies of 1–7%, suggesting that this may be another malaria-related polymorphism. HA secondary to PK deficiency is an autosomal recessive disease (Fig. 100-5).

The clinical picture of homozygous (or biallelic) PK deficiency is that of an HA that often presents in the newborn with neonatal jaundice, requiring nearly always phototherapy and frequently exchange transfusion; the jaundice persists, and it is often associated with reticulocytosis. The anemia is of variable severity; sometimes it is so severe as to require regular blood transfusion treatment, whereas sometimes it is mild, bordering on a nearly compensated hemolytic disorder. As a result, the diagnosis may be delayed: in some cases, it is made, for instance, in a young woman during her first pregnancy, when the anemia may get worse. The delay in diagnosis may be caused in part by the fact that the anemia is often remarkably well tolerated because

TABLE 100-4 Red Cell Enzyme Abnormalities Causing Hemolysis

ENZYME (ACRONYM)	GENE SYMBOL; CHROMOSOMAL LOCATION	PREVALENCE OF ENZYME DEFICIENCY (RANK)	CLINICAL MANIFESTATIONS EXTRA-RED CELL	COMMENTS
Glycolytic Pathway				
Hexokinase (HK)	<i>HK1</i> ; 10q22	Very rare		May benefit from splenectomy; BMT
Glucose 6-phosphate isomerase (G6PI)	<i>GPI</i> ; 19q31.1	Rare (4); at least 60 cases reported ^a	NM, CNS	May benefit from splenectomy
Phosphofructokinase (PFK) ^b	<i>PFKM</i> ; 12q13	Very rare	Myopathy; myoglobinuria	
Aldolase	<i>ALDOA</i> ; 16q22-24	Very rare	Myopathy	
Triose phosphate isomerase (TPI)	<i>TPI1</i> ; 12p13.31	Very rare	CNS (severe), NM	
Glyceraldehyde 3-phosphate dehydrogenase (GAPD)	<i>GAPDH</i> ; 12p13.31	Very rare	Myopathy	
Bisphosphoglycerate mutase (BPGM)	<i>BPGM</i> ; 7q33	Very rare		Erythrocytosis rather than hemolysis; some of the rare mutations are in the enzyme active site
Phosphoglycerate kinase (PGK)	<i>PGK1</i> ; Xq21.1	Very rare	CNS, NM	May benefit from splenectomy; BMT
Pyruvate kinase (PK)	<i>PKLR</i> ; 1q22	Rare (2) ^a		May benefit from splenectomy; BMT
Redox				
Glucose 6-phosphate dehydrogenase (G6PD)	<i>G6PD</i> ; Xq28	Common (1) ^a	Very rarely granulocytes	In almost all cases, only AHA from exogenous trigger
Glutathione synthase	<i>GSS</i> ; 20q11.22	Very rare	CNS	
Glutathione reductase	<i>GSR</i> ; 8p12	Very rare	Cataracts	AHA from exogenous trigger (favism)
γ -Glutamylcysteine synthetase	<i>GCLC</i> ; 6p12.1	Very rare	CNS	Mutations affect catalytic subunit
Cytochrome b5 reductase	<i>CYB5R3</i> ; 22q13.2	Rare	CNS	Methemoglobinemia rather than hemolysis
Nucleotide Metabolism				
Adenylate kinase (AK)	<i>AK1</i> ; 9q34.11	Very rare	CNS	May benefit from splenectomy
Pyrimidine 5' nucleotidase (P5N)	<i>NTSC3A</i> ; 7p14.3	Rare (3) ^a		May benefit from splenectomy

^aThe numbers from (1) to (4) indicate the ranking order of these enzymopathies in terms of frequency. ^bPFK deficiency is associated with increased glycogen in muscle, and it is also known as glycogen storage disease type VII or Tarui's disease. ^cOccasional report of successful treatment of the hematologic manifestations by BMT.

Abbreviations: AHA, acquired hemolytic anemia; BMT, bone marrow transplantation; CNS, central nervous system; NM, neuromuscular.

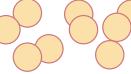
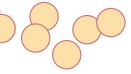
	PK deficiency (autosomal)	G6PD deficiency (X-linked)
Homozygous normal		
Heterozygous		
Homozygous deficient		

FIGURE 100-5 Different phenotypes of heterozygotes for red cell enzymopathies. In a heterozygote for deficiency of PK, encoded by an autosomal gene (see Table 100-4), the level of enzyme is about one-half of normal in all red cells. Because this level of enzyme is sufficient, there are no clinical consequences, i.e., PK deficiency is recessive. In a heterozygote for deficiency of G6PD, encoded by an X-linked gene, the situation is quite different: X-chromosome inactivation generates red cell mosaicism, whereby some red cells are entirely normal and others are G6PD deficient. Therefore, G6PD deficiency is expressed in heterozygotes: it is not recessive.

the metabolic block at the last step in glycolysis causes an increase in 2,3-bisphosphoglycerate (or DPG; Fig. 100-1), a major effector of the hemoglobin-oxygen dissociation curve; thus the oxygen delivery to the tissues is enhanced, a remarkable compensatory feat.

TREATMENT

Pyruvate Kinase Deficiency

The management of PK deficiency is mainly supportive. In view of the marked increase in red cell turnover, oral folic acid supplements should be given constantly. Blood transfusion should be used as necessary, and iron chelation may be required even in some patients who, though not receiving blood transfusion, may be developing iron overload (see “General Pathophysiology” above). About one-half of patients sooner or later undergo splenectomy, which usually provides a modest but significant increase in hemoglobin (paradoxically, often reticulocytes also increase considerably). Cholecystectomy may also be required. Some patients with severe disease have received bone marrow transplantation (BMT) from an HLA-identical PK-normal sibling. Prenatal diagnosis has been carried out in a mother who had already had an affected child. A clinical trial of a small molecule that is a specific PK ligand and may increase the stability and/or catalytic efficiency of mutant PK is currently ongoing. Rescue of inherited PK deficiency through lentiviral-mediated human PK gene transfer has been successful in mice. An oral small molecule allosteric activator of PK called *mitapivat* raised hemoglobin levels in about half of PK deficient patients in a small phase 2 study.

Other Glycolytic Enzyme Abnormalities All of these defects are rare to very rare (Table 100-4), and most of them cause HA with varying degrees of severity. It is not unusual for the presentation to be in the guise of severe neonatal jaundice, which may require exchange transfusion; if the anemia is less severe, it may present later in life, or it may even remain asymptomatic and be detected incidentally when a blood count is done for unrelated reasons. The spleen is often enlarged. When other systemic manifestations occur, they can involve the central nervous system (sometimes entailing severe mental retardation, particularly in the case of triose phosphate isomerase deficiency), the neuromuscular system, or both (see Table 100-4). This is not altogether surprising if we consider that these are housekeeping genes, i.e., expressed in all tissues. The diagnosis of HA is usually not difficult, thanks to the triad of normo-macrocytic anemia, reticulocytosis, and hyperbilirubinemia. Enzymopathies should be considered in the differential diagnosis of any chronic Coombs-negative HA. Unlike with membrane disorders, in most

cases of glycolytic enzymopathies, morphologic abnormalities are conspicuous by their absence. A definitive diagnosis can be made only by demonstrating the deficiency of an individual enzyme by quantitative assays; these are carried out in only a few specialized laboratories. If a particular molecular abnormality is already known in the family, then one could test directly for that defect at the DNA level, thus bypassing the need for enzyme assays. Of course the time may be getting nearer when a patient will present with her or his exome already sequenced, and we will need to concentrate on which genes to look up within the file. The principles for the management of these conditions are similar as for PK deficiency. In isolated cases of glycolytic enzyme abnormalities, BMT has been carried out successfully, although unfortunately nonhematologic manifestations, if any, are not reversed.

ABNORMALITIES OF REDOX METABOLISM Glucose-6-phosphate Dehydrogenase (G6PD) Deficiency G6PD is a housekeeping enzyme critical in the redox metabolism of all aerobic cells (Fig. 100-1). In red cells, its role is even more critical because it is the only source of NADPH, which directly and via glutathione (GSH) defends these cells against oxidative stress (Fig. 100-6). G6PD deficiency-related HA is a prime example of an HA due to interaction between an intracorporeal cause and an extracorporeal cause: indeed, in the vast majority of cases hemolysis is triggered by an exogenous agent. Although the G6PD activity is decreased in most tissues of G6PD-deficient subjects, in other cells the decrease is much less pronounced than in red cells, and it does not seem to impact on clinical expression.

■ GENETIC CONSIDERATIONS

 The G6PD gene is X-linked, and this has important implications. First, because males have only one G6PD gene (i.e., they are hemizygous for this gene), they must be either normal or G6PD deficient. By contrast, females, who have two G6PD genes, can be either normal or deficient (homozygous) or intermediate (heterozygous). Second, as a result of the phenomenon of X chromosome inactivation, heterozygous females are genetic mosaics (see Fig. 100-5), with a highly variable ratio of G6PD-normal to G6PD-deficient cells and an equally variable degree of clinical expression; some heterozygotes can be just as affected as hemizygous males. The enzymatically active form of G6PD is either a dimer or a tetramer of a single protein subunit of 514 amino acids. G6PD-deficient subjects have been found invariably to have mutations in the coding region of the G6PD gene. Almost all of the 230 different mutations known are single missense point mutations, entailing single amino acid replacements in the G6PD protein. In most cases, these mutations cause G6PD deficiency by decreasing the *in vivo* stability of the protein; thus the physiologic decrease in G6PD activity that takes place with red cell aging is greatly accelerated. In some cases, an amino acid replacement can also affect the catalytic function of the enzyme.

Among these mutations, those underlying chronic nonspherocytic hemolytic anemia (CNSHA; see below) are a discrete subset. This much more severe clinical phenotype can be ascribed in some cases to adverse qualitative changes (for instance, a decreased affinity for the substrate glucose-6-phosphate) or simply to the fact that the enzyme deficit is more extreme because of a more severe instability of the enzyme. For instance, a cluster of mutations map at or near the dimer interface, and clearly they compromise severely the formation of the dimer.

Epidemiology G6PD deficiency is widely distributed in tropical and subtropical parts of the world (Africa, Southern Europe, the Middle East, Southeast Asia, and Oceania) (Fig. 100-7) and wherever people from those areas have migrated. A conservative estimate is that at least 500 million people have a G6PD deficiency gene. In several of these areas, the frequency of a G6PD deficiency gene may be as high as 20% or more. It would be quite extraordinary for a trait that causes significant pathology to spread widely and reach high frequencies in many populations without conferring some biologic advantage. Indeed, G6PD is one of the best-characterized examples of genetic polymorphisms in the human species. Clinical field studies and in

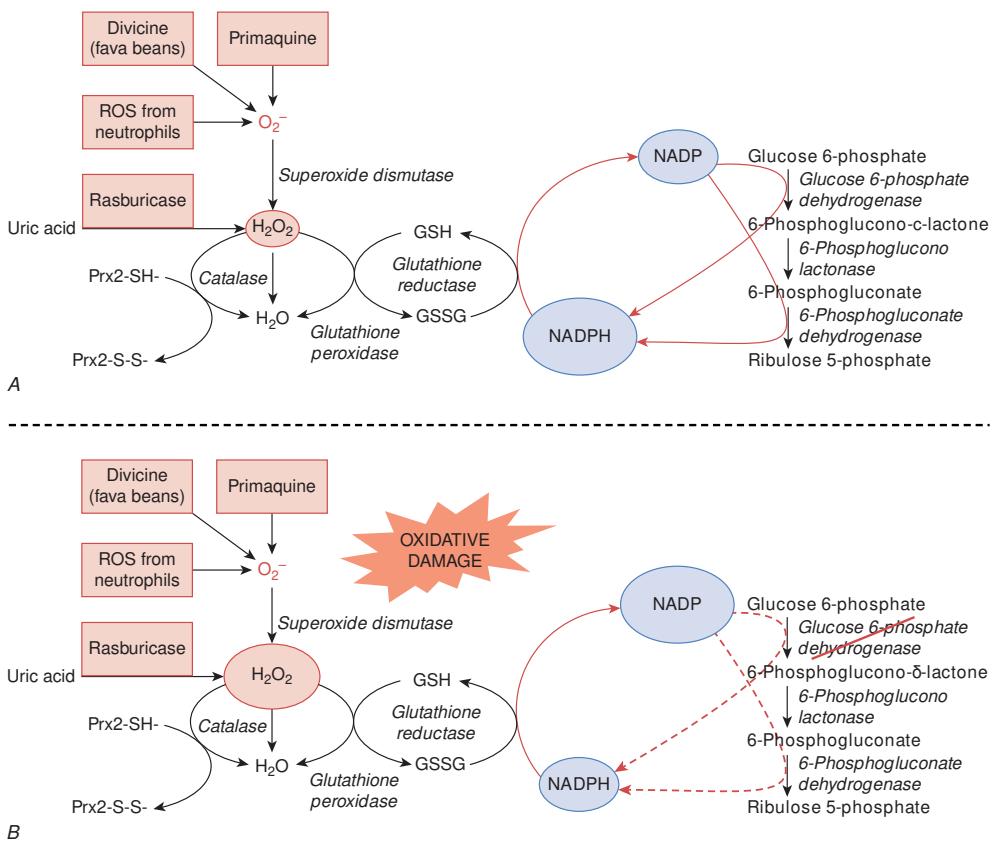


FIGURE 100-6 The role of G6PD in protecting red cells from oxidative damage. **A.** In G6PD-normal red cells, G6PD and 6-phosphogluconate dehydrogenase—two of the enzymes of the pentose phosphate pathway—provide ample supply of NADPH, which in turn regenerates GSH when this is oxidized by reactive oxygen species (e.g., O_2^- and H_2O_2). Thus when O_2^- (meant here to represent itself and other reactive oxygen species or ROS) is produced by pro-oxidant compounds such as primaquine, or the glucosides in fava beans (divicine), or the oxidative burst of neutrophils, these ROS are rapidly neutralized; similarly, when rasburicase administered to degrade uric acid produces an equimolar amount of hydrogen peroxide, this is rapidly degraded by the combined action of glutathione peroxidase, catalase, and Prx2 (peroxiredoxin-2: all three mechanisms are NADPH dependent). **B.** In G6PD-deficient red cells, where the enzyme activity is reduced, NADPH production is limited, and it may not be sufficient to cope with the excess ROS generated by pro-oxidant compounds, and the consequent excess hydrogen peroxide. This diagram also explains why a defect in glutathione reductase has very similar consequences to G6PD deficiency.

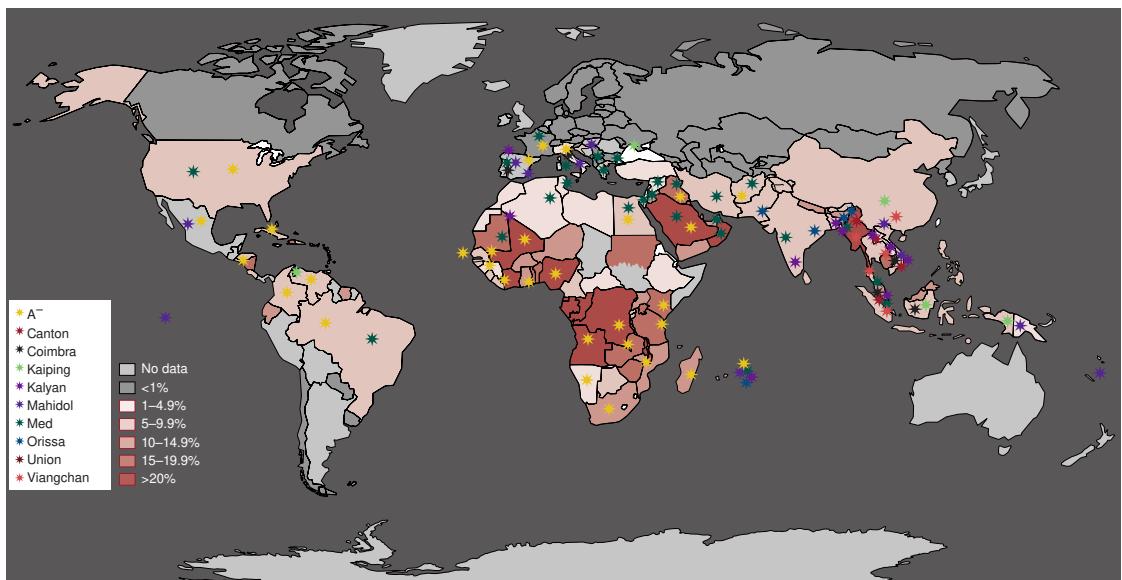


FIGURE 100-7 Epidemiology of G6PD deficiency throughout the world. Each country on the map is shaded in a color based on the best estimate of the mean frequency of G6PD deficiency allele(s) in that country (this is the same as the frequency of G6PD deficient males). The small panel on the left gives the key to color shadings corresponding to each country. The larger panel gives a color-coded list of ten common G6PD variants associated with G6PD deficiency: asterisk-shaped symbols in the corresponding colors are shown in the countries where these variants have been observed (for graphic reasons symbols could not be inserted in all countries). (Republished with permission of American Society of Hematology, from *Glucose-6-phosphate dehydrogenase deficiency*, L Luzzatto et al. 136:1225, 2020 permission conveyed through Copyright Clearance Center, Inc.)

vitro experiments strongly support the view that G6PD deficiency has been selected by *Plasmodium falciparum* malaria because it confers a relative resistance against this highly lethal infection. As in other cases of balanced polymorphism, it is heterozygotes, therefore females, who are protected. Different G6PD variants underlie G6PD deficiency in different parts of the world. Examples of widespread variants are G6PD Mediterranean on the shores of that sea, in the Middle East, and elsewhere; G6PD A– in Africa, in the Middle East, and in Southern Europe; G6PD Orissa in India; G6PD Viangchan and G6PD Mahidol in Southeast Asia; G6PD Kaiping and G6PD Canton in China; and G6PD Union worldwide. The heterogeneity of polymorphic G6PD variants is proof of their independent origin, further supporting the notion of selection by a common environmental agent, namely malaria, in keeping with the concept of convergent evolution (Fig. 100-7).

Clinical Manifestations The vast majority of people with G6PD deficiency remain clinically asymptomatic throughout their lifetime; however, all of them have an increased risk of developing neonatal jaundice (NNJ) and a risk of developing acute HA (AHA) when challenged by a number of oxidative agents. NNJ related to G6PD deficiency is rarely present at birth; the peak incidence of clinical onset is between day 2 and day 3, and in most cases the anemia is not severe. However, NNJ can be very severe in some G6PD-deficient babies, especially in association with prematurity, infection, and/or environmental factors (such as naphthalene-camphor balls, which may be used in babies' bedding and clothing); and the risk of severe NNJ is also increased by the coexistence of a monoallelic or biallelic mutation in the uridyl transferase gene (*UGT1A1*; the same mutations are associated with Gilbert's syndrome). It is imperative to manage promptly NNJ associated with G6PD deficiency, because it can produce kernicterus and permanent neurologic damage.

AHA can develop as a result of three types of triggers: (1) fava beans, (2) infections, and (3) drugs (Table 100-5). Typically, a hemolytic attack starts with malaise, weakness, and abdominal or lumbar pain. Within a timeframe of several hours to 2–3 days, the patient develops jaundice and often dark urine. The onset can be extremely abrupt, especially with favism in children. The anemia is moderate to extremely severe, usually normocytic and normochromic, and due partly to intravascular hemolysis; hence, it is associated with hemoglobinemia, hemoglobinuria, high LDH, and low or absent plasma haptoglobin. The blood film shows anisocytosis, polychromasia, and spherocytes; in addition, the most typical feature of G6PD deficiency is the presence of bizarre

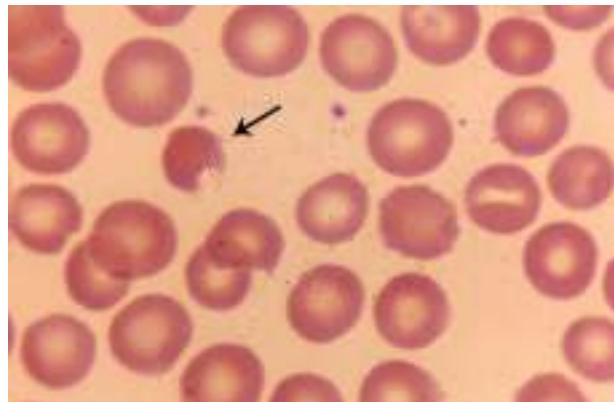


FIGURE 100-8 Peripheral blood smear from a glucose-6-phosphate dehydrogenase (G6PD)-deficient boy experiencing hemolysis. Note the red cells that are misshapen and called "bite" cells. (From MA Lichtman et al: *Lichtman's Atlas of Hematology*; <http://www.accessmedicine.com>. Copyright © The McGraw-Hill Companies, Inc. All rights reserved.)

poikilocytes, with red cells that appear to have unevenly distributed hemoglobin ("hemighosts") and red cells that appear to have had parts of them bitten away ("bite cells" or "blister cells") (Fig. 100-8). A classical test, now rarely carried out, is supravital staining with methyl violet, which, if done promptly, reveals the presence of Heinz bodies (consisting of precipitates of denatured hemoglobin and hemichromes), which are regarded as a signature of oxidative damage to red cells (they are also seen with unstable hemoglobins). Since there is also a substantial component of extravascular hemolysis, unconjugated bilirubin is high and there is often clinical icterus. The most serious threat from AHA in adults is the development of acute renal failure (this is exceedingly rare in children). Once the threat of acute anemia is over and in the absence of comorbidity, full recovery from AHA associated with G6PD deficiency is the rule.

It was primaquine (PQ)-induced AHA that led to the discovery of G6PD deficiency, but this drug has not been very prominent subsequently because it is not necessary for the treatment of life-threatening *P.falciparum* malaria. Today there is a revival in the use of PQ for two reasons. First, it is the only effective agent for eliminating the gametocytes of *P.falciparum* (thus preventing further transmission): a small single dose (0.25 mg/kg) is required, and it is safe for G6PD-deficient persons. Second, a 14-day course of PQ is needed for eliminating the hypnozoites of *Plasmodium vivax* (thus preventing endogenous relapse). In countries aiming to eliminate malaria, there may be a call for mass administration of PQ; this ought to be associated with G6PD testing. At the other end of the historic spectrum, the latest additions to the list of potentially hemolytic drugs (Table 100-5) are rasburicase and pegloticase; again G6PD testing ought to be made mandatory before giving either of these drugs, because fatal cases have been reported upon using one of these drugs, which generate hydrogen peroxide, in newborns with kidney injury and in adults with tumor lysis syndrome.

Although drug-induced AHA has been prominent in the study of G6PD deficiency, the most common clinical manifestations are in fact NNJ and favism, both of which are of public health importance in many populations. Contrary to beliefs that are still widespread, fava bean pollen inhalation does not cause favism, and other beans are safe.

A very small minority of subjects with G6PD deficiency have CNSHA of variable severity. The patient is nearly always a male, usually with a history of NNJ, who may present with anemia, unexplained jaundice, or gallstones later in life. The spleen may be enlarged. The severity of anemia ranges in different patients from borderline to transfusion dependent. The anemia is usually normo-macrocytic, with reticulocytosis. Bilirubin and LDH are increased. Although hemolysis is, by definition, chronic in these patients, they are also vulnerable to acute oxidative damage, and therefore the same agents that can cause AHA in people with the ordinary type of G6PD deficiency will cause

TABLE 100-5 Drugs That Carry Risk of Clinical Hemolysis in Persons with Glucose 6-Phosphate Dehydrogenase Deficiency

	DEFINITE RISK	POSSIBLE RISK	DOUBTFUL RISK
Antimalarials	Primaquine	Chloroquine; hydroxychloroquine	Quinine
	Dapsone/ chlorproguanil ^a		
Sulphonamides/ sulphones	Dapsone	Sulfamethoxazole Sulfasalazine Sulfadimidine	Sulfisoxazole Sulfadiazine
Antibacterial/ antibiotics	Cotrimoxazole Nalidixic acid	Ciprofloxacin Norfloxacin	Chloramphenicol <i>p</i> -Aminosalicylic acid
	Nitrofurantoin Niridazole		
Antipyretic/ analgesics	Acetanilide	Acetylsalicylic acid high dose (>3 g/d)	
	Phenazopyridine		Acetaminophen Phenacetin
Other	Rasburicase Naphthalene Methylene blue	Vitamin K analogues Ascorbic acid (>1 g)	Doxorubicin Probenecid

^aMarketed as Lapdap from 2003 to 2008.

severe exacerbations in people with CNSHA associated with G6PD deficiency. In some cases of CNSHA, the deficiency of G6PD is so severe in granulocytes that it limits their capacity to produce an oxidative burst, with consequent increased susceptibility to some bacterial infections.

Laboratory Diagnosis The suspicion of G6PD deficiency can be confirmed by semiquantitative methods often referred to as screening tests, which are suitable for population studies and can correctly classify male subjects, in the steady state, as G6PD normal or G6PD deficient. However, in clinical practice, a diagnostic test is usually needed when the patient has had a hemolytic attack: whereby the oldest, most G6PD-deficient red cells have been selectively destroyed, and young red cells, having higher G6PD activity, are being released into the circulation. Under these conditions, only a quantitative test can give a definitive result. In males, this test will identify normal hemizygotes and G6PD-deficient hemizygotes; among females, some heterozygotes will be missed, but those who are at most risk of hemolysis will be identified. Of course, G6PD deficiency also can be diagnosed by DNA testing. Currently easy-to-use “point of care” tests for G6PD deficiency are becoming available, geared especially to the prospect of mass administration of PQ or of the newly introduced derivative tafenoquine.

TREATMENT

G6PD Deficiency

The AHA of G6PD deficiency is largely preventable by avoiding exposure to triggering factors of previously screened subjects. Of course, the practicability and cost-effectiveness of screening depend on the prevalence of G6PD deficiency in each individual community. Favism is entirely preventable in G6PD-deficient subjects by not eating fava beans. Drug-induced hemolysis can be prevented by testing for G6PD deficiency before prescribing; in many cases one can use alternative drugs. When AHA develops and once its cause is recognized, no specific treatment is needed in most cases. However, if the anemia is severe, it may be a medical emergency, especially in children, requiring immediate action, including blood transfusion. This has been the case with an antimalarial drug combination containing dapsone (called Lapdap, introduced in 2003) that has caused severe acute hemolytic episodes in children with malaria in several African countries; after a few years, the drug was taken off the market. If there is acute renal failure, hemodialysis may be necessary, but if there is no previous kidney disease, recovery is the rule. The management of NNJ associated with G6PD deficiency is no different from that of NNJ due to other causes.

In cases with CNSHA, if the anemia is not severe, regular folic acid supplements and regular hematologic surveillance will suffice. It will be important to avoid exposure to potentially hemolytic drugs, and blood transfusion may be indicated when exacerbations occur, mostly in concomitance with intercurrent infection. In rare patients, regular blood transfusions may be required, in which case appropriate iron chelation should be instituted. Unlike in HS, there is no evidence of selective red cell destruction in the spleen; however, in practice, splenectomy has proven beneficial in severe cases.

Other Abnormalities of the Redox System As mentioned previously, GSH is a key player in the defense against oxidative stress. Inherited defects of GSH metabolism are exceedingly rare, but each one can give rise to chronic HA (Table 100-4). A rare, peculiar, and severe but usually self-limited HA occurring in the first month of life, called *infantile poikilocytosis*, may be associated with deficiency of glutathione peroxidase (GSHPX) due not to an inherited abnormality, but to transient nutritional deficiency of selenium, an element essential for the activity of GSHPX.

PYRIMIDINE 5' NUCLEOTIDASE P5N DEFICIENCY P5N is a key enzyme in the catabolism of nucleotides arising from the degradation of nucleic acids that takes place in the final stages of erythroid cell maturation. How exactly its deficiency causes HA is not well understood,

but a highly distinctive feature of this condition is a morphologic abnormality of the red cells known as *basophilic stippling*. The condition is rare, but it probably ranks third in frequency among red cell enzyme defects (after G6PD deficiency and PK deficiency). The anemia is lifelong, of variable severity, and may benefit from splenectomy.

Familial (Atypical) Hemolytic-Uremic Syndrome (aHUS) This term is used to designate a group of rare disorders, mostly affecting children, characterized by microangiopathic HA with presence of fragmented erythrocytes in the peripheral blood smear, thrombocytopenia (usually mild), and acute renal failure. (The word *atypical* in this phrase should be consigned to history: it was introduced originally to distinguish this condition from the hemolytic-uremic syndrome [HUS] caused by infection with *Escherichia coli* producing the Shiga toxin, regarded as *typical*.) The genetic basis of atypical HUS (aHUS) has been elucidated. Studies of >100 families have revealed that those family members who developed HUS had mutations in any one of several genes encoding complement regulatory proteins: complement factor H (CFH), CD46 or membrane cofactor protein (MCP), complement factor I (CFI), complement component C3, complement factor B (CFB), thrombomodulin, and others. Thus, whereas all other inherited HAs are due to intrinsic red cell abnormalities, this group is unique in that hemolysis results from an inherited defect external to red cells (Table 100-1). Because the regulation of the complement cascade has considerable redundancy, in the steady state any of the above abnormalities can be tolerated. However, when an intercurrent infection or some other trigger briskly activates complement the deficiency of one of the complement regulators becomes critical. Endothelial cells get damaged, especially in the kidney; at the same time, and partly as a result of this, there will be brisk hemolysis (thus, the more common Shiga toxin-related HUS (Chap. 166) can be regarded as a phenotype of aHUS). aHUS is a severe disease, with up to 15% mortality in the acute phase and up to 50% of cases progressing to end-stage renal disease (ESRD). Not infrequently, aHUS undergoes spontaneous remission. Because it is an inherited abnormality, it is not surprising that, given renewed exposure to a trigger, the syndrome will tend to recur; when it does, the prognosis is always serious. The traditional treatment has been plasma exchange, which will supply the deficient complement regulator. This has changed since the introduction of the anti-C5 complement inhibitor eculizumab (see ‘Paroxysmal Nocturnal Hemoglobinuria’) was found to greatly ameliorate the microangiopathic picture, with improvement in platelet counts and in renal function, thus abrogating the need for plasma exchange, which is not always effective and not free of complications. Because the basis of aHUS is genetic, and relapses are always possible even after complete remission, there is a rationale for continuing eculizumab indefinitely, especially in order to prevent ESRD. Patients who relapsed after discontinuing eculizumab have responded again. Discontinuation of eculizumab might be reasonable especially in patients heterozygous for a MCP mutation. However, there is no evidence base at the moment for balancing the pros and cons of lifetime eculizumab (a very expensive drug).

■ ACQUIRED HEMOLYTIC ANEMIA

Mechanical Destruction of Red Cells Although red cells are characterized by the remarkable deformability that enables them to squeeze through capillaries narrower than themselves for thousands of times in their lifetime, there are at least two situations in which they succumb to shear, if not to wear and tear; the result is intravascular hemolysis, resulting in hemoglobinuria (Table 100-6). One situation is acute and self-inflicted, *march hemoglobinuria*. Why sometimes a marathon runner may develop this complication, whereas on another occasion, this does not happen, we do not know (perhaps her or his footwear needs attention). A similar syndrome may develop after prolonged barefoot ritual dancing or intense playing of bongo drums. The other situation is chronic and iatrogenic (it has been called *microangiopathic hemolytic anemia*). It takes place in patients with prosthetic heart valves, especially when paraprosthetic regurgitation is present. If the hemolysis consequent on mechanical trauma to the red cells is mild, and if the supply of iron is adequate, the loss may be largely

TABLE 100-6 Diseases and Clinical Situations in Which Hemolysis Is Largely Intravascular

	ONSET/TIME COURSE	MAIN MECHANISM	APPROPRIATE DIAGNOSTIC PROCEDURE	COMMENTS
Mismatched blood transfusion	Abrupt	Nearly always ABO incompatibility	Repeat cross-match	
Paroxysmal nocturnal hemoglobinuria (PNH)	Chronic with acute exacerbations	Complement (C)-mediated destruction of CD59(-) red cells	Flow cytometry to display a CD59(-) red cell population	Exacerbations due to C activation through any pathway
Paroxysmal cold hemoglobinuria (PCH)	Acute	Immune lysis of normal red cells	Test for Donath-Landsteiner antibody	Often triggered by viral infection
Septicemia	Very acute	Exotoxins produced by <i>Clostridium perfringens</i>	Blood cultures	Other organisms may be responsible
Microangiopathic	Acute or chronic	Red cell fragmentation	Red cell morphology on blood smear	Different causes ranging from endothelial damage to hemangioma to leaky prosthetic heart valve
March hemoglobinuria	Abrupt	Mechanical destruction	Targeted history taking	Has been reported after extreme ritual dancing
Favism	Acute	Destruction of older fraction of G6PD-deficient red cells	G6PD assay	Triggered by ingestion of large dish of fava beans ^a

^aThe trigger of acute hemolytic anemia, often with hemoglobinuria, can be infection or a drug (see Table 100-5) rather than fava beans. Hemoglobinuria may or may not be reported by patient; but it is often macroscopic, i.e., recognizable by simple inspection of urine.

Abbreviation: G6PD, glucose 6-phosphate dehydrogenase.

compensated; if more than mild anemia develops, reintervention to correct regurgitation may be required.

Infection By far the most frequent infectious cause of HA in endemic areas is malaria (Chap. 224). In other parts of the world, the most frequent direct cause is probably Shiga toxin-producing *E. coli* O157:H7, now recognized as the main etiologic agent of HUS, which is more common in children than in adults (Chap. 161). Life-threatening intravascular hemolysis, due to a toxin with lecithinase activity, occurs with *Clostridium perfringens* sepsis, particularly following open wounds, septic abortion, or as a disastrous accident due to a contaminated blood unit. Rarely, and if at all in children, HA is seen with sepsis or endocarditis from a variety of organisms. In addition, bacterial and viral infections can cause HA by indirect mechanisms (see Table 100-6).

Immune Hemolytic Anemias These can arise through at least two distinct mechanisms. First, when an antibody directed against a certain molecule (e.g., a drug) reacts with that molecule, red cells may get caught in the reaction (the so-called innocent bystander mechanism: see section below on Hemolytic Anemia from Toxic Agents and Drugs), whereby they are damaged or destroyed. Second, and more frequently, a true autoantibody is directed against a red cell antigen, i.e., a molecule present on the surface of red cells. Autoimmune hemolytic anemias have been originally classified into two types, depending on the thermal amplitude of the autoantibodies involved: this classification is valid, because the two types have different pathophysiological and clinical features.

AUTOIMMUNE HEMOLYTIC ANEMIA, WARM TYPE WAIHA: FOR SIMPLICITY WE WILL USE THE ACRONYM AIHA This type has an estimated incidence in the United States of about 1–3:100,000 per year, and a prevalence of 17:100,000. AIHA can be serious since even with appropriate management the mortality is of the order of 5–10%.

Clinical Features and Diagnosis The onset is often abrupt and can be dramatic. The hemoglobin level may drop, within days, to as low as 4 g/dL; the massive red cell removal will produce jaundice, and sometimes the spleen is enlarged. When this triad is present, the suspicion of AIHA must be high. The reticulocyte count is typically elevated, except when erythroid precursors are also targeted by the autoantibody attack. LDH may also be elevated. In some cases, AIHA can be associated, on first presentation or subsequently, with autoimmune thrombocytopenia. This double autoimmune condition, referred to as Evans syndrome, may be a manifestation of common variable immune deficiency, and in children it may suggest one of several primary immune deficiency syndromes. Evans syndrome signals high-risk disease. Other predictors of the outcome and of the probability of relapse of AIHA are severe

anemia (Hb <6 g/dL), certain characteristics of the antibody, acute renal failure, and infection.

There are few situations in hematology where one laboratory test is as informative as the direct antiglobulin test developed in 1945 by R. R. A. Coombs and known since then by this name. The currently recommended version of this test uses in the first instance a “broad-spectrum” reagent, i.e., one that will detect not only immunoglobulins (Ig) but also complement (C) components (usually C3 fragments) bound to the surface of the patient's red cells. If the test is positive (and barring special circumstances such as previous blood transfusion), it is practically diagnostic of AIHA, and one can then determine, by using specific reagents, whether Ig or C or both are implicated. The sensitivity of the Coombs test varies depending on the techniques that are used: in general, the test is positive if there are an average of at least 400 molecules of Ig and/or C on each red cell; but with more advanced techniques involving flow cytometry analysis or enzyme-linked radiolabeled tests allowing the detection of ~30–40 antibody molecules per erythrocyte, the sensitivity can be pushed to as low as 30–40 molecules per red cell. Therefore liaison with a specialized laboratory is desirable; a dual direct antiglobulin test has also been developed. In the past the diagnosis of “Coombs-negative AIHA” was regarded as a last resort, but it is important to know that a patient with this label may have severe AIHA, because if the antibody is powerful (high affinity/avidity), few molecules may be sufficient to opsonize red cells. Based on the Coombs test findings as well as on the thermal characteristics and the antigenic specificities of the autoantibodies (Table 100-7), AIHA has been classified into subtypes.

In AIHA the autoantibody reacts best at 37°C and it is usually Rhesus-specific (sometimes specifically anti-e). The main mechanism of hemolysis in AIHA is that the Fc portion of the IgG antibody bound to red cells is recognized by the Fc receptor of macrophages: this will trigger erytrophagocytosis wherever macrophages are abundant, i.e., in the liver, in the bone marrow, but especially in red pulp of the spleen (see Fig. 100-9) that, also because of its special anatomy, is often the predominant site of red cell destruction.

AIHA may be seen in isolation (and it is then called *idiopathic*) or as secondary to other disorders such as systemic autoimmune disorders (systemic lupus erythematosus [SLE]: sometimes AIHA may be the first manifestation that leads to a diagnosis of SLE) or lymphoproliferative disorders (Table 100-7). Like all autoimmune diseases, AIHA must arise from a dysregulation of immunity. It is therefore not surprising that it is increasingly being recognized in chronic lymphocytic leukemia (CLL), whether treated or untreated; after BMT; and after solid organ transplantation entailing immunosuppressive treatment. Recently, warm antibody AIHA has also occurred as a side effect of the use of immune checkpoint inhibitors, such as nivolumab, in patients with various types of cancer.

CLINICAL SETTING	TYPE OF ANTIBODY	
	COLD, MOSTLY IgM, OPTIMAL TEMPERATURE 4°C–30°C	WARM, MOSTLY IgG, OPTIMAL TEMPERATURE 37°C; OR MIXED
Primary	CAD	AIHA (idiopathic)
Secondary to viral infection	EBV	Parvovirus B19
	CMV	HIV
	Other	HCV EBV Viral vaccines
Secondary to other infection	Mycoplasma infection: paroxysmal cold hemoglobinuria	Babesia
Secondary to/ associated with other disease	CAD in: Waldenström's disease Lymphoma	AIHA in: SLE, scleroderma, RA CLL Lymphoproliferative disorders Multiple myeloma Other malignancy Chronic inflammatory disorders (e.g., IBD) Thyroiditis (including Hashimoto) After allogeneic HSCT Common variable immunodeficiency After immune checkpoint modulating drugs
Secondary to drugs: drug-induced immune hemolytic anemia	Small minority (e.g., with lenalidomide)	Majority: currently most common culprit drugs are cefotetan, ceftriaxone, piperacillin, methyldopa, fludarabine
		Drug-dependent: antibody destroys red cells only when drug present (e.g., rarely penicillin)
		Drug-independent: antibody can destroy red cells even when drug no longer present (e.g., methyldopa)
Associated with	Pregnancy	

Abbreviations: AIHA, autoimmune hemolytic anemia; CAD, cold agglutinin disease; CLL, chronic lymphocytic leukemia; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HSCT, hematopoietic stem cell transplantation; IBD, inflammatory bowel disease; SLE, systemic lupus erythematosus; RA: rheumatoid arthritis.

TREATMENT

Warm Antibody Autoimmune Hemolytic Anemia

Severe acute AIHA can be a medical emergency. The immediate treatment almost invariably includes transfusion of red cells. This may pose a special problem because many or all of the blood units cross-matched may be incompatible. In these cases, it is often correct, if paradoxical, to transfuse ABO-matched but incompatible blood: the rationale being that the transfused red cells will be destroyed no less—but no more—than the patient's own red cells, and in the meantime the patient stays alive. A situation like this requires close liaison and understanding between the clinical unit treating the patient and the blood transfusion/serology lab. Whenever the anemia is not immediately life-threatening, blood transfusion should be withheld (because compatibility problems may increase with each unit of blood transfused), and medical treatment started immediately with prednisone (1 mg/kg per day), which will produce a remission promptly in at least one-half of patients. Rituximab (anti-CD20), previously regarded as second-line treatment, is increasingly being used at a relatively low dose (100 mg/week × 4), together with prednisone as part of first-line treatment. It is especially encouraging that this approach seems to reduce the rate of relapse, a common occurrence in AIHA.

For patients who do relapse or are refractory to medical treatment, additional therapeutic strategies are now available. Splenectomy does not cure the disease, but it can produce significant benefit by removing a major site of hemolysis, thus improving the anemia and/or reducing the need for other therapies (e.g., the dose of prednisone); of course, splenectomy is not free of risk, as it entails increased risk of sepsis and of thrombosis. The response rate to splenectomy and to rituximab are similar. Since the introduction of rituximab, azathioprine, cyclophosphamide, cyclosporine, mycophenolate and intravenous immunoglobulin have become second- or third-line agents. In very rare severe refractory cases, one may have to consider a high dose of cyclophosphamide (50 mg/kg/d for 4 days) followed by a myelo-stimulating agent to support bone marrow or the anti-CD52 agent, alemtuzumab. When severe anemia is associated with reticulocytopenia, the use of erythropoietin may help to reduce or avoid the requirement for transfusion of red cells.

PAROXYSMAL COLD HEMOGLOBINURIA PCH PCH is a rare form of AIHA occurring mostly in children, usually triggered by a viral infection, usually self-limited, and characterized by the so-called Donath-Landsteiner antibody. In vitro, this antibody has unique serologic features; it has usually anti-P specificity and it binds to red cells only at a low temperature (optimally at 4°C), but when the temperature is shifted to 37°C, lysis of red cells takes place in the presence of complement. Consequently, in vivo there is intravascular hemolysis, resulting in hemoglobinuria. Clinically the differential diagnosis must include other causes of hemoglobinuria (Table 100-6), but the presence of the Donath-Landsteiner antibody will prove PCH. Active supportive treatment, including blood transfusion, may be needed to control the anemia; subsequently, recovery is the rule.

COLD AGGLUTININ DISEASE This designation indicates the other main type of AIHA, which has quite different features when compared with wAIHA. First, cold agglutinin disease (CAD) is a chronic and more frequently indolent condition—in contrast to the abrupt onset of warm antibody AIHA. Second, the term *cold* refers to the fact that the autoantibody involved reacts with red cells poorly or not at all at 37°C, whereas it reacts strongly at lower temperatures. As a result, hemolysis is more prominent the more the body is exposed to the cold. Third, the antibody is produced by a clone of autoreactive B lymphocytes. Sometimes the antibody concentration in the serum is high enough to show up as a spike in plasma protein electrophoresis, thus qualifying CAD as an IgM monoclonal gammopathy; however, it differs from Waldenström macroglobulinemia by not having the characteristic *MYD88* mutation (see Chap. 111): there is instead, in the B-cell clone of a majority of CAD patients, a somatic mutation in the *KMT2D* gene, encoding a lysine histone methylase that seems to favor proliferation. The antibody produced by the B-cell clone is IgM; usually it has an anti-I specificity (the I antigen is present on the red cells of almost everybody), and it may have a very high titer (1:100,000 or more has been observed). IgM, when bound to red cells, is a powerful activator of the complement cascade, with ultimate formation of the membrane attack complex (see Fig. 100-9): this will directly cause destruction of red cells (*intravascular hemolysis*: indeed, CAD patients may present with hemoglobinuria). In addition, once complement is activated C3b will bind to red cells that, thus opsonized, will be destroyed by macrophages (*extravascular hemolysis*): unlike in AIHA, there is no predominance of the spleen in this process.

In mild forms of CAD, avoidance of exposure to cold may be all that is needed to enable the patient to have a reasonably comfortable quality of life; but in more severe forms, the management of CAD is not easy. Plasma exchange will remove antibodies and is, therefore, in theory, a rational approach in severe cases. However, the management of CAD has changed significantly with the advent of the anti-CD20 antibody rituximab: up to 60% of patients respond. If remission is followed by relapse, a new course of rituximab may be again effective, and remissions may be more durable with a rituximab-fludarabine combination, in particular in CAD associated with lymphoproliferative disorders. Therefore, even

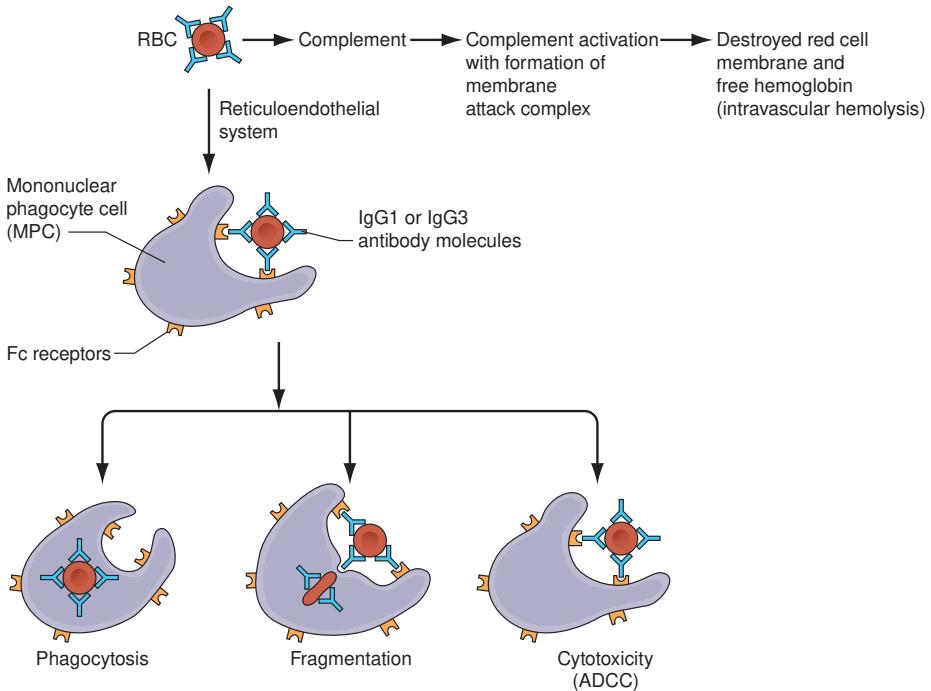


FIGURE 100-9 Mechanism of antibody-mediated immune destruction of red blood cells (RBCs). The three bottom images illustrate three different modalities of extravascular hemolysis. ADCC, antibody-dependent cell-mediated cytotoxicity. (Reproduced with permission from N Young et al: *Clinical Hematology*. Philadelphia, Elsevier, 2006.)

in the absence of a formal trial, rituximab has become de facto first-line treatment: especially since previously used immunosuppressive/cytotoxic agents, although they can reduce the antibody titer, have limited clinical efficacy and, in view of the chronic nature of CAD, their side effects may prove unacceptable. Unlike in AIHA, prednisone and splenectomy are ineffective. In the management of CAD in relapse, there is an emerging role for the B-cell receptor inhibitors venetoclax and ibrutinib, as well as for the proteasome inhibitor bortezomib. A different approach targeting complement inhibitors has been also explored by using eculizumab (anti-C5) or sutimlimab (anti-C1s): a limitation of this approach is that hemolysis will be curbed only for as long as these agents are administered.

In terms of supportive treatment, blood transfusion may be helpful—in spite of the fact that red cells from the donor, being I-positive, will survive no longer than those of the patient: both the blood bag and the patient's extremities must be kept warm during transfusion.

Hemolytic Anemia from Toxic Agents and Drugs A number of chemicals with oxidative potential, whether medicinal or not, can cause hemolysis even in people who are not G6PD deficient (for which, see above). Examples are hyperbaric oxygen (or 100% oxygen), nitrates, chlorates, methylene blue, dapsone, cisplatin, and numerous aromatic (cyclic) compounds. Other chemicals may be hemolytic through nonoxidative, largely unknown mechanisms; examples include arsine, stibine, copper, and lead. The HA caused by lead poisoning is characterized by basophilic stippling; it is in fact a phenocopy of that seen in P5N deficiency (see above), suggesting it is mediated at least in part by lead inhibiting this enzyme.

In these cases, hemolysis appears to be mediated by a direct chemical action on red cells. But drugs can cause hemolysis through at least two other mechanisms. (1) A drug can behave as a hapten and induce antibody production; in rare subjects, this happens, for instance, with penicillin. Upon a subsequent exposure, red cells are caught, as innocent bystanders, in the reaction between penicillin and antipenicillin antibodies. Hemolysis will subside as soon as penicillin administration is stopped. (2) A drug can trigger, perhaps through mimicry, the production of an antibody against a red cell antigen. The best-known

example is methyldopa, an antihypertensive agent no longer in use, which in a small fraction of patients stimulated the production of the Rhesus antibody anti-e. In patients who have this antigen, the anti-e is a true autoantibody, which then causes true AIHA (see above). Usually this will gradually subside once methyldopa is discontinued.

Severe intravascular hemolysis can be caused by the venom of certain snakes (cobras and vipers), and HA can also follow spider bites.

Paroxysmal Nocturnal Hemoglobinuria (PNH) PNH is an acquired chronic HA characterized by persistent intravascular hemolysis with occasional or frequent recurrent exacerbations. In addition to (i) hemolysis, there may be (ii) pancytopenia and (iii) a distinct tendency to venous thrombosis. This triad makes PNH a truly unique clinical condition; however, when not all of these three features are manifest on presentation, the diagnosis is often delayed, although it can always be made by appropriate laboratory investigations (see below).

PNH is encountered in all populations throughout the world, but it is a rare disease, with an estimated prevalence of ~5 per million (it may be somewhat less rare in Southeast Asia and in the Far East). PNH has about the same frequency in men and women. PNH is not inherited, and it has never been reported as a congenital disease, but it can present in small children or as late as in the seventies, although most patients are young adults.

CLINICAL FEATURES When seeking medical attention, the patient may report that one morning, she or he “passed blood instead of urine.” This distressing or frightening event may be regarded as the classic presentation; however, more frequently, this symptom is not noticed or not reported. Indeed, the patient often presents simply as a problem in the differential diagnosis of anemia, whether symptomatic or discovered incidentally. Sometimes the anemia is associated from the outset with neutropenia, thrombocytopenia, or both, thus signaling an element of bone marrow failure (see below). Some patients may present with recurrent attacks of severe abdominal pain eventually found to be related to thrombosis in abdominal veins, or attributable to NO depletion associated with intravascular hemolysis. When thrombosis affects the hepatic vein, it may produce acute hepatomegaly and ascites,

i.e., a full-fledged Budd-Chiari syndrome, which, in the absence of liver disease, ought to raise the suspicion of PNH.

The natural history of PNH can extend over decades. In the past, with supportive treatment only, the median survival was estimated to be about 10–20 years, with the most common cause of death being venous thrombosis, followed by infection secondary to severe neutropenia and hemorrhage secondary to severe thrombocytopenia. Rarely (estimated 1–2% of all cases), PNH may terminate in acute myeloid leukemia. On the other hand, full spontaneous recovery from PNH has been documented, albeit rarely.

LABORATORY INVESTIGATIONS AND DIAGNOSIS The most consistent blood finding is anemia, which may range from mild to moderate to very severe. The anemia is usually normo-macrocytic, with unremarkable red cell morphology. If the MCV is high, it is usually largely accounted for by reticulocytosis, which may be quite marked (up to 20%, or up to 400,000/ μ L). The anemia may become microcytic if the patient is allowed to become iron-deficient as a result of chronic iron loss through hemoglobinuria. Unconjugated bilirubin is mildly or moderately elevated; LDH is typically markedly elevated (values in the thousands are common); and haptoglobin is usually undetectable. All of these findings make the diagnosis of HA compelling. Hemoglobinuria may be overt in a random urine sample; if it is not, it may be helpful to obtain serial urine samples (Fig. 100-9) because hemoglobinuria can vary dramatically from day to day and even from hour to hour. The bone marrow is usually cellular, with marked to massive erythroid hyperplasia, often with mild to moderate dyserythropoietic features (these overlap with those seen in myelodysplastic syndromes, but PNH remains a separate entity). At some stage of the disease, the marrow may become hypocellular or even frankly aplastic (see below).

The definitive diagnosis of PNH must be based on the demonstration that a substantial proportion of the patient's red cells have an increased susceptibility to complement (C), due to the deficiency on their surface of proteins (particularly CD59 and CD55) that normally protect the red cells from activated C. The sucrose hemolysis test is unreliable; in contrast, the acidified serum (Ham) test is highly reliable but is carried out only in a few laboratories. The gold standard today is flow cytometry, which can be carried out on granulocytes as well as on red cells and has a very high sensitivity. In PNH, characteristically, one sees a bimodal distribution of cells, with a discrete population that is CD59 and CD55 negative. Although very small populations of CD59(−) cells are of interest in terms of pathophysiology (particularly of aplastic anemia [AA]), no patient should be diagnosed with PNH unless the proportion is substantial: in first approximation at least 5% of the total red cells and at least 20% of the total granulocytes.

PATOPHYSIOLOGY Hemolysis in PNH is mainly intravascular and is due to an intrinsic abnormality of the red cell, which makes it exquisitely sensitive to activated C, whether C is activated through the alternative pathway or through an antigen-antibody reaction (classic pathway). The former mechanism is mainly responsible for chronic hemolysis in PNH; the latter explains why the hemolysis can be dramatically exacerbated in the course of a viral or bacterial infection. Hypersusceptibility to C is due to deficiency in the red cell membrane of several protective proteins (Fig. 100-10), among which CD59 is the most important because it is able to hinder the insertion into the membrane of C9 polymers (the so-called membrane attack complex, or MAC). The molecular basis for the deficiency of these proteins has been pinpointed not to a defect in any of the respective genes, but rather to the shortage of a unique glycolipid molecule, GPI (Fig. 100-2), which, through a peptide bond, anchors these proteins to the surface membrane of cells. The shortage of GPI is due in turn to a somatic mutation in an X-linked gene, called *PIGA*, required for an early step in GPI biosynthesis. As a result, the patient's marrow is a mosaic of mutant and nonmutant cells, and the peripheral blood always contains both GPI-negative (PNH) cells and GPI-positive (non-PNH) cells: in most cases the former prevail. Thrombosis is one of the most immediately life-threatening complications of PNH, and yet one of the least understood in its pathogenesis. It could be that deficiency of CD59 on the PNH platelet causes inappropriate platelet activation; however,

other mechanisms are possible. In very rare cases PNH can be caused by biallelic mutations of the *PIGT* gene, in the absence of a *PIGA* mutation. In these cases, because GPI is produced but cannot bind to proteins, the clinical picture is further complicated by the coexistence of a chronic inflammatory state.

BONE MARROW FAILURE BMF AND RELATIONSHIP BETWEEN PNH AND APLASTIC ANEMIA AA It is not unusual that patients with firmly established PNH have a previous history of AA, sometimes well documented; indeed, BMF preceding overt PNH is probably the rule rather than the exception. On the other hand, sometimes a patient with PNH becomes less hemolytic and more pancytopenic and ultimately has the clinical picture of AA. The relationship between PNH and AA manifested in the clinical course of patients may reflect a close link in pathogenesis. AA is thought to be an organ-specific autoimmune disease, in which T cells cause damage to hematopoietic stem cells via an as yet unidentified molecular target. The same may be true of PNH, and in this condition the target might be the GPI molecule itself. This would explain why GPI-negative (PNH) stem cells are spared; *PIGA* mutations can be demonstrated in normal people. Thus, PNH results from the combined action of two factors: failure of normal hematopoiesis and massive expansion of a PNH clone. There is evidence from mouse models that PNH stem cells do not expand on their own, and there is evidence from human patients that expansion is associated with negative selection against GPI-positive cells by GPI-specific T cells. Thus, PNH is a prime example of a clonal disease that is not malignant.

TREATMENT

Paroxysmal Nocturnal Hemoglobinuria

Until some 15 years ago there were essentially two treatment options for PNH: either allogeneic BMT, providing a definitive cure at the cost of nonnegligible risks; or continued supportive treatment for what, unlike other acquired HAs, may be a lifelong condition. A major advance has been the introduction in 2007 of a humanized monoclonal antibody, eculizumab, which binds to the complement component C5 near the site that, when cleaved, will trigger the distal part of the complement cascade leading to formation of the MAC. With C5 blocked by anti-C5, the patient is relieved of intravascular hemolysis and of its attendant consequences, including hemoglobinuria; with a substantial decrease in the rate of thrombosis. In the majority of those patients who needed regular blood transfusion, the transfusion requirement is either abolished or significantly reduced. For many PNH patients, eculizumab has meant a real improvement in the quality of life, as well as a decrease in complications, particularly thrombosis. At the same time, it is important to know that in patients on eculizumab the PNH red cells, now protected from being lysed through the MAC, do still bind C3 fragments and thus become opsonized. Therefore, hemolysis continues, but it is now extravascular. The extent to which this happens depends in part on a genetic polymorphism of the complement receptor CR1. Those patients who, on eculizumab, are still receiving blood transfusion are at risk of iron overload. Based on its half-life, eculizumab must be administered intravenously every 14 days. Ravulizumab, a long-lived anti-C5 derivative of eculizumab, is administered at 8-week rather than 2-week intervals: it provides similar benefit with obvious practical advantage.

Eculizumab and ravulizumab are very expensive and for this reason not accessible to patients in many parts of the world. Therefore, the management of PNH by supportive treatment is still very important. Folic acid supplements (at least 3 mg/d) are mandatory; the serum iron should be checked periodically, and iron supplements should be administered as appropriate. Transfusion of white cell-free red cells should be used whenever necessary, which, for some patients, means quite frequently. Long-term glucocorticoids are not indicated because there is no evidence that they have any effect on chronic hemolysis; in fact, they are contraindicated

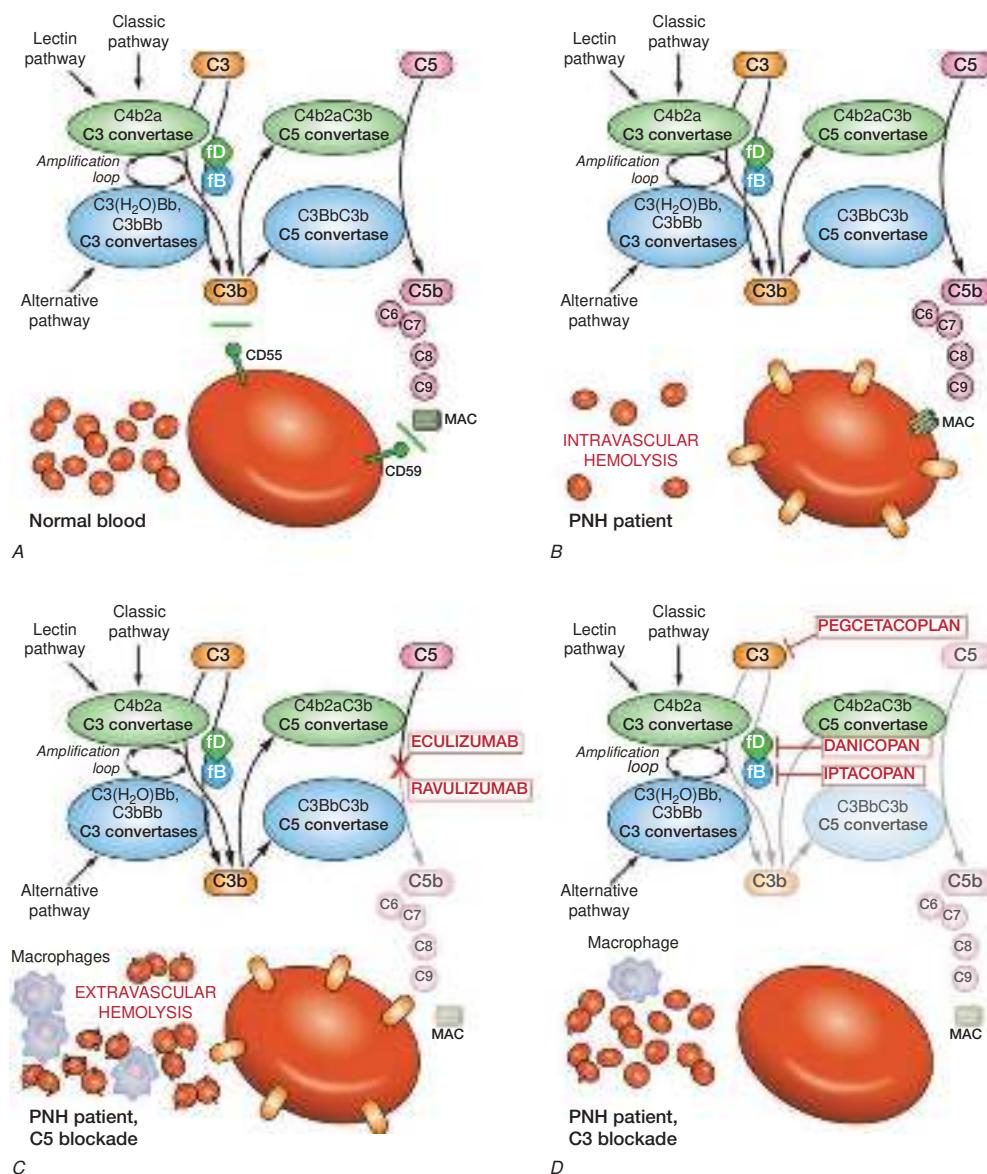


FIGURE 100-10 The complement cascade and the fate of red cells. **A.** In normal blood, when complement is activated, red cells are protected from lysis in several ways: primarily by the 2 glycosylphosphatidylinositol (GPI)-linked surface proteins CD55 (prevents binding of C3 fragments) and CD59 (prevents the membrane attack complex [MAC] from inserting into the membrane). **B.** PNH red cells are deficient in CD55 and CD59 because the GPI biosynthetic pathway is blocked as a result of a PIGA mutation; therefore, C3 fragments, particularly C3d, bind to their surface, and the red cells are rapidly lysed by the action of the MAC. **C.** With drugs (monoclonal antibodies) that bind to C5 and prevent it splitting into C5a and C5b, the entire distal pathway from C5 onward is blocked, MAC is not formed, and IVH is abrogated. However, red cells opsonized by C3d will be destroyed in the spleen and elsewhere; this drug-induced EVH varies in severity between patients. The Coombs test, which is characteristically negative in PNH, becomes positive (provided that a "broad spectrum" or an anticomplement reagent is used). **D.** With a drug that targets C3, C3b formation is inhibited, and the distal pathway is not triggered by C3b. Therefore, again, no MAC is formed (abrogating IVH), and, at the same time, opsonization of red cells by C3d is prevented, so that EVH is also curbed. The same is largely true for drugs that target factor B or factor D, although C3b can still be formed through the classical pathway. (Reproduced with permission from L Luzzatto: Control of hemolysis in patients with PNH. *Blood* 138:1909, 2021.)

because their side effects are considerable. A short course of prednisone may be useful when an inflammatory process exacerbates hemolysis. Any patient who has had venous thrombosis or who has a genetically determined thrombophilic state in addition to PNH should be on regular anticoagulant prophylaxis. With thrombotic complications that do not resolve otherwise, thrombolytic treatment with tissue plasminogen activator may be indicated.

Where anti-C5 therapy is available the proportion of PNH patients receiving BMT has decreased significantly. However, when an HLA-identical sibling is available, BMT should be taken into

consideration for any young patient with severe PNH; and for patients with the so-called PNH-AA syndrome, since eculizumab has no effect on BMF. For these patients immunosuppressive treatment with antithymocyte globulin and cyclosporine A may be an alternative, and it may be compatible with concurrent administration of eculizumab.

In view of persistent extravascular hemolysis, and sometimes persistent blood transfusion requirement in PNH patients on C5 blockade therapy, there has been great stimulus to developing agents that may inhibit complement activation more upstream. Several compounds that inhibit either the convertase function of C3 or plasma factors required for this function are currently in clinical trials (see Fig. 100-11).

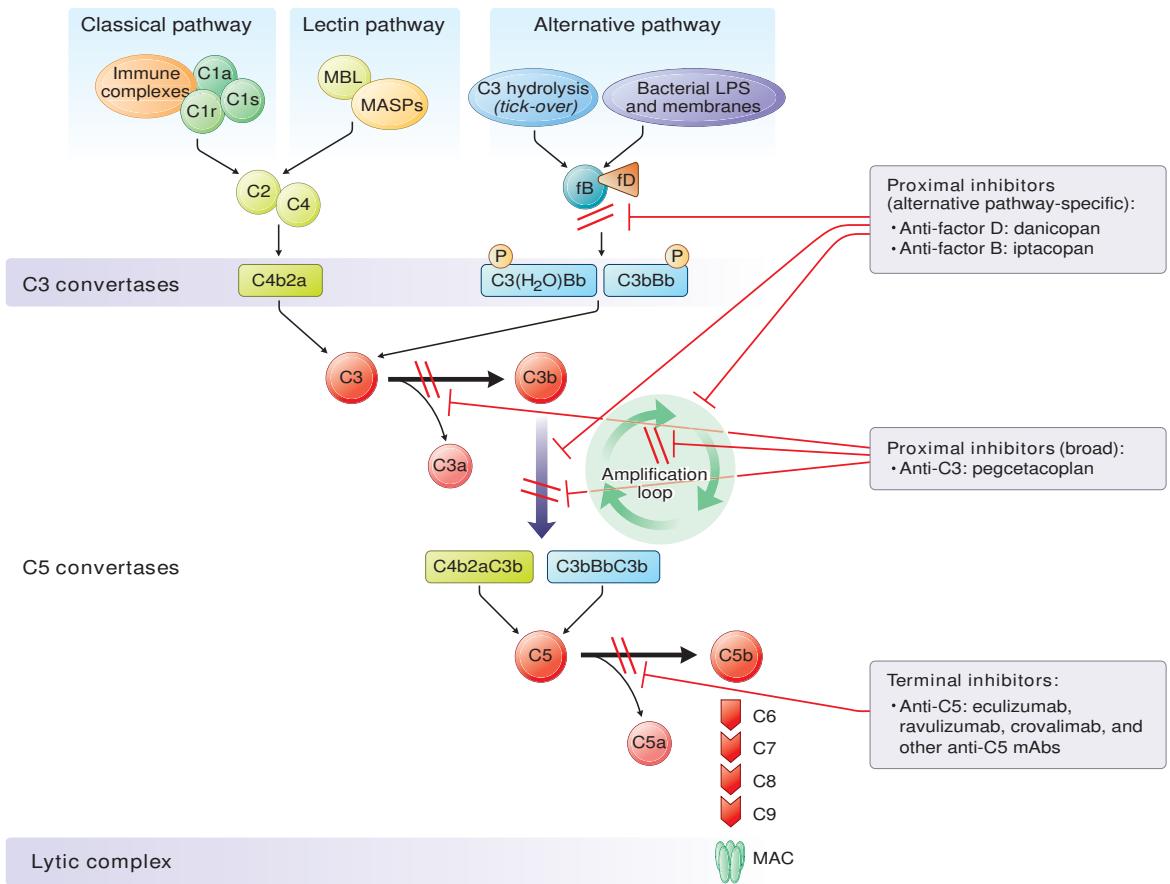


FIGURE 100-11 Monoclonal antibodies and small molecules in use or in development for the management of PNH and other complement-related disorders. Complement components are indicated by C followed by a number. MBL stands for mannose-binding lectin; MASPI for mannose-binding lectin-associated serine protease 1. P is properdin. Of the inhibitors shown on the right, only eculizumab and ravulizumab, which bind to C5 and are therefore inhibitors of the distal pathway, are already licensed drugs: both effectively abrogate MAC formation but they do not interfere with the formation of either the C3 convertase or the C5 convertase: in contrast, this can be achieved with the upstream inhibitors danicopan, iptacopan, and pegcetacoplan.

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101

Anemia Due to Acute Blood Loss

Dan L. Longo



Blood loss causes anemia by two main mechanisms: (1) by the direct loss of red cells; and (2) if the loss of blood is protracted, it will gradually deplete iron stores, eventually resulting in iron deficiency. The latter type of anemia is covered in Chap. 97. Here, we are concerned with the former type, that is, *posthemorrhagic anemia*, which follows *acute* blood loss. This can be *external* (e.g., after trauma or obstetric hemorrhage) or *internal* (e.g., from bleeding in the gastrointestinal tract, rupture of the spleen, rupture of an ectopic pregnancy, subarachnoid hemorrhage, leaking aneurysm). In any of these cases, after the sudden loss of a large amount of blood, there are three clinical/pathophysiologic stages. (1) At first, the dominant feature is hypovolemia, which poses a threat particularly to organs that normally have a high blood supply, like the brain and the kidneys; therefore, loss of consciousness and acute renal failure are major threats. It is important to note that at this stage an ordinary blood count will not show anemia because the hemoglobin concentration is not affected. On physical exam, tachycardia, tachypnea, decreased pulse pressure, cold skin that appears pale and mottled, and decreased urine output may be noted. (2) Next, as

an emergency response, baroreceptors and stretch receptors will cause release of vasopressin and other peptides, and the body will shift fluid from the extravascular to the intravascular compartment, producing hemodilution; thus, the hypovolemia gradually converts to anemia. The degree of anemia will reflect the amount of blood lost. If after 3 days the hemoglobin is, for example, 7 g/dL, it means that about half of the entire blood has been lost. (3) Provided bleeding does not continue, the bone marrow response will gradually ameliorate the anemia. In this phase of the process, the reticulocyte count and erythropoietin levels will be elevated. The physiologic increase in marrow red cell production reflected by the increase in reticulocytes is similar to the marrow response to hemolysis.

The diagnosis of acute posthemorrhagic anemia (APHA) is usually straightforward, although sometimes internal bleeding episodes (e.g., after a traumatic injury), even when large, may not be immediately obvious. Look for physical findings that may help localize the bleeding. Grey Turner sign (flank ecchymosis) may reflect retroperitoneal bleeding. Cullen sign (umbilical ecchymosis) may suggest intraperitoneal or retroperitoneal bleeding. Dullness to chest percussion may suggest intrapleural bleeding. Whenever an abrupt fall in hemoglobin has taken place, whatever history is given by the patient, APHA should be suspected. Supplementary history may have to be obtained by asking the appropriate questions, and appropriate investigations (e.g., a sonogram or an endoscopy) may have to be carried out.

TREATMENT

Anemia Due to Acute Blood Loss

In patients who are hemodynamically unstable, the usual airway, breathing, and circulation assessments take priority. In the face of bleeding associated with hypotension, pharmacologic support with vasopressors is critical. With respect to anemia treatment, a two-pronged approach is imperative. (1) In many cases, the blood lost needs to be replaced promptly. Unlike with many chronic anemias, when finding and correcting the cause of the anemia is the first priority and blood transfusion may not be even necessary because the body is adapted to the anemia, with acute blood loss, the reverse is true; because the body is not adapted to the anemia, blood transfusion takes priority. (2) While the emergency is being confronted, it is imperative to stop the hemorrhage and to eliminate its source.

In an acute hemorrhage situation, plasma may be preferred to saline for volume expansion since dilution of clotting factors with crystalloid may interfere with hemostasis.

A special type of APHA is blood loss during and immediately after surgery, which can be substantial (e.g., up to 2 L in the case of a radical prostatectomy). Of course with elective surgical procedures, the patient's own stored blood may be available (through preoperative autologous blood donation), and in any case, blood loss ought to have been carefully monitored/measured. The fact that this blood loss is iatrogenic dictates that ever more effort should be invested in optimizing its management. The special features of transfusion medicine are discussed in [Chap. 113](#).

A Holy Grail of emergency medicine for a long time has been the idea of a blood substitute that would be universally available, suitable for all recipients, easy to store and to transport, safe, and as effective as blood itself. Two main paths have been pursued: (1) fluorocarbon synthetic chemicals that bind oxygen reversibly, and (2) artificially modified hemoglobins, known as hemoglobin-based oxygen carriers (HBOCs). Although there are numerous anecdotal reports of the use of both approaches in humans, and although HBOCs have reached the stage of phase 2–3 clinical trials, no “blood substitute” has yet become standard treatment.

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102

Bone Marrow Failure Syndromes Including Aplastic Anemia and Myelodysplasia

Neal S. Young



Bone marrow failure diseases include aplastic anemia, myelodysplastic syndrome (MDS), pure red cell aplasia (PRCA), and myelophthysis. Hypoproliferative anemia is a cardinal feature of these disorders, but more frequent is *pancytopenia*: anemia, leukopenia, and thrombocytopenia. Low blood counts in marrow failure result from deficient hematopoiesis, as distinguished from blood count depression due to peripheral destruction of red cells (hemolytic anemias), platelets (idiopathic thrombocytopenic purpura [ITP] or due to splenomegaly), and granulocytes (as in the immune leukopenias). Marrow damage and dysfunction also may be secondary to infection, inflammation, or cancer.

Hematopoietic failure syndromes are classified by dominant morphologic features of the bone marrow ([Table 102-1](#)). Although practical distinction among these syndromes usually is clear from the marrow pathology, some processes are so closely related that the diagnosis may be complex. Separation between aplastic anemia and hypocellular MDS can be particularly difficult. Mutations on genomic screens may be etiologic or interpreted as risk factors. Patients may seem to suffer from two or three related diseases simultaneously, or one diagnosis may appear to evolve into another. Many of these syndromes share an immune-mediated mechanism of marrow destruction and some element of genomic instability resulting in a higher rate of malignant transformation.

It is important that the internist and general practitioner recognize the marrow failure syndromes because quality of life and ultimate prognosis may be poor if the patient is untreated; effective therapies are often available but sufficiently complicated in their choice and delivery so as to warrant the care of a hematologist or oncologist. While the identification of pathogenic mutations on genomic screen, often on testing ordered by the internist and pediatrician, has revolutionized the diagnosis of the marrow failure syndromes, these results often require the interpretation of the hematologist and oncologist.

APLASTIC ANEMIA

DEFINITION

Aplastic anemia is pancytopenia with bone marrow hypocellularity. Acquired aplastic anemia is distinguished from iatrogenic aplasia, from marrow hypocellularity after intensive cytotoxic chemotherapy for cancer, and from usually accidental physical and chemical injury, as in radiation poisoning. Aplastic anemia can also be constitutional. Genetic diseases such as Fanconi anemia and dyskeratosis congenita usually (but not always) present in early childhood and have typical physical anomalies. Telomere diseases (see [Chap. 469](#)) and hematologic manifestations of mutations in genes such as *GATA2*, *RUNX1*, and *MPL* can present as marrow failure in normal-appearing adults.

TABLE 102-1 Differential Diagnosis of Pancytopenia

Pancytopenia with Hypocellular Bone Marrow**Acquired aplastic anemia**

Constitutional aplastic anemia (Fanconi anemia, dyskeratosis congenita, and others)

Hypocellular myelodysplastic syndrome

Rare aleukemic leukemia

Some acute lymphoid leukemia

Rare lymphomas of bone marrow

Copper deficiency

Pancytopenia with Cellular Bone Marrow**Primary bone marrow diseases**

Myelodysplastic syndromes

Paroxysmal nocturnal hemoglobinuria (PNH)

Myelofibrosis

Aleukemic leukemia

Myelophthisis

Bone marrow lymphoma

Hairy cell leukemia

Secondary to systemic diseases

Systemic lupus erythematosus

Hypersplenism

B₁₂, folate deficiency

Copper deficiency

Alcohol

HIV infection

Brucellosis

Sarcoidosis

Tuberculosis

Leishmaniasis

Sepsis

Hypocellular Bone Marrow ± Pancytopenia

Q fever

Legionnaires' disease

Anorexia nervosa, starvation

Mycobacterium

Acquired aplastic anemia is often stereotypical in its manifestations, with the abrupt onset of low blood counts in a previously well young adult; seronegative hepatitis or a course of an incriminated medical drug may precede the onset. The diagnosis in these instances is uncomplicated. Sometimes blood count depression is moderate or incomplete, resulting in anemia, leukopenia, and thrombocytopenia in some combination. Aplastic anemia is related to both paroxysmal nocturnal hemoglobinuria (PNH; Chap. 100) and to MDS, and a clear distinction among these disorders may not be possible.

■ EPIDEMIOLOGY

The incidence of acquired aplastic anemia in Europe and Israel is two cases per million persons annually. In Thailand and China, rates of five to seven per million have been established. Men and women are affected with equal frequency, but the age distribution is biphasic, with the major peak in the teens and twenties and a second rise in older adults.

■ ETIOLOGY

The origins of aplastic anemia have been inferred from several recurring clinical associations (Table 102-2); unfortunately, these relationships are not reliable in an individual patient and may not be etiologic. In addition, although most cases of aplastic anemia are idiopathic, little other than history separates these cases from those with a presumed etiology such as a drug exposure.

Radiation Marrow aplasia is a major acute sequela of radiation. Radiation damages DNA; tissues dependent on active mitosis are particularly susceptible. Nuclear accidents involve not only power plant workers but also employees of hospitals, laboratories, and industry (food sterilization, metal radiography, etc.), as well as innocents exposed to stolen, misplaced, or misused sources. Whereas the radiation dose can be approximated from the rate and degree of decline in blood counts, dosimetry by reconstruction of the exposure can help to estimate the patient's prognosis and also to protect medical personnel

TABLE 102-2 Classification of Aplastic Anemia and Single Cytopenias

ACQUIRED	INHERITED/CONSTITUTIONAL
Aplastic Anemia	
Secondary	Fanconi anemia
Radiation	Dyskeratosis congenita/telomere disease
Drugs and chemicals	Shwachman-Diamond syndrome
Regular effects	Familial aplastic anemia/leukemia predisposition syndromes: <i>GATA2</i> , <i>RUNX1</i> , <i>CTLA4</i> , and others
Idiosyncratic reactions	
Viruses	Nonhematologic syndromes (Down, Dubowitz, Seckel)
Epstein-Barr virus (infectious mononucleosis)	
Hepatitis (non-A, non-B, non-C hepatitis)	
Parvovirus B19 (transient aplastic crisis, pure red cell aplasia [PRCA])	
HIV-1 (AIDS)	
Immune diseases	
Eosinophilic fasciitis	
Hypoimmunoglobulinemia	
Large granular lymphocytosis (LGL)	
Thymoma/thymic carcinoma	
Graft-versus-host disease in immunodeficiency	
Paroxysmal nocturnal hemoglobinuria (PNH)	
Pregnancy	
Idiopathic (immune)	
Cytopenias	
PRCA (see Table 102-4)	Congenital PRCA (Diamond-Blackfan anemia)
Neutropenia/agranulocytosis	Kostmann syndrome
Idiopathic	Shwachman-Diamond syndrome
Drugs, toxins	Reticular dysgenesis
LGL	
Pure white cell aplasia (+/- thymoma)	
Thrombocytopenia	Amegakaryocytic thrombocytopenia
Drugs, toxins	Thrombocytopenia with absent radii
Acquired amegakaryocytic thrombocytopenia	Other rare germline mutations

from contact with radioactive tissue and excreta. MDS and leukemia, but probably not aplastic anemia, are late effects of radiation.

Chemicals Benzene is a notorious cause of bone marrow failure: epidemiologic, clinical, and laboratory data link benzene to aplastic anemia, acute leukemia, and blood and marrow abnormalities. For leukemia, incidence is correlated with cumulative exposure, but susceptibility must also be important because only a minority of even heavily exposed workers develop myelotoxicity. The employment history is important, especially in industries where benzene is used for a secondary purpose, usually as a solvent. Benzene-related blood diseases have declined with regulation of industrial exposure. Although benzene is no longer generally available as a household solvent, exposure to its metabolites occurs in the normal diet and in the environment. The association between marrow failure and other chemicals is much less well substantiated. Further, there is scant direct evidence of marrow failure as a late effect of exposure, even to benzene.

Drugs (Table 102-3) Many chemotherapeutic drugs have marrow suppression as a major toxicity; effects are dose dependent and will

TABLE 102-3 Some Drugs and Chemicals Associated with Aplastic Anemia

Agents that regularly produce marrow depression as major toxicity in commonly used doses or normal exposures:
Cytotoxic drugs used in cancer chemotherapy: <i>alkylating agents, antimetabolites, antimitotics</i> , some antibiotics
Agents that frequently but not inevitably produce marrow aplasia:
Benzene
Agents associated with aplastic anemia but with a relatively low probability:
Chloramphenicol
Insecticides
Antiprotozoals: <i>quinacrine</i> and chloroquine, meprazine
Nonsteroidal anti-inflammatory drugs (including <i>phenylbutazone, indomethacin, ibuprofen, sulindac, aspirin</i>)
Anticonvulsants (<i>hydantoins, carbamazepine, phenacetamide, felbamate</i>)
Heavy metals (<i>gold, arsenic, bismuth, mercury</i>)
Sulfonamides: some antibiotics, antithyroid drugs (<i>methimazole, methylthiouracil, propylthiouracil</i>), antidiabetes drugs (<i>tolbutamide, chlorpropamide</i>), carbonic anhydrase inhibitors (<i>acetazolamide and methazolamide</i>)
Antihistamines (<i>cimetidine, chlorpheniramine</i>)
D-Penicillamine
Estrogens (in pregnancy and in high doses in animals)
Agents whose association with aplastic anemia is more tenuous:
Other antibiotics (<i>streptomycin, tetracycline, methicillin, mebendazole, trimethoprim/sulfamethoxazole, flucytosine</i>)
Sedatives and tranquilizers (<i>chlorpromazine, prochlorperazine, piperacetazine, chlordiazepoxide, meprobamate, methyprylon</i>)
Allopurinol
Methyldopa
Quinidine
Lithium
Guanidine
Potassium perchlorate
Thiocyanate
Carbamazole

Note: Terms set in italics show the most consistent association with aplastic anemia.

occur in all recipients. In contrast, idiosyncratic reactions to a large and diverse group of drugs may lead to aplastic anemia without a clear dose-response relationship. A large international study in Europe in the 1980s quantitated drug relationships, especially for nonsteroidal analgesics, sulfonamides, thyrostatic drugs, some psychotropics, penicillamine, allopurinol, and gold. Association does not equal causation: a drug may have been used to treat the first symptoms of bone marrow failure (antibiotics for fever or a preceding viral illness) or provoked the first symptom of a preexisting disease (petechiae by nonsteroidal anti-inflammatory agents administered to the thrombocytopenic patient). In the context of total drug use, idiosyncratic reactions, although individually devastating, are rare events. Risk estimates are usually lower when determined in population-based studies. Furthermore, the low absolute risk is also made more obvious: even a 10- or 20-fold increase in risk translates, in a rare disease, to just a handful of drug-induced aplastic anemia cases among hundreds of thousands of exposed persons.

Infections Transient, mild blood count depression is frequent in the course of many viral and bacterial infections. Aplastic anemia can rarely follow infectious mononucleosis. Parvovirus B19 does not usually cause generalized bone marrow failure.

Immunologic Diseases Aplasia is a major consequence and the inevitable cause of death in *transfusion-associated graft-versus-host disease* (GVHD) that can occur after infusion of nonirradiated blood

products to an immunodeficient recipient. Aplastic anemia is strongly associated with the rare collagen vascular syndrome eosinophilic fascitis that is characterized by painful induration of subcutaneous tissues (Chap. 360). Thymoma and hypoimmunoglobulinemia are occasional associations with aplastic anemia. Pancytopenia with marrow hypoplasia can also occur in systemic lupus erythematosus (SLE).

Hepatitis Posthepatitis marrow failure accounts for 5% of etiologies in most series. Patients are usually young men who have recovered from a bout of liver inflammation 1–2 months earlier; the subsequent pancytopenia is very severe. The hepatitis is seronegative (non-A, non-B, non-C); intensive laboratory efforts including deep sequencing have not disclosed an infectious agent, and the hepatitis is presumed to be immune-mediated. Fulminant liver failure in childhood can follow seronegative hepatitis, and marrow failure occurs at a high rate in these patients.

Pregnancy Aplastic anemia very rarely may occur and recur during pregnancy and resolve with delivery or with spontaneous or induced abortion.

Paroxysmal Nocturnal Hemoglobinuria An acquired mutation in the *PIG-A* gene in a hematopoietic stem cell is required for the development of PNH, but *PIG-A* mutations probably occur commonly in normal individuals. If the *PIG-A* mutant stem cell proliferates, the result is a clone of progeny deficient in glycosylphosphatidylinositol-linked cell surface membrane proteins (Chap. 100). Small clones of deficient cells can be detected by sensitive flow cytometry tests in one-half or more of patients with aplastic anemia at the time of presentation. Functional studies of bone marrow from PNH patients, even those with mainly hemolytic manifestations, show evidence of defective hematopoiesis. Patients with an initial clinical diagnosis of PNH, especially younger individuals, may later develop frank marrow aplasia and pancytopenia; patients with an initial diagnosis of aplastic anemia may suffer later from hemolytic PNH years after recovery of blood counts.

Constitutional Syndromes Fanconi anemia, an autosomal recessive disorder, manifests as congenital developmental anomalies, progressive pancytopenia, and an increased risk of malignancy. Chromosomes in Fanconi anemia are susceptible to DNA cross-linking agents, the basis for a diagnostic assay. Patients with Fanconi anemia typically have short stature, café au lait spots, and anomalies involving the thumb, radius, and genitourinary tract. At least 17 different genetic defects (all but one with an identified gene) have been defined; the most common, type A Fanconi anemia, is due to a mutation in *FANCA*. Most of the Fanconi anemia gene products form a protein complex that activates *FANCD2* by monoubiquitination to play a role in the cellular response to DNA damage and especially interstrand cross-linking.

Diamond-Blackfan anemia (see below) and Shwachman-Diamond syndrome are ribosomopathies, genetic defects in ribosome assembly that are tissue specific. In Shwachman-Diamond syndrome, presentation is early in life with neutropenia, pancreatic insufficiency, and malabsorption; most patients have compound heterozygous mutations in *SBDS*.

In the telomeropathies, inherited genetic defects alter telomere repair or one of the shelterin protein components of the telomere. The pediatric syndrome dyskeratosis congenita is characterized by the triad of mucous membrane leukoplakia, dystrophic nails, reticular hyperpigmentation, and early development of aplastic anemia (Chap. 469). Dyskeratosis congenita is due to mutations in genes of the telomere repair complex, which acts to maintain telomere length in replicating cells: the X-linked variety is due to mutations in the *DKC1* (*dyskerin*) gene; the more unusual autosomal dominant type is due to mutation in *TERC*, which encodes an RNA template. Rarely, mutations can also occur in genes such as *TNF2* that encode shelterin proteins, which bind telomere DNA.

Mutations in *TERC* and *TERT*, which encode the catalytic reverse transcriptase telomerase, have subtle and milder effects on hematopoietic function, and presentation in adults is not unusual. It manifests as moderate aplastic anemia, which can be chronic and not progressive,

and isolated macrocytic anemia or thrombocytopenia. Physical anomalies are usually not present, but early hair graying is a clue to the diagnosis. A detailed personal and family history may disclose pulmonary fibrosis and hepatic cirrhosis. Variable penetrance means that *TERT* and *TERC* mutations represent risk factors for marrow failure, as family members with the same mutations may have normal or only slight hematologic abnormalities but more subtle evidence of (compensated) hematopoietic insufficiency. Measurement of telomere length of peripheral blood leukocytes is a commercially available functional test.

■ PATHOPHYSIOLOGY

Bone marrow failure results from severe damage to the hematopoietic cell compartment. In aplastic anemia, replacement of the bone marrow by fat is apparent in the morphology of the biopsy specimen (Fig. 102-1) and magnetic resonance imaging (MRI) of the spine. Cells bearing the CD34 antigen, a marker of early hematopoietic cells, are greatly diminished, and in functional studies, committed and primitive progenitor cells are virtually absent; *in vitro* assays have suggested that the stem cell pool is reduced to $\leq 1\%$ of normal in severe disease at the time of presentation.

Constitutional Genetic Syndromes

An intrinsic stem cell defect exists for the constitutional aplastic anemias: in a critical DNA repair pathway in Fanconi anemia, manifested in the laboratory as chromosome damage and cell death on exposure to certain chemical agents. In the telomeropathies, inability to repair telomeres or to protect chromosome ends is the result of mutations in genes of the telomerase complex or the shelterin proteins; telomere defects limit the cell's capacity to proliferate. Mutations in the *GATA* and *RUNX* genes affect signal transduction and transcriptional regulation in hematopoietic gene networks.

Chemical and Drug Injury Extrinsic damage to the marrow follows massive physical or chemical insults such as high doses of radiation and toxic chemicals. For the more common idiosyncratic reaction to modest doses of medical drugs, altered drug metabolism has been invoked as a mechanism. The metabolic pathways of many drugs and chemicals, especially if they are polar and have limited water solubility, involve enzymatic degradation to highly reactive electrophilic compounds; these intermediates are toxic because of their propensity to bind to cellular macromolecules. For example, derivative hydroquinones and quinolones are responsible for benzene-induced tissue injury. Excessive generation of toxic intermediates or failure to detoxify the intermediates may be genetically determined and apparent only on specific drug challenge; the complexity and specificity of the pathways imply multiple susceptibility loci and would provide an explanation for the rarity of idiosyncratic drug reactions.

Immune-Mediated Stem Cell Destruction The recovery of marrow function in some patients prepared for bone marrow transplantation with antilymphocyte globulin first suggested that aplastic anemia might be immune mediated. Laboratory data, including animal models, support an important role for the immune system in aplastic anemia. Blood and bone marrow cells of patients can suppress normal hematopoietic progenitor cell growth, and removal of T cells from aplastic anemia bone marrow improves hematopoiesis *in vitro*. Increased numbers of activated cytotoxic T-cell clones usually

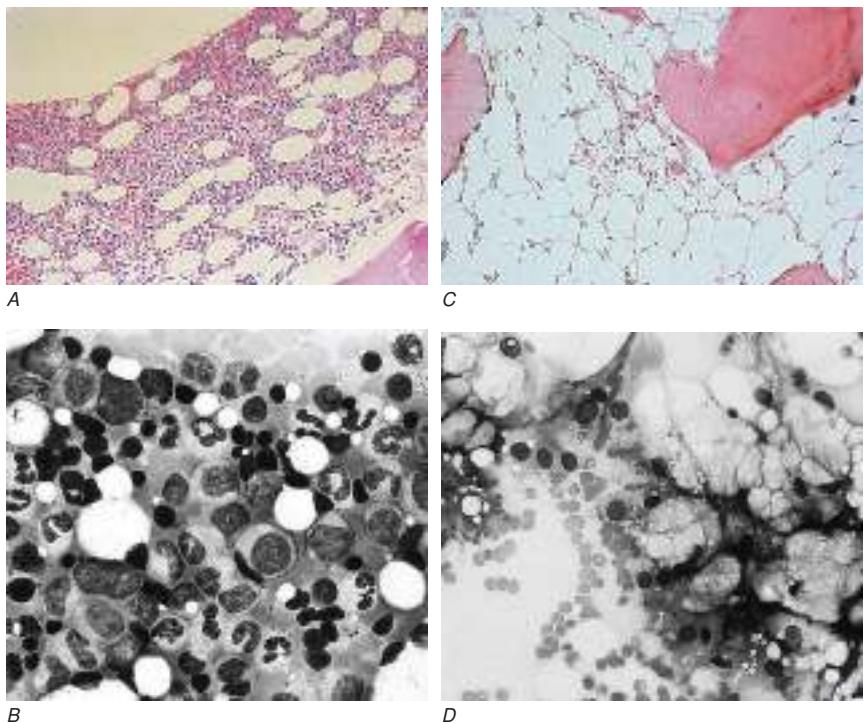


FIGURE 102-1 Normal and aplastic bone marrow. *A*, Normal bone marrow biopsy. *B*, Normal bone marrow aspirate smear. The marrow is normally 30–70% cellular, and there is a heterogeneous mix of myeloid, erythroid, and lymphoid cells. *C*, Aplastic anemia biopsy. *D*, Marrow smear in aplastic anemia. The marrow shows replacement of hematopoietic tissue by fat and only residual stromal and lymphoid cells.

decline with successful immunosuppressive therapy; type 1 cytokines are implicated; and interferon γ (IFN- γ) induces Fas expression on CD34 cells, leading to apoptotic cell death. Hematopoietic stem cells that have lost human leukocyte antigen (HLA) expression may be selectively expanded. The rarity of aplastic anemia despite common exposures (medicines, seronegative hepatitis) suggests that genetically determined features of the immune response can convert a normal physiologic response into a sustained abnormal autoimmune process, including polymorphisms in histocompatibility antigens, cytokine genes, and genes that regulate T-cell polarization (maturation toward helper or cytotoxic phenotypes) and effector function.

■ CLINICAL FEATURES

History Aplastic anemia can appear abruptly or insidiously. Bleeding is the most common early symptom; a complaint of days to weeks of easy bruising, oozing from the gums, nose bleeds, heavy menstrual flow, and sometimes petechiae will have been noticed. With thrombocytopenia, massive hemorrhage is unusual, but small amounts of bleeding in the central nervous system can result in catastrophic intracranial or retinal hemorrhage. Symptoms of anemia are also frequent, including lassitude, weakness, shortness of breath, and a pounding sensation in the ears. Infection is an unusual first symptom in aplastic anemia (unlike in agranulocytosis, where pharyngitis, anorectal infection, or frank sepsis occurs early). Patients often feel and look remarkably well despite drastically reduced blood counts. Systemic complaints and weight loss should point to other etiologies of pancytopenia. Prior medical drug use, chemical exposure, and preceding viral illnesses must often be elicited with directed questioning. A family history of hematologic diseases or blood abnormalities, of pulmonary or liver fibrosis, or of early hair graying points to a telomeropathy; a family history of unusual infections and warts points to *GATA2* deficiency.

Physical Examination Petechiae and ecchymoses are typical, and retinal hemorrhages may be present. Pelvic and rectal examinations

can often be deferred but, when performed, should be undertaken with great gentleness to avoid trauma; these may show bleeding from the cervical os and blood in the stool. Pallor of the skin and mucous membranes is common. Infection on presentation is unusual but may occur if the patient has been symptomatic for a few weeks. Lymphadenopathy and splenomegaly are highly atypical of aplastic anemia. Café au lait spots and short stature suggest Fanconi anemia; peculiar nails and leukoplakia suggest dyskeratosis congenita; early graying (and use of hair dyes to mask it!) suggests a telomerase defect.

■ LABORATORY STUDIES

Blood The smear shows large erythrocytes and a paucity of platelets and granulocytes. Mean corpuscular volume (MCV) is commonly increased. Reticulocytes are absent or few, and lymphocyte numbers may be normal or reduced. The presence of immature myeloid forms suggests leukemia or MDS; nucleated red blood cells (RBCs) suggest marrow fibrosis or tumor invasion; abnormal platelets suggest either peripheral destruction or MDS.

Bone Marrow The bone marrow is usually readily aspirated but dilute on smear, and the fatty biopsy specimen may be grossly pale on withdrawal; a ‘dry tap’ instead suggests fibrosis or myelophthisis. In severe aplasia, the smear of the aspirated specimen shows only red cells, residual lymphocytes, and stromal cells; the biopsy (which should be >1 cm in length) is superior for determination of cellularity and shows mainly fat under the microscope, with hematopoietic cells occupying <25% of the marrow space; sometimes, the biopsy is virtually all fat. The correlation between marrow cellularity and disease severity is imperfect; patients with moderate disease by blood counts can have empty iliac crest biopsies, whereas ‘hot spots’ of hematopoiesis may be seen in severe cases. Residual hematopoietic cells should have normal morphology, except for mildly megaloblastic erythropoiesis; megakaryocytes are greatly reduced and usually absent. Granulomas may indicate an infectious etiology of the marrow failure.

Ancillary Studies Chromosome breakage studies of peripheral blood using diepoxybutane or mitomycin C should be performed on children and younger adults to exclude Fanconi anemia. Very short telomere length strongly suggests the presence of a telomerase or shelterin mutation, which can be pursued by family studies and nucleotide sequencing. Chromosome studies of bone marrow cells are often revealing in MDS but should be negative in typical aplastic anemia. Flow cytometry offers a sensitive diagnostic test for PNH. Serologic studies may show evidence of recent viral infection, such as Epstein-Barr virus and HIV. Posthepatitis aplastic anemia is seronegative.

Genomics Next-generation sequencing allows for large number of genes to be tested for the presence of pathogenic mutations. Panels are available commercially and in certified academic laboratories. While expensive, they are very useful and sometimes critical in establishing the correct diagnosis. Germline gene panels examine 50 or more genes etiologic in constitutional bone marrow failure, including many for which functional assays (described above) are not available. A germline panel should be considered for all children and those adults with suggestive clinical features or family histories. Somatic mutations are sought when MDS is suspected. Myeloid neoplasm gene panels can query about 100 genes that are recurrently mutated in MDS and acute myeloid leukemia (AML). Pathogenic mutations in spliceosome genes and genes in the cohesion family are frequent in MDS and unexpected in aplastic anemia.

■ DIAGNOSIS

The diagnosis of aplastic anemia is usually straightforward, based on the combination of pancytopenia with a fatty bone marrow. Aplastic anemia is a disease of the young and should be a leading diagnosis in the pancytopenic adolescent or young adult. When pancytopenia is secondary, the primary diagnosis is usually obvious from either history or physical examination: the massive spleen of alcoholic cirrhosis, the history of metastatic cancer or SLE, or miliary tuberculosis on chest radiograph (Table 102-1).

Diagnostic problems can occur with atypical presentations and among related hematologic diseases. Patients with bone marrow hypocellularity may have depression of only one or two of three blood lines, with later progression to pancytopenia. The most important differential diagnoses are between acquired and constitutional aplastic anemia, and between aplastic anemia and MDS. The bone marrow in constitutional aplastic anemia is usually morphologically indistinguishable from the aspirate in acquired disease (an exception is *GATA2* deficiency with its characteristic megakaryocyte atypia). The diagnosis can be suggested by family history, abnormal blood counts since childhood, or the presence of associated, sometimes subtle physical anomalies. Genomic testing for pathogenic mutations in genes etiologic in constitutional marrow failure syndromes can discriminate acquired from inherited aplastic anemia (but results may not return for several weeks, a problem in the severely pancytopenic patient). Acute myeloid leukemia (AML) and MDS in a pedigree should prompt screening for an inherited predisposition syndrome, such as *RUNX1* mutations. Aplastic anemia may be difficult to distinguish from the hypocellular variety of MDS: MDS is favored by finding morphologic abnormalities, particularly of megakaryocytes and myeloid precursor cells, and typical cytogenetic abnormalities and somatic mutations on genomic screening of myeloid neoplasm genes (see above). There remains an unclear boundary between immune aplastic anemia and low-risk MDS: patients with deletion of 13q and 20q may respond well to immunosuppression, and mutations in genes such as *DNMT3A* and *ASXL1* occur in both diseases.

■ PROGNOSIS

The natural history of severe aplastic anemia is rapid deterioration and death. Historically, provision first of RBCs and later of platelet transfusions and effective antibiotics were of some benefit, but few patients show spontaneous recovery. The major prognostic determinant is the blood count. Severe disease historically has been defined by the presence of two of three parameters: absolute neutrophil count <500/ μ L, platelet count <20,000/ μ L, and corrected reticulocyte count <1% (or absolute reticulocyte count <60,000/ μ L). In the era of effective immunosuppressive therapies, absolute numbers of reticulocytes (>25,000/ μ L) and lymphocytes (>1000/ μ L) may be better predictors of response to treatment and long-term outcome.

Other prognostic factors include the presence of a PNH clone, short telomeres on presentation, and somatically mutated white cells. Even small PNH clones may indicate an immune pathophysiology and responsiveness to immunosuppressive therapies. Telomere shortening in most patients likely reflects stem cell reserve, regenerative stress, and susceptibility to chromosomal instability. Collectively, the presence of mutations in the same myeloid neoplasia genes that are mutated in clonal hematopoiesis of indeterminate potential (*ASXL1*, *DNMT3A*) is associated with worse prognosis and clonal evolution.

TREATMENT

Aplastic Anemia

Severe acquired aplastic anemia can be cured by replacement of the absent hematopoietic cells (and the immune system) by stem cell transplant, or it can be ameliorated by suppression of the immune system to allow recovery of the patient’s residual bone marrow function. Glucocorticoids are not of value as primary therapy. Suspected exposures to drugs or chemicals should be discontinued; however, spontaneous recovery of severe blood count depression is rare, and a waiting period before beginning treatment may not be advisable unless the blood counts are only modestly depressed.

HEMATOPOIETIC STEM CELL TRANSPLANTATION

This is the first choice for the younger patient with a fully histocompatible sibling donor (Chap. 114). HLA typing should be ordered as soon as the diagnosis of aplastic anemia is established in a child or younger adult. In transplant candidates, transfusion of blood from family members should be avoided so as to prevent sensitization to histocompatibility antigens. In general, limited numbers of blood

products probably do not greatly affect outcome, especially when blood products are depleted of leukocytes. For allogeneic transplant from fully matched siblings, long-term survival rates for children are 90%. Transplant morbidity and mortality are increased among adults, due to the higher risk of chronic GVHD and infections. Nevertheless, transplant should be considered early in all but the most elderly, including from alternative donors.

Most patients do not have a suitable sibling donor. Occasionally, a full phenotypic match can be found within the family and serve as well. Matched unrelated donors in large registries are available for the majority of Caucasian patients. With high-resolution matching at HLA, outcomes are similar to those with sibling donors, although complications (mainly GVHD and infection) are more frequent. Cord blood also can be a source of stem cells, especially for children. Matched unrelated donor transplants are often considered as initial treatment in children and as salvage therapy for adults after failed immunosuppression. Transplantation from an HLA haploidentical family donor is increasingly popular, as a donor is almost always quickly available. There is large experience in China, where lymphocyte depletion is usually performed before donor cell infusion. Posttransplant cyclophosphamide appears effective in preventing GVHD. Transplant protocols for marrow failure now usually do not include radiation in order to avoid late occurrence of cancer.

IMMUNOSUPPRESSION

The standard regimen of antithymocyte globulin (ATG) in combination with cyclosporine induces hematologic recovery (independence from transfusion and a leukocyte count adequate to prevent infection) in 60–70% of patients. Children do especially well, whereas older adult patients can suffer complications due to the presence of comorbidities. An early robust hematologic response correlates with long-term survival. Improvement in granulocyte number is generally apparent within 2 months of treatment. Most recovered patients continue to have some degree of blood count depression, the MCV remains elevated, and bone marrow cellularity returns toward normal very slowly if at all. Relapse (recurrent pancytopenia) is frequent, often occurring as cyclosporine is tapered or discontinued; most, but not all, patients respond to reinstitution of immunosuppression, but some responders become dependent on continued cyclosporine administration. “Clonal evolution,” isolated chromosomal abnormalities or the development of MDS, with typical cytogenetic aberrations and abnormal marrow morphology, occurs in 15% of treated patients over a decade following initiation of ATG, usually but not invariably associated with a return of pancytopenia, and some patients develop leukemia. A laboratory diagnosis of PNH can generally be made at the time of presentation of aplastic anemia by flow cytometry; recovered patients may have frank hemolysis if the PNH clone expands. Bone marrow examinations should be performed if there is an unfavorable change in blood counts.

Horse ATG is administered as intravenous infusions and requires hospitalization. Rabbit ATG is much less effective, perhaps because it reduces T-regulatory cell numbers in patients. Serum sickness, a flulike illness with a characteristic cutaneous eruption and arthralgia, may develop 10 days after initiating treatment. Methylprednisolone is administered with ATG to ameliorate the immune consequences of heterologous protein infusion. (Excessive or extended glucocorticoid therapy is associated with avascular joint necrosis.) Cyclosporine is administered orally at an initial high dose, with subsequent adjustment according to blood levels. Its most important side effects are nephrotoxicity, hypertension, and seizures.

Most patients with aplastic anemia lack a suitable marrow donor, and immunosuppression is the treatment of choice. Overall survival is equivalent with transplantation and immunosuppression. However, successful transplant cures marrow failure, whereas patients who recover adequate blood counts after immunosuppression remain at risk of relapse and malignant evolution. Increasing age

and the severity of neutropenia are the most important factors weighing in the decision between transplant and immunosuppression in adults who have a matched family donor: older patients do better with ATG and cyclosporine, whereas transplant is preferred if neutropenia is profound.

ELTROMBOPAG

Hematopoietic growth factors (HGFs) such as erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF) are not effective in aplastic anemia, probably because endogenous blood levels in patients are extremely high. Circulating thrombopoietin is also elevated, but a thrombopoietin mimetic showed unexpected activity in refractory disease, producing robust, trilineage, and usually durable hematologic responses. Likely the mechanism of action of thrombopoietin mimetics is stimulation of the hematopoietic stem cell, but iron chelation and increased regulatory T cells are also possibly beneficial effects. Eltrombopag added to first-line immunosuppression with horse ATG markedly increased overall and complete response rates, to about 80% and 50%, respectively. Eltrombopag is approved by the U.S. Food and Drug Administration (FDA) as monotherapy for refractory aplastic anemia and in combination with horse ATG and cyclosporine as initial therapy.

Transplant from a suitable donor is preferred in the young patient, whereas immunosuppression is preferred in the older adult. Even heavily transfused and infected patients in whom immunosuppression has failed can be salvaged by stem cell transplant later.

ANDROGENS

The effectiveness of androgens has not been verified in controlled trials, but occasional patients will respond or even demonstrate blood count dependence on continued therapy. Sex hormones upregulate telomerase gene activity in vitro, which is possibly also their mechanism of action in improving marrow function. For patients with moderate disease, especially if a telomere gene defect is present, a 3- to 4-month trial may improve all blood counts (Chap. 470).

SUPPORTIVE CARE

Meticulous medical attention is required so that the patient may survive to benefit from definitive therapy or, having failed treatment, to maintain a reasonable existence in the face of pancytopenia. First and most important, infection in the presence of severe neutropenia must be aggressively treated by prompt institution of parenteral, broad-spectrum antibiotics. Therapy is empirical and must not await results of culture, although specific foci of infection such as oropharyngeal or anorectal abscesses, pneumonia, sinusitis, and typhlitis (necrotizing colitis) should be sought on physical examination and with radiographic studies. When indwelling plastic catheters become contaminated, vancomycin should be added. Persistent or recrudescent fever implies fungal disease: *Candida* and *Aspergillus* are common, especially after several courses of antibacterial antibiotics. A major reason for the improved prognosis in aplastic anemia has been the development of better antifungal drugs and the timely institution of such therapy when infection is suspected. Granulocyte transfusions can be effective when bacterial or fungal infection is progressive or refractory to antibiotics. Hand washing, the single best method of preventing the spread of infection, remains a neglected practice. Nonabsorbed antibiotics for gut decontamination are poorly tolerated and unproven, nor does reverse isolation reduce mortality from infections.

Both platelet and erythrocyte numbers can be maintained by transfusion. Alloimmunization historically limited the usefulness of platelet transfusions and is now minimized by several strategies, including use of single donors to reduce exposure and physical or chemical methods to diminish leukocytes in the product; HLA-matched platelets are usually effective in patients refractory to random donor products. Inhibitors of fibrinolysis such as aminocaproic acid have not been shown to relieve mucosal oozing; the use of low-dose glucocorticoids to induce “vascular stability” is unproven and not recommended. With prophylactic platelet transfusions, the

goal is to maintain the platelet count >10,000/ μ L (oozing from the gut increases sharply at counts <5000/ μ L). Menstruation should be suppressed either by oral estrogens or nasal follicle-stimulating hormone/luteinizing hormone antagonists. Aspirin and other nonsteroidal anti-inflammatory agents must be avoided in the presence of thrombocytopenia.

RBCs should be transfused so as to allow patient a normal level of activity, usually at a hemoglobin value of 70 g/L (90 g/L if there is underlying cardiac or pulmonary disease); a regimen of 2 units every 2 weeks will replace normal losses in a patient without a functioning bone marrow. In chronic anemia, the iron chelators deferoxamine and deferasirox should be added at approximately the fiftieth transfusion to avoid secondary hemochromatosis.

PURE RED CELL APLASIA

Other more restricted forms of marrow failure occur, in which only a single cell type is affected and the marrow shows corresponding absence or decreased numbers of specific precursor cells: aregenerative anemia as in PRCA (see below), thrombocytopenia with amegakaryocytosis (*Chap. 115*), and neutropenia without marrow myeloid cells in agranulocytosis (*Chap. 64*). In general, and in contrast to aplastic anemia and MDS, the unaffected lineages appear quantitatively and qualitatively normal. Agranulocytosis, the most frequent of these syndromes, is usually a complication of medical drug use, either by a mechanism of direct chemical toxicity or by immune destruction. Agranulocytosis has an incidence similar to aplastic anemia (but geographically more frequent in Europe than in Asia); in contrast to aplastic anemia, agranulocytosis is more prevalent among older adults and in women. Agranulocytosis should resolve with discontinuation of exposure, but significant mortality is attached to neutropenia in the older and often previously unwell patient. Both pure white cell aplasia (agranulocytosis without incriminating drug exposure) and amegakaryocytic thrombocytopenia are exceedingly rare and, like PRCA, appear to be due to a destructive immune response. In all of the single-lineage failure syndromes, progression to pancytopenia or leukemia is unusual.

DEFINITION AND DIFFERENTIAL DIAGNOSIS

PRCA is characterized by anemia, reticulocytopenia, and absent or rare erythroid precursor cells in the bone marrow. The classification of PRCA is shown in *Table 102-4*. In adults, PRCA is acquired. An identical syndrome can occur constitutionally: Diamond-Blackfan anemia, or congenital PRCA, is diagnosed at birth or in early childhood and often responds to glucocorticoid treatment; mutations in ribosome protein genes are etiologic. Temporary red cell failure occurs in transient aplastic crisis of hemolytic anemias due to acute parvovirus infection (*Chap. 197*) and in transient erythroblastopenia of childhood, which occurs in normal children.

CLINICAL ASSOCIATIONS AND ETIOLOGY

PRCA has important associations with immune system diseases. A minority of cases occur with a thymoma. More frequently, red cell aplasia can be the major manifestation of large granular lymphocytosis or complicates chronic lymphocytic leukemia. Some patients may be hypogammaglobulinemic. A ribosomal protein gene is deleted in the 5q- syndrome, such that the MDS may manifest as an acquired red cell aplasia. Occasionally (as compared to agranulocytosis), PRCA can be due to an idiosyncratic drug reaction. Subcutaneous administration of EPO has provoked PRCA mediated by neutralizing antibodies to the hormone. PRCA due to antibodies to blood group antigens (isoagglutins) is a complication of allogeneic stem cell transplant. For most PRCAs, T-cell inhibition is probably the prevalent immune mechanism.

PERSISTENT PARVOVIRUS B19 INFECTION

Chronic parvovirus infection is a treatable cause of red cell aplasia. This common virus causes a benign exanthem of childhood (fifth disease) and a polyarthralgia/arthritis syndrome in adults. In patients

TABLE 102-4 Classification of Pure Red Cell Aplasia

Self-limited	
	Transient erythroblastopenia of childhood
	Transient aplastic crisis of hemolysis (acute B19 parvovirus infection)
Fetal red blood cell aplasia	
	Nonimmune hydrops fetalis (in utero B19 parvovirus infection)
Constitutional pure red cell aplasia	
	Congenital pure red cell aplasia (Diamond-Blackfan anemia)
Acquired pure red cell aplasia	
	MDS (5q- syndrome)
	Cancer
	Thymoma
	Lymphoid malignancies (and more rarely other hematologic diseases)
	Paraneoplastic to solid tumors
Connective tissue disorders with immunologic abnormalities	
	Systemic lupus erythematosus, juvenile rheumatoid arthritis, rheumatoid arthritis
	Multiple endocrine gland insufficiency
Viruses	
	Persistent B19 parvovirus, hepatitis, adult T-cell leukemia virus, Epstein-Barr virus
Pregnancy	
Drugs	
	Especially phenytoin, azathioprine, chloramphenicol, procainamide, isoniazid
Antibodies to erythropoietin	
Idiopathic (immune)	

with underlying hemolysis (or any condition that increases demand for RBC production), parvovirus infection can cause a transient aplastic crisis and an abrupt but temporary worsening of the anemia due to failed erythropoiesis. In normal individuals, acute infection is resolved by production of neutralizing antibodies to the virus, but in the setting of congenital, acquired, or iatrogenic immunodeficiency, persistent viral infection may occur. The bone marrow shows red cell aplasia and the presence of giant pronormoblasts (*Fig. 102-2*), which is the cytopathic sign of B19 parvovirus infection. Viral tropism for human erythroid progenitor cells is due to its use of erythrocyte P antigen as a cellular receptor for entry. Direct cytotoxicity of virus causes anemia if demands on erythrocyte production are high; in normal individuals, the temporary cessation of red cell production is not clinically apparent, and skin and joint symptoms are mediated by immune complex deposition.

TREATMENT

Pure Red Cell Aplasia

History, physical examination, and routine laboratory studies may disclose an underlying disease or a drug exposure. Thymoma should be sought by radiographic procedures; tumor excision is indicated, but anemia does not necessarily improve with surgery. The diagnosis of parvovirus infection requires detection of viral DNA sequences in the blood (IgG and IgM antibodies are commonly absent). The presence of erythroid colonies has been considered predictive of response to immunosuppressive therapy in idiopathic PRCA.

Red cell aplasia is compatible with long-term survival with supportive care alone: a combination of erythrocyte transfusions and iron chelation. For persistent B19 parvovirus infection, almost all patients respond to intravenous immunoglobulin therapy. The majority of patients with acquired PRCA respond favorably to immunosuppression: glucocorticoids, cyclosporine, ATG, azathioprine, and cyclophosphamide are effective.

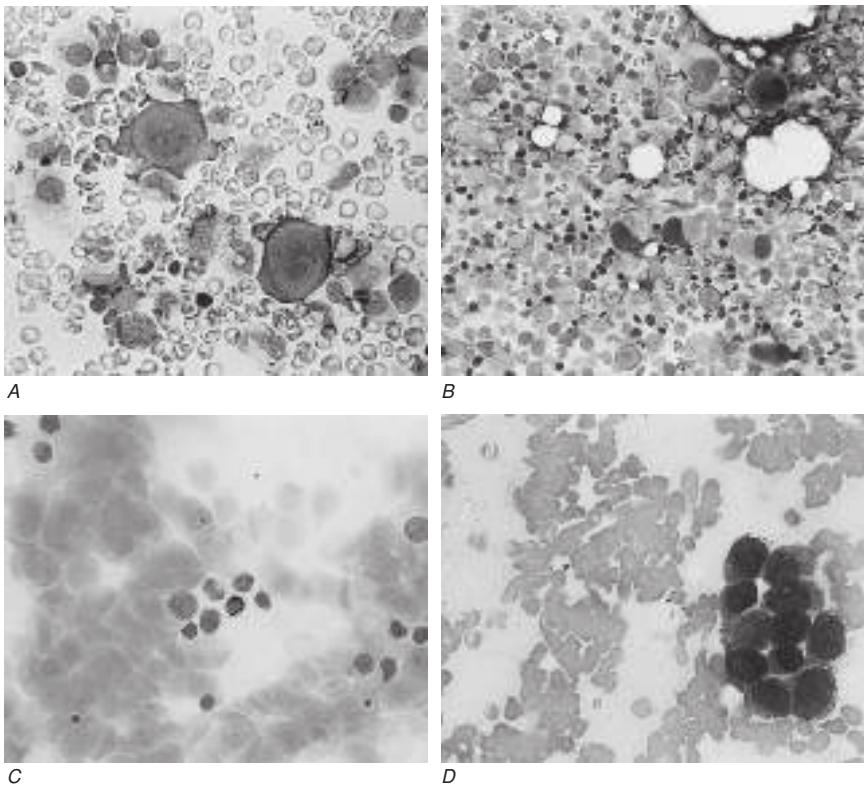


FIGURE 102-2 Pathognomonic cells in marrow failure syndromes. *A*, Giant pronormoblast, the cytopathic effect of B19 parvovirus infection of the erythroid progenitor cell. *B*, Uninuclear megakaryocyte and microblastic erythroid precursors typical of the 5q- myelodysplasia syndrome. *C*, Ringed sideroblast showing perinuclear iron granules. *D*, Tumor cells present on a touch preparation made from the marrow biopsy of a patient with metastatic carcinoma.

MYELODYSPLASTIC SYNDROMES

■ DEFINITION

The MDS are a heterogeneous group of hematologic disorders characterized by both (1) cytopenias due to bone marrow failure and (2) a high risk of development of AML. Anemia due to ineffective erythropoiesis, often with thrombocytopenia and neutropenia, occurs with dysmorphic (abnormal appearing) and usually cellular bone marrow, or with specific chromosome abnormalities or acquired mutations. In patients with “low-risk” MDS, marrow failure dominates the clinical course. In other patients, myeloblasts are present at diagnosis, chromosomes are abnormal, and the “high risk” is due to leukemic progression. MDS may be fatal due, most often, to complications of pancytopenia or to progression to leukemia, but a large proportion of patients will die of concurrent disease, the comorbidities typical in an elderly population. A useful nosology of these often-confusing entities was first developed by the French-American-British Cooperative Group in 1983. Five subtypes were defined then: refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-t), and chronic myelomonocytic leukemia (CMML). The World Health Organization (WHO) classification (2002) recognized that the distinction between RAEB-t and AML was arbitrary, grouped them together as acute leukemia, and clarified that CMML behaves as a myeloproliferative disease. The current WHO classification of 2016 is more refined but also more complicated (**Table 102-5**): blast percentage remains critical in defining MDS categories; erythroid predominant leukemias are now largely regarded as MDS; defining cytogenetic abnormalities are reaffirmed; and a single somatic mutation, in *SF3B1*, is now a feature of sideroblastic anemias. Identification of somatically mutated genes and their correlation with

clinical outcomes will be increasingly important in defining classification, prognosis, and targeting therapy.

The diagnosis of MDS can be a challenge, even for the expert, because sometimes subtle clinical and pathologic features must be distinguished, and precise diagnostic categorization requires a hematopathologist knowledgeable in the latest classification scheme. Unfortunately, agreement among pathologists on morphologic features and classification is imperfect; changes in the appearance of megakaryocytes are more reliable than loss of granules in neutrophil precursors or dyserythropoiesis. Further, dysplastic changes can be observed in normal individuals, and they can occur with vitamin deficiencies and as drug effects. Genomic testing is increasingly routine and can be difficult to interpret, as in differences between somatic and germline mutations, pathogenic mutations versus those of unknown significance (clonal hematopoiesis increases in frequency with age and involves genetic changes that may be clinically silent or convey an increased risk of hematologic malignancy), and clone size and changes over time. It is important that the internist and primary care physician be sufficiently familiar with MDS to expedite referral to a hematologist because many new therapies are now available to improve hematopoietic function and the judicious use of supportive care can improve the patient's quality of life.

■ EPIDEMIOLOGY

MDS is a disease of the elderly; the mean age at onset is older than 70 years. There is a slight male predominance. MDS is a relatively common form of bone marrow failure, with reported incidence rates of 35 to >100 per million persons in the general population and 120 to >500 per million in older adults. Estimates of incidence in the United States range from 30,000 to 40,000 new cases annually and a prevalence of 60,000–120,000 in the population. Rates of MDS have increased over time due to better recognition of the syndrome by physicians and an aging population.

MDS is rare in children, in whom it often has a constitutional genetic basis that can be identified on genomic screens of myeloid cancer predisposition panels.

Secondary or therapy-related MDS, usually related to previous iatrogenic exposure to alkylating agents and other chemotherapy as well as radiation, is not age related.

■ ETIOLOGY AND PATHOPHYSIOLOGY

MDS is associated with environmental exposures such as radiation and benzene; other risk factors have been reported inconsistently. Secondary, therapy-related MDS occurs as a late toxicity of cancer treatment; radiation and the radiomimetic alkylating agents such as busulfan, nitrosourea, or procarbazine (with a latent period of 5–7 years); or the DNA topoisomerase inhibitors (2-year latency). Acquired aplastic anemia, Fanconi anemia, and other constitutional marrow failure diseases can evolve into MDS; occasionally, MDS in adults is recognized as due to germline *GATA2*, *RUNX1*, or telomere gene mutations. The typical MDS patient does not have a suggestive environmental exposure history or a preceding hematologic disease. MDS is a disease of aging, consistent with accumulation of mutations within a hematopoietic stem cell in an aging marrow environment.

TABLE 102-5 World Health Organization (WHO) Classification of Myelodysplastic Syndromes (MDS)/Neoplasms

NAME	RING SIDEROBLASTS	MYELOBLASTS	KARYOTYPE
MDS with single lineage dysplasia (MDS-SLD)	<15% (<5%) ^a	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia (MDS-MLD)	<15% (<5%) ^a	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with ring sideroblasts (MDS-RS)			
MDS-RS with single lineage dysplasia (MDS-RS-SLD)	≥15% / ≥5% ^a	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS with multilineage dysplasia (MDS-RS-MLD)	≥15% / ≥5% ^a	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	None or any	BM <5%, PB <1%, no Auer rods	del(5q) alone or with 1 additional abnormality except -7 or del(7q)
MDS with excess blasts (MDS-EB)			
MDS-EB-1	None or any	BM 5–9% or PB 2–4%, no Auer rods	Any
MDS-EB-2	None or any	BM 10–19% or PB 5–19% or Auer rods	Any
MDS, unclassifiable (MDS-U)			
• with 1% blood blasts	None or any	BM <5%, PB = 1%, no Auer rods	Any
• with single lineage dysplasia and pancytopenia	None or any	BM <5%, PB = 1%, no Auer rods	Any
• based on defining cytogenetic abnormality	<15%	BM <5%, PB = 1%, no Auer rods	MDS-defining abnormality
Refractory cytopenia of childhood	None	BM <5%, PB <2%	Any

^aIf *SF3B1* mutation is present.

Abbreviations: BM, bone marrow; PB, peripheral blood.

MDS is a clonal hematopoietic stem cell disorder characterized by disordered cell proliferation, impaired differentiation, and aberrant hematopoiesis, resulting in cytopenias and risk of progression to leukemia. Both chromosomal and genetic instability have been implicated; both are aging-related. Cytogenetic abnormalities are found in approximately one-half of patients, and some of the same specific lesions are also seen in leukemia; aneuploidy (chromosome loss or gain) is more frequent than translocations. Accelerated telomere attrition may destabilize the genome in marrow failure and predispose to acquisition of chromosomal lesions. Cytogenetic abnormalities are not random (loss of all or part of 5, 7, and 20, trisomy of 8) and may be related to etiology (11q23 following topoisomerase II inhibitors). The type and number of cytogenetic abnormalities strongly correlate with the probability of leukemic transformation and survival.

Genomics has illuminated the role of specific mutations and distinct molecular pathways in the pathophysiology of MDS. Somatic mutations in about 100 genes, which are recurrently present in myeloid neoplasms and are acquired in about 100 genes, are arise in the abnormal marrow cells (and are absent in the germline). Many of the same genes are mutated in AML and in MDS, whereas others are distinctive in subtypes of MDS. A prominent example is *SF3B1*, in which mutations strongly associate with sideroblastic anemia. Some mutations correlate with prognosis: spliceosome defects (like *SF3B1*) correlate with favorable outcome, and mutations in *EZH2*, *TP53*, *RUNX1*, and *ASXL1* with poor outcome. Correlation and exclusion in the pattern of mutations indicate a functional genomic architecture. Driver genes mutated early are consistent with normal blood counts and marrow morphology, but these expanded clones of cells containing them are susceptible to malignant transformation with the acquisition of additional mutations. Deep sequencing results in patients whose MDS evolved to AML have shown clonal succession, with founder clones acquiring additional mutations to produce clonal dominance. Mutations and cytogenetic abnormalities are not independent: *TP53* mutations associate with complex cytogenetic abnormalities and *TET2* mutations with normal cytogenetics. The prevalence of abnormal cells by morphology underestimates bone marrow involvement by MDS clones, as cells normal in appearance are derived from the abnormal clones. Presenting and evolving hematologic manifestations result from the accumulation of multiple genetic lesions: loss of tumor-suppressor genes, activating oncogene, epigenetic pathways that affect mRNA processing and methylation status, or other harmful alterations. Pathophysiology has been linked to mutations and chromosome abnormalities in some specific

MDS syndromes. The 5q- deletion leads to heterozygous loss of a ribosomal protein gene which mimics constitutional red cell aplasia. An immune pathophysiology may be important in lower risk MDS, as cytopenias can respond to immunosuppressive therapy as administered for aplastic anemia. In general for MDS, the role of the immune system and its cells and cytokines; the role of the hematopoietic stem cell niche, the microenvironment, and cell-cell interactions; the fate of normal cells in the Darwinian competitive environment of the dysplastic marrow; and how mutant cells produce marrow failure in MDS are still not completely understood.

CLINICAL FEATURES

Anemia dominates the early course. Most symptomatic patients complain of the gradual onset of fatigue and weakness, dyspnea, and pallor, but at least one-half of patients are asymptomatic, and their MDS is discovered only incidentally on routine blood counts. Previous chemotherapy or radiation exposure is an important historic fact. Fever and weight loss are more often features of a myeloproliferative rather than myelodysplastic process. MDS in childhood is rare and, when diagnosed, implicates an underlying genetic disease. Children with Down syndrome are susceptible to MDS as well as leukemia. A family history may indicate a hereditary form of sideroblastic anemia, Fanconi anemia, or a telomeropathy. Inherited *GATA2* mutations, as in the MonoMAC syndrome (with increased susceptibility to viral, mycobacterial, and fungal infections, as well as deficient numbers of monocytes, natural killer cells, and B lymphocytes), predispose to MDS. Germline *RUNX1* mutations also confer a high risk of MDS and leukemia, often preceded by years of modest thrombocytopenia. A family history is important in all MDS patients, as constitutional mutations may not result in manifest disease until adulthood.

The physical examination in MDS is remarkable for signs of anemia; approximately 20% of patients have splenomegaly. Some unusual skin lesions, including Sweet's syndrome (febrile neutrophilic dermatosis), occur with MDS. Accompanying autoimmune syndromes are not infrequent. In the younger patient, stereotypical anomalies point to a constitutional syndrome (short stature, abnormal thumbs in Fanconi anemia; early graying in the telomeropathies; cutaneous warts in *GATA2* deficiency).

LABORATORY STUDIES

Blood Anemia is present in most cases, either alone or as part of bi- or pancytopenia; isolated neutropenia or thrombocytopenia is

more unusual. Macrocytosis is common, as in most marrow failure disease. Platelets also are large and lack granules. In functional studies, they may show marked abnormalities, and patients may have bleeding symptoms despite seemingly adequate numbers. Neutrophils are hypo-granulated; have hyposegmented, ringed, or abnormally segmented nuclei; contain Döhle bodies; and may be functionally deficient. Circulating myeloblasts usually correlate with marrow blast numbers, and their quantity is important for classification and prognosis. The total white blood cell count (WBC) is usually normal or low, except in CMML. As in aplastic anemia, MDS can be associated with a clonal population of PNH cells. Genetic testing is commercially available for constitutional syndromes.

Bone Marrow The bone marrow is usually normal or hypercellular, but in about 20% of cases, it is sufficiently hypocellular to lead to confusion with aplastic anemia. No single characteristic feature of marrow morphology distinguishes MDS, but the following are commonly observed: dyserythropoietic changes (especially nuclear abnormalities) and ringed sideroblasts in the erythroid lineage; hypogranulation and hyposegmentation in granulocytic precursors, with an increase in myeloblasts; and megakaryocytes showing reduced numbers or disorganized nuclei. Megaloblastic nuclei and defective hemoglobinization in the erythroid lineage are common. Prognosis strongly correlates with the proportion of marrow blasts, which should be enumerated manually on the marrow smear and by flow cytometry of an aspirate. Flow cytometry can also reveal characteristically aberrant hematopoietic differentiation. Cytogenetics and fluorescent *in situ* hybridization can identify chromosomal abnormalities.

DIFFERENTIAL DIAGNOSIS

Deficiencies of vitamin B₁₂ or folate should be excluded by appropriate blood tests; vitamin B₆ deficiency can be assessed by a therapeutic trial of pyridoxine if the bone marrow shows ringed sideroblasts. Copper deficiency can lead to cytopenias and dysplastic marrows of varying cellularity. Marrow dysplasia can be observed in acute viral infections, drug reactions, or chemical toxicity but should be transient. More difficult are the distinctions between hypocellular MDS and aplasia or between RA with excess blasts and acute leukemia: the WHO considers 20% blasts in the marrow as the criterion that separates AML from MDS. In young patients, underlying, predisposing genetic diseases should be considered and appropriate genomic testing performed (see above).

PROGNOSIS

The median survival varies greatly from years for patients with 5q- or sideroblastic anemia to a few months in RA with excess blasts or severe pancytopenia associated with monosomy 7. The International Prognostic Scoring System (IPSS), revised in 2012 (Table 102-6), assists in making predictions. Even “lower-risk” MDS has significant morbidity and mortality. More refined (and also more complicated) prognostic

scoring systems can separate those with intermediate-1 risk who have relatively poor prognoses. Prognostic systems have been developed based on survival from diagnosis, but prognosis changes over time, and hazard ratios for survival and leukemic transformation converge over time among risk categories, consistent with dynamic changes in clonal architecture.

Most patients die as a result of complications of pancytopenia and not due to leukemic transformation; perhaps one-third succumb to diseases unrelated to their MDS. Precipitous worsening of pancytopenia, acquisition of new chromosomal abnormalities on serial cytogenetic determination, increase in the number of blasts, and marrow fibrosis are all poor prognostic indicators. The outlook in therapy-related MDS, regardless of type, is extremely poor, and most patients progress within a few months to refractory AML.

TREATMENT

Myelodysplasia

Historically, therapy of MDS has been unsatisfactory, but several drugs may not only improve blood counts but also delay onset of leukemia and improve survival. The choice of therapy for an individual patient, administration of treatment, and management of toxicities are complicated and require hematologic expertise.

Only hematopoietic stem cell transplantation offers cure of MDS. The survival rate in selected patient cohorts is ~50% at 3 years but improving. Results using unrelated matched donors are similar to those with siblings, and patients in their fifties and older have been successfully transplanted. Nevertheless, treatment-related mortality and morbidity increase with recipient age. The transplant conundrum is that the high-risk patient (by IPSS score and presence of monosomal karyotype), for whom the procedure is most obviously indicated, has a high probability of a poor outcome from transplant-related mortality or disease relapse, whereas the low-risk patient, who is more likely to tolerate transplant, also may do well for years with less aggressive therapies. In practice, only a small proportion of MDS patients undergo transplantation.

MDS has been regarded as particularly refractory to cytotoxic chemotherapy regimens, and as in AML in the older adult, drug toxicity is frequent and often fatal, and remissions, if achieved, are brief. Low doses of cytotoxic drugs have been administered for their “differentiation” potential, and from this experience, drug therapies have emerged based on pyrimidine analogues. These drugs are classified as epigenetic modulators, believed to act through a demethylating mechanism to alter gene regulation and allow differentiation to mature blood cells from the abnormal MDS stem cell. The hypomethylating agents azacitidine and decitabine are frequently used in bone marrow failure clinics. Azacitidine improves blood counts and survival in MDS, compared to best supportive care. Azacitidine is usually administered subcutaneously, daily for 7 days, at 4-week intervals, for at least four cycles before assessing for response. Overall, generally improved blood counts with a decrease in transfusion requirements occurred in ~50% of patients in published trials. Response is dependent on continued drug administration, and most patients eventually become refractory to drug intervention and experience recurrent cytopenias or progression to AML. Decitabine is closely related to azacitidine; 30–50% of patients show responses in blood counts, with a duration of response of almost a year. Decitabine is usually administered by continuous intravenous infusion in regimens of varying doses and durations of 3–10 days in repeating cycles. The major toxicity of azacitidine and decitabine is myelosuppression, leading to worsening blood counts. Hypomethylating agents are frequently used in the high-risk patient who is not a candidate for stem cell transplant. In the lower risk patient, they are also effective, but alternative therapies should be considered.

Lenalidomide, a thalidomide derivative with a more favorable toxicity profile, is particularly effective in reversing anemia in MDS patients with 5q- syndrome; not only do a high proportion of these

TABLE 102-6 Revised International Prognostic Scoring System (IPSS-R)

1. New marrow blast categories
≤2%, >2%–<5%, 5–10%, >10–30%
2. Refined cytogenetic abnormalities and risk groups
16 (vs 6) specific abnormalities, 5 (vs 3) subgroups^a
3. Evaluation of depth of cytopenias^b
Clinically and statistically relevant cut points used
4. Inclusion of differentiating features
Age, performance status, serum ferritin, LDH, β₂-microglobulin
5. Prognostic model with 5 (vs 4) risk categories
Improved predictive power

^aGood, normal, –Y, del(5q), del (20q); poor, complex (≥3 abnormalities) or chromosome 7 abnormalities; intermediate, all other abnormalities. ^bCytopenias at baseline, cut points: hemoglobin <80, 80–<100, or ≥100 g/L; platelet count <50, 50–100, or ≥100,000/µL, and absolute neutrophil count <800 versus ≥800/µL.

Abbreviation: LDH, lactate dehydrogenase.

patients become transfusion independent with normal or near-normal hemoglobin levels, but their cytogenetics also become normal. The drug has many biologic activities, and it is unclear which is critical for clinical efficacy. Lenalidomide is administered orally. Most patients will improve within 3 months of initiating therapy. Toxicities include myelosuppression (worsening thrombocytopenia and neutropenia, necessitating blood count monitoring) and an increased risk of deep vein thrombosis and pulmonary embolism.

Immunosuppression also may produce sustained independence from transfusion and improve survival. ATG, cyclosporine, and the anti-CD52 monoclonal antibody alemtuzumab are especially effective in younger MDS patients (<60 years old) with more favorable IPSS. In a consortium retrospective review, about 50% of patients with mainly refractory anemia responded to ATG, usually combined with cyclosporine, particularly patients with hypocellular marrow.

HGFs can improve blood counts but, as in most other marrow failure states, have been most beneficial to patients with the least severe pancytopenia. EPO alone or in combination with G-CSF can improve hemoglobin levels, particularly in those with low serum EPO levels who have no or a modest need for transfusions. Survival may be enhanced by EPO and amelioration of anemia. G-CSF treatment alone failed to improve survival in a controlled trial. Thrombopoietin mimetics appear to improve platelet counts in some MDS patients, with no clear evidence that they increase the rate of leukemic transformation.

New drugs for MDS are entering the clinic or are in late development. Luspatercept, which affects transforming growth factor β -mediated suppression of erythropoiesis, has been approved by the FDA for anemia in MDS. Novel targeted therapies in trials include inhibitors of hypoxia-inducible factor and spliceosome genes, drugs that act to restore TP53 activity, and venetoclax, an inhibitor of the bcl2 protein that increases programmed cell death (and is approved for use or employed off-label in other hematologic malignancies).

The same principles of supportive care described for aplastic anemia apply to MDS. Many patients will be anemic for years. RBC transfusion support should be accompanied by iron chelation to prevent secondary hemochromatosis.

MYELOPHTHISIS ANEMIAS

Fibrosis of the bone marrow (see Fig. 100-2), usually accompanied by a characteristic blood smear picture called *leukoerythroblastosis*, can occur as a primary hematologic disease, called *myelofibrosis* or *myeloid metaplasia* (Chap. 103), and as a secondary process, called *myelophthisis*. Myelophthisis, or secondary myelofibrosis, is reactive. Fibrosis can be a response to invading tumor cells, usually an epithelial cancer of breast, lung, or prostate origin or neuroblastoma. Marrow fibrosis may occur with infection of mycobacteria (both *Mycobacterium tuberculosis* and *Mycobacterium avium*), fungi, or HIV and in sarcoidosis. Intracellular lipid deposition in Gaucher disease and obliteration of the marrow space related to absence of osteoclast remodeling in congenital osteopetrosis also can produce fibrosis. Secondary myelofibrosis is a late consequence of radiation therapy or treatment with radiomimetic drugs. Usually the infectious or malignant underlying processes are obvious. Marrow fibrosis can also be a feature of a variety of hematologic syndromes, especially chronic myeloid leukemia, multiple myeloma, lymphomas, myeloma, and hairy cell leukemia.

The pathophysiology has three distinct features: proliferation of fibroblasts in the marrow space (myelofibrosis); the extension of hematopoiesis into the long bones and into extramedullary sites, usually the spleen, liver, and lymph nodes (myeloid metaplasia); and ineffective erythropoiesis. The etiology of the fibrosis is unknown but most likely involves dysregulated production of growth factors: platelet-derived growth factor and transforming growth factor β have been implicated. Abnormal regulation of other hematopoietins would lead to localization of blood-producing cells in nonhematopoietic tissues and uncoupling of the usually balanced processes of stem cell proliferation and differentiation. Myelofibrosis is remarkable for

pancytopenia despite very large numbers of circulating hematopoietic progenitor cells.

Anemia is dominant in secondary myelofibrosis, usually normocytic and normochromic. The diagnosis is suggested by the characteristic leukoerythroblastotic smear (see Fig. 100-1). Erythrocyte morphology is highly abnormal, with circulating nucleated RBCs, teardrops, and shape distortions. WBC numbers are often elevated, sometimes mimicking a leukemoid reaction, with circulating myelocytes, promyelocytes, and myeloblasts. Platelets may be abundant and are often of giant size. Inability to aspirate the bone marrow, the characteristic “dry tap,” can allow a presumptive diagnosis in the appropriate setting before the biopsy is decalcified.

The course of secondary myelofibrosis is determined by its etiology, usually a metastatic tumor or an advanced hematologic malignancy. Treatable causes must be excluded, especially tuberculosis and fungus. Transfusion support can relieve symptoms.

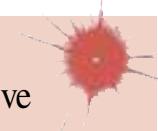
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Polycythemia Vera and Other Myeloproliferative Neoplasms

Jerry L. Spivak



The World Health Organization (WHO) classification of the chronic myeloproliferative neoplasms (MPNs) includes eight disorders, some of which are rare or poorly characterized (Table 103-1) but all of which share an origin in a hematopoietic cell; overproduction of one or more of the formed elements of the blood without significant dysplasia; and a predilection to extramedullary hematopoiesis, myelofibrosis, and transformation at varying rates to acute leukemia. Within this broad classification, however, significant phenotypic heterogeneity exists. Some diseases such as chronic myelogenous leukemia (CML), chronic neutrophilic leukemia (CNL), and chronic eosinophilic leukemia (CEL) express primarily a myeloid phenotype, whereas in other diseases, such as polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocythemia (ET), erythroid or megakaryocytic

TABLE 103-1 World Health Organization Classification of Chronic Myeloproliferative Neoplasms

Chronic myeloid leukemia, <i>BCR-ABL</i> -positive
Chronic neutrophilic leukemia
Chronic eosinophilic leukemia, not otherwise specified
Polycythemia vera
Primary myelofibrosis
Essential thrombocythemia
Mastocytosis
Myeloproliferative neoplasms, unclassifiable

hyperplasia predominates. The latter three disorders, in contrast to the former three, also appear capable of transforming into each other.

Such phenotypic heterogeneity has a genetic basis; CML is the consequence of the balanced translocation between chromosomes 9 and 22 ($t[9;22][q34;11]$); CNL has been associated with a $t(15;19)$ translocation; and CEL occurs with a deletion or balanced translocations involving the *PDGFRα* gene. By contrast, PV, PMF, and ET are characterized by driver mutations that directly or indirectly constitutively activate JAK2, a tyrosine kinase essential for the function of the erythropoietin and thrombopoietin receptors and also utilized by the granulocyte colony-stimulating factor receptor. This important distinction is reflected in the natural histories of CML, CNL, and CEL, which are usually measured in years, with a high rate of leukemic transformation. The natural histories of PV, PMF, and ET, by contrast, are usually measured in decades, and transformation to acute leukemia is uncommon in the absence of chemotherapy. This chapter focuses only on PV, PMF, and ET because their clinical features and driver mutation overlap are substantial, although their disease duration varies.

The other chronic MPNs will be discussed in Chaps. 105 and 110.

POLYCYTHEMIA VERA

PV is a clonal hematopoietic stem cell disorder in which phenotypically normal red cells, granulocytes, and platelets accumulate in the absence of a recognizable physiologic stimulus. The most common of the MPNs, PV occurs in 2.5 per 100,000 persons, sparing no adult age group and increasing with age to rates $>10/100,000$. Familial transmission is infrequent, and women under age 50 predominate among sporadic cases.

ETIOLOGY

Nonrandom chromosome abnormalities such as deletion 20q and deletion 13q or trisomy 9 occur in up to 30% of untreated PV patients, but unlike CML, no consistent cytogenetic abnormality has been associated with the disorder. However, a mutation in the autoinhibitory pseudokinase domain of the tyrosine kinase JAK2 that replaces valine with phenylalanine (V617F), causing constitutive kinase activation, has a central role in PV pathogenesis.

JAK2 is a member of an evolutionarily well-conserved, nonreceptor tyrosine kinase family and serves as the cognate tyrosine kinase for the erythropoietin and thrombopoietin receptors. It also functions as an obligate chaperone for these receptors in the Golgi apparatus and is responsible for their cell-surface expression. The conformational change induced in the erythropoietin and thrombopoietin receptors following binding to their respective cognate ligands, erythropoietin or thrombopoietin, leads to JAK2 autophosphorylation, receptor phosphorylation, and phosphorylation of proteins involved in cell proliferation, differentiation, and resistance to apoptosis. Transgenic animals lacking JAK2 die as embryos from severe anemia. Constitutive activation of JAK2, on the other hand, explains the erythropoietin hypersensitivity, erythropoietin-independent erythroid colony formation, rapid terminal differentiation, increased *Bcl-X_L* expression, and apoptosis resistance in the absence of erythropoietin that characterize the in vitro behavior of PV erythroid progenitor cells.

More than 95% of PV patients express this mutation, as do 50% of PMF and ET patients. Importantly, the *JAK2* gene is located on

the short arm of chromosome 9, and loss of heterozygosity on chromosome 9p involving the segment containing the *JAK2* locus over time due to mitotic recombination (uniparental disomy) is the most common cytogenetic abnormality in PV. Loss of heterozygosity in this region leads to homozygosity for *JAK2* V617F and occurs in 60% of PV patients and to a lesser extent in PMF but is rare in ET. Most PV patients who do not express *JAK2* V617F express a mutation in exon 12 of the gene and are not clinically different from those who do, with the exception of a higher frequency of isolated erythrocytosis, nor do *JAK2* V617F heterozygotes differ clinically from homozygotes. Importantly, the predisposition to acquire *JAK2* mutations appears to be associated with a specific *JAK2* gene haplotype, GGCC. *JAK2* V617F is the basis for many of the phenotypic and biochemical characteristics of PV such as increased blood cell production and increased inflammatory cytokine production; however, it cannot solely account for the entire PV phenotype and is probably not the initiating lesion in any of the MPNs. First, PV patients with the same phenotype and documented clonal disease can have mutations in *LNK*, a JAK2 inhibitor, or rarely, calreticulin (*CALR*), an ER chaperone. Second, ET and PMF patients have the same mutation but different clinical phenotypes. Third, familial PV can occur without the mutation, even when other members of the same family express it. Fourth, inhibition of *JAK2* V617F-expressing hematopoietic progenitor cells by the nonspecific JAK1/2 kinase inhibitor, ruxolitinib, does not affect the behavior of the involved hematopoietic stem cells. Finally, in some *JAK2* V617F-positive PV or ET patients, acute leukemia can occur in a *JAK2* V617F-negative progenitor cell, suggesting the presence of an ancestral precursor cell.

CLINICAL FEATURES

Although PV is a panmyelopathy, isolated thrombocytosis, leukocytosis, or splenomegaly may be its initial presenting manifestation, but most often, the disorder is first recognized by the incidental discovery of a high hemoglobin, hematocrit, or red cell count. With the exception of aquagenic pruritus, or erythromelalgia, no symptoms distinguish PV from other causes of erythrocytosis.

Uncontrolled erythrocytosis causes hyperviscosity, leading to neurologic symptoms such as vertigo, tinnitus, headache, visual disturbances, and transient ischemic attacks (TIAs). Systolic hypertension is also a feature of the red cell mass elevation. In some patients, venous or arterial thrombosis may be the presenting manifestation of PV. Any vessel can be affected, but cerebral, cardiac, and mesenteric vessels are most commonly involved. Hepatic venous thrombosis (Budd-Chiari syndrome) is particularly common in young women and may be catastrophic if sudden and complete obstruction of the hepatic vein occurs. Indeed, PV should be suspected in any patient who develops hepatic vein thrombosis, since this is the only type of thrombosis associated with *JAK2* V617F expression. Digital ischemia, easy bruising, epistaxis, acid-peptic disease, or gastrointestinal hemorrhage may occur due to vascular stasis or thrombocytosis. In the latter instance, absorption and proteolysis of high-molecular-weight von Willebrand multimers by the large platelet mass cause acquired von Willebrand's disease. Erythema, burning, and pain in the extremities, a symptom complex known as erythromelalgia, is another complication of thrombocytosis in PV due to increased platelet stickiness. Given the large turnover of hematopoietic cells, hyperuricemia with secondary gout, uric acid stones, and symptoms due to hypermetabolism can also complicate the disorder.

DIAGNOSIS

When PV presents with erythrocytosis in combination with leukocytosis, thrombocytosis, or splenomegaly or any combination of these, the diagnosis is apparent. However, when patients present with an elevated hemoglobin, hematocrit, or red cell count alone, the diagnostic evaluation is more complex because of the many diagnostic possibilities (Table 103-2). Furthermore, unless the hemoglobin level is ≥ 20 g/dL (hematocrit $\geq 60\%$), it is not possible to distinguish true erythrocytosis from disorders causing plasma volume contraction. This is because uniquely in PV, in contrast to other causes of true erythrocytosis, there is expansion of the plasma volume, which can mask the elevated red cell mass, particularly in women; thus, red cell mass and plasma

TABLE 103-2 Causes of Erythrocytosis

Relative Erythrocytosis	
Hemoconcentration secondary to dehydration, diuretics, ethanol abuse, androgens, or tobacco abuse	
Absolute Erythrocytosis	
Hypoxia	Tumors
Carbon monoxide intoxication	Hypernephroma
High-oxygen-affinity hemoglobins	Hepatoma
High altitude	Cerebellar hemangioblastoma
Pulmonary disease	Uterine myoma
Right-to-left cardiac or vascular shunts	Adrenal tumors
Sleep apnea syndrome	Meningioma
Hepatopulmonary syndrome	Pheochromocytoma
Renal Disease	Drugs
Renal artery stenosis	Androgens
Focal sclerosing or membranous glomerulonephritis	Recombinant erythropoietin
Postrenal transplantation	Familial (with normal hemoglobin function)
Renal cysts	Erythropoietin receptor mutations
Bartter's syndrome	VHL mutations (Chuvash polycythemia)
	2,3-BPG mutation
	PHD2 and HIF2α mutations
	Polycythemia vera

Abbreviations: 2,3-BPG, 2,3-bisphosphoglycerate; VHL, von Hippel-Lindau.

volume determinations are necessary to establish the presence of an absolute erythrocytosis and distinguish this from relative erythrocytosis due to a reduction in plasma volume alone (also known as *stress* or *spurious erythrocytosis* or *Gaisböck's syndrome*). Figure 63-18 illustrates a diagnostic algorithm for the evaluation of suspected erythrocytosis. Assay for JAK2 mutations in the presence of a normal arterial oxygen saturation provides an alternative diagnostic approach to erythrocytosis when red cell mass and plasma volume determinations are not available; a normal serum erythropoietin level does not exclude the presence of PV, but an elevated erythropoietin level is more consistent with a secondary cause for the erythrocytosis.

Other laboratory studies that may aid in diagnosis include the red cell count, mean corpuscular volume, and red cell distribution width (RDW), particularly when the hematocrit or hemoglobin levels are less than 60% or 20 g/dL, respectively. Only three situations cause microcytic erythrocytosis: β-thalassemia trait, hypoxic erythrocytosis, and PV. With β-thalassemia trait, the RDW is usually normal, whereas with hypoxic erythrocytosis and PV, the RDW may be elevated due to associated iron deficiency. Today, however, the assay for JAK2 V617F has superseded other tests for establishing the diagnosis of PV. Of course, in patients with associated acid-peptic disease, occult gastrointestinal bleeding may lead to a presentation with hypochromic, microcytic anemia, masking the presence of PV.

A bone marrow aspirate and biopsy provide no specific diagnostic information because these may be normal or indistinguishable from ET or PMF. Similarly, no specific cytogenetic abnormality is associated with the disease, and the absence of a cytogenetic marker does not exclude the diagnosis.

COMPLICATIONS

Many of the clinical complications of PV relate directly to the increase in blood viscosity associated with red cell mass elevation and indirectly to the increased turnover of red cells, leukocytes, and platelets with the attendant increase in uric acid and inflammatory cytokine production. The latter appears to be responsible for constitutional symptoms. Peptic ulcer disease can also be due to *Helicobacter pylori* infection, the incidence of which is increased in PV, while the pruritus associated with this disorder may be a consequence of mast cell activation by JAK2 V617F. A sudden increase in spleen size can be associated with

painful splenic infarction. Myelofibrosis appears to be part of the natural history of the disease but is a reactive, reversible process that does not itself impede hematopoiesis and by itself has no prognostic significance. In 15% of patients, however, myelofibrosis is associated with hematopoietic stem cell failure, manifested by substantial extramedullary hematopoiesis in the liver and spleen and transfusion-dependent anemia. The organomegaly can cause significant mechanical discomfort, portal hypertension, and progressive cachexia. Although the incidence of acute myeloid leukemia is increased in PV, the incidence of acute leukemia in patients not exposed to chemotherapy or radiation therapy is low. Interestingly, chemotherapy, including hydroxyurea, has been associated with acute leukemia in JAK2 V617F-negative stem cells in some PV patients. *Erythromelalgia* is a curious syndrome of unknown etiology associated with thrombocytosis, primarily involving the lower extremities and usually manifested by erythema, warmth, and pain of the affected appendage and occasionally digital infarction. It occurs with a variable frequency and is usually responsive to salicylates. Some of the central nervous system symptoms observed in patients with PV, such as ocular migraine, appear to represent a variant of erythromelalgia.

Left uncontrolled, erythrocytosis can lead to thrombosis involving vital organs such as the liver, heart, brain, or lungs. Patients with massive splenomegaly are particularly prone to thrombotic events because the associated increase in plasma volume masks the true extent of the red cell mass elevation measured by the hematocrit or hemoglobin level. A "normal" hematocrit or hemoglobin level in a PV patient with massive splenomegaly should be considered indicative of an elevated red cell mass until proven otherwise.

TREATMENT

Polycythemia Vera

PV is generally an indolent disorder, the clinical course of which is measured in decades, and its management should reflect its tempo. Thrombosis due to erythrocytosis is the most significant complication and often the presenting manifestation; maintenance of the hemoglobin level at ≤ 140 g/L (14 g/dL; hematocrit $<45\%$) in men and ≤ 120 g/L (12 g/dL; hematocrit $<42\%$) in women is mandatory to avoid thrombotic complications. Phlebotomy serves initially to reduce viscosity by reducing the red cell mass to normal while further expanding the plasma volume. Periodic phlebotomies thereafter serve to maintain the red cell mass within the normal range and induce a state of iron deficiency that prevents accelerated reexpansion of the red cell mass. In most PV patients, once an iron-deficient state is achieved, phlebotomy is usually only required at 3-month intervals. Neither phlebotomy nor iron deficiency increases the platelet count relative to the effect of the disease itself, and neither thrombocytosis nor leukocytosis is correlated with thrombosis in PV, in contrast to the strong correlation between erythrocytosis and thrombosis. The use of salicylates to prevent thrombosis in PV patients is not only potentially harmful if the red cell mass is not controlled by phlebotomy but also an unproven remedy, particularly in patients over age 70.

Anticoagulation is indicated when a thrombosis has occurred, and the newer oral anticoagulants may be preferable to a vitamin K antagonist since they do not require monitoring. Asymptomatic hyperuricemia (<10 mg/dL) requires no therapy, but allopurinol should be administered to avoid further elevation of the uric acid when chemotherapy is used to reduce splenomegaly or leukocytosis or to treat pruritus. Generalized pruritus intractable to antihistamines or antidepressants such as doxepin can be a major problem in PV; the JAK1/2 inhibitor ruxolitinib, pegylated interferon α (IFN-α), psoralens with ultraviolet light in the A range (PUVA) therapy, and hydroxyurea are other methods of palliation. Asymptomatic thrombocytosis requires no therapy unless the platelet count is sufficiently high to cause bleeding due to acquired von Willebrand's disease, but bleeding in this situation is not usually spontaneous and is responsive to tranexamic acid or ε-aminocaproic acid. Symptomatic

splenomegaly can be treated with either ruxolitinib or pegylated IFN- α . PEGylated IFN- α has the advantage over recombinant IFN- α of being better tolerated and requiring only weekly administration and produced complete hematologic and molecular remissions in

20% of PV patients; its role in this disorder is currently under investigation. Anagrelide, a phosphodiesterase inhibitor, can reduce the platelet count and, if tolerated, is preferable to hydroxyurea because it lacks marrow toxicity and is also protective against venous thrombosis while hydroxyurea is not.

A reduction in platelet number may be necessary for the treatment of erythromelalgia or ocular migraine if salicylates are not effective or if the platelet count is sufficiently high to increase the risk of hemorrhage but only to the degree that symptoms are alleviated. Alkylating agents and radioactive sodium phosphate (^{32}P) are leukemogenic in PV, and their use should be avoided. If a cytotoxic agent must be used, hydroxyurea is preferred, but this drug does not prevent either thrombosis or myelofibrosis in PV, is itself leukemogenic, and should be used for as short a time as possible. Previously, PV patients with massive splenomegaly unresponsive to reduction by chemotherapy or interferon required splenectomy. However, with the introduction of the nonspecific JAK2 inhibitor ruxolitinib, it has been possible in the majority of patients with PV complicated by myelofibrosis and myeloid metaplasia to reduce spleen size while at the same time alleviating constitutional symptoms and pruritus due to cytokine release and reducing the phlebotomy requirement. However, in contrast to PMF, these patients have a more chronic course. In contrast to other malignancies, PV patients have a low rate of mutation accumulation, and the acquisition of deleterious mutations such as *TP53* mutations as detected by next-generation sequencing is usually associated with leukemic transformation. Since hydroxyurea antagonizes *TP53* and also causes del17p, leading to *TP53* haploinsufficiency, its use should be constrained in PV.

Ruxolitinib has also been demonstrated in a phase 3 clinical trial to be effective in PV patients without myelofibrosis who are intolerant or refractory to hydroxyurea or best available supportive therapy. In some patients with end-stage disease, pulmonary hypertension may develop due to fibrosis or extramedullary hematopoiesis. A role for bone marrow transplantation, either allogeneic or haploidentical, in PV has not been defined.

Most patients with PV can live long lives without functional impairment when their red cell mass is effectively managed with phlebotomy alone. Chemotherapy is never indicated to control the red cell mass in PV, but when venous access is an issue, ruxolitinib or pegylated interferon is the preferred therapy.

■ PRIMARY MYELOFIBROSIS

Chronic PMF (other designations include *idiopathic myelofibrosis*, *agnogenic myeloid metaplasia*, or *myelofibrosis with myeloid metaplasia*) is a clonal hematopoietic stem cell disorder associated with mutations in *JAK2*, *MPL*, or *CALR* and characterized by marrow fibrosis, extramedullary hematopoiesis, and splenomegaly. PMF is the least common MPN, and establishing its diagnosis in the absence of a specific clonal marker is difficult because myelofibrosis and splenomegaly are also features of both PV and CML. Furthermore, myelofibrosis and splenomegaly also occur in a variety of benign and malignant disorders (Table 103-3), many of which are amenable to specific therapies not effective in PMF. In contrast to the other MPNs and so-called acute or malignant myelofibrosis, which can occur at any age, PMF primarily afflicts men in their sixth decade or later.

■ ETIOLOGY

Nonrandom chromosome abnormalities such as 9p-, 20q-, 13q-, trisomy 8 or 9, or partial trisomy 1q are common in PMF, but no cytogenetic abnormality specific to the disease has been identified. *JAK2* V617F is present in 55% of PMF patients, and mutations in the thrombopoietin receptor, *MPL*, occur in ~4%. Most of the rest have mutations in the calreticulin gene (*CALR*) that alter the carboxy-terminal portion of the protein, permitting it to bind and activate *MPL*.

TABLE 103-3 Disorders Causing Myelofibrosis

MALIGNANT	NONMALIGNANT
Acute leukemia (lymphocytic, myelogenous, megakaryocytic)	HIV infection
Chronic myeloid leukemia	Hyperparathyroidism
Hairy cell leukemia	Renal osteodystrophy
Hodgkin's disease	Systemic lupus erythematosus
Primary myelofibrosis	Tuberculosis
Lymphoma	Vitamin D deficiency
Multiple myeloma	Thorium dioxide exposure
Myelodysplasia	Gray platelet syndrome
Metastatic carcinoma	
Polycythemia vera	
Systemic mastocytosis	

The degree of myelofibrosis and the extent of extramedullary hematopoiesis are not related. Fibrosis in this disorder is associated with overproduction of transforming growth factor β and tissue inhibitors of metalloproteinases, while osteosclerosis is associated with overproduction of osteoprotegerin, an osteoclast inhibitor. Marrow angiogenesis occurs due to increased production of vascular endothelial growth factor. Importantly, fibroblasts in PMF are polyclonal and not part of the neoplastic clone but can be induced by it to produce inflammatory cytokines.

■ CLINICAL FEATURES

No signs or symptoms are specific for PMF. Many patients are asymptomatic at presentation, and the disease is often detected by the discovery of splenic enlargement and/or abnormal blood counts during a routine examination. In contrast to its companion MPN, night sweats, fatigue, and weight loss are common presenting complaints. A blood smear will show the characteristic features of extramedullary hematopoiesis: teardrop-shaped red cells, nucleated red cells, myelocytes, and promyelocytes; myeloblasts may also be present (Fig. 103-1). Anemia, usually mild initially, is common, whereas the leukocyte and platelet counts are either normal or increased, but either can be depressed. Mild hepatomegaly may accompany the splenomegaly but is unusual in its absence; isolated lymphadenopathy should suggest another diagnosis. Both serum lactate dehydrogenase and alkaline phosphatase levels can be elevated. Marrow is usually inaspirable due to the myelofibrosis (Fig. 103-2), and bone x-rays may reveal osteosclerosis. Exuberant extramedullary hematopoiesis can cause ascites; portal, pulmonary, or

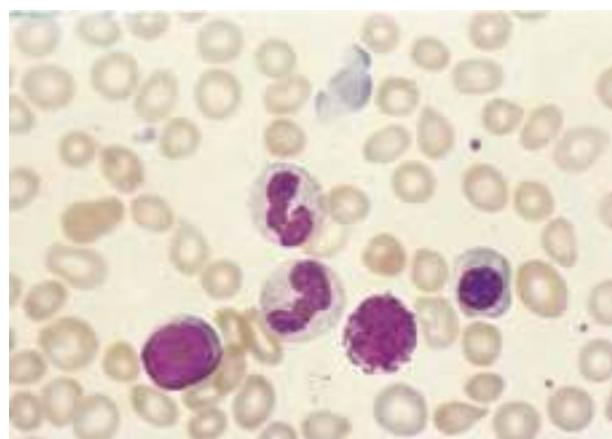


FIGURE 103-1 Teardrop-shaped red blood cells indicative of membrane damage from passage through the spleen, a nucleated red blood cell, and immature myeloid cells indicative of extramedullary hematopoiesis are noted. This peripheral blood smear is related to any cause of extramedullary hematopoiesis.

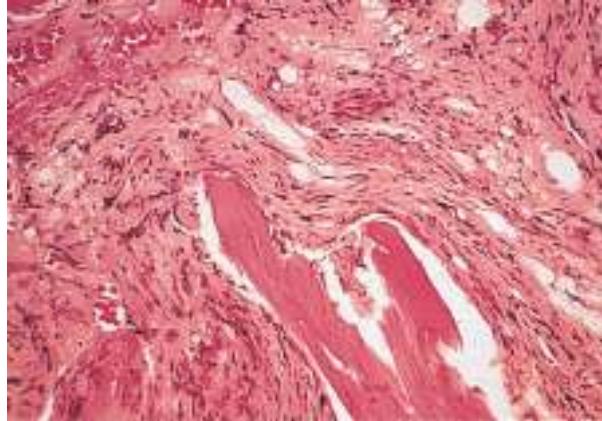


FIGURE 103-2 This marrow section shows the marrow cavity replaced by fibrous tissue composed of reticulin fibers and collagen. When this fibrosis is due to a primary hematologic process, it is called *myelofibrosis*. When the fibrosis is secondary to a tumor or a granulomatous process, it is called *myelophthisis*.

intracranial hypertension; intestinal or ureteral obstruction; pericardial tamponade; spinal cord compression; or skin nodules. Splenic enlargement can be sufficiently rapid to cause splenic infarction with fever and pleuritic chest pain. Hyperuricemia and secondary gout may ensue.

■ DIAGNOSIS

While the clinical picture described above is characteristic of PMF, all of these clinical features can be observed in PV or CML. Massive splenomegaly commonly masks erythrocytosis in PV, and reports of intraabdominal thrombosis in PMF most likely represent instances of unrecognized PV. In some PMF patients, erythrocytosis has developed during the course of the disease. Furthermore, because many other disorders have features that overlap with PMF but respond to distinctly different therapies, the diagnosis of PMF is one of exclusion, which requires that the disorders listed in Table 103-3 be ruled out.

The presence of teardrop-shaped red cells, nucleated red cells, myelocytes, and promyelocytes establishes the presence of extramedullary hematopoiesis, while the presence of leukocytosis, thrombocytosis with large and bizarre platelets, and circulating myelocytes suggests the presence of an MPN as opposed to a secondary form of myelofibrosis (Table 103-3). Marrow is usually inaspirable due to increased marrow reticulin, but marrow biopsy will reveal a hypercellular marrow with trilineage hyperplasia and, in particular, increased numbers of megakaryocytes in clusters and with large, dysplastic nuclei. However, there are no characteristic bone marrow morphologic abnormalities that distinguish PMF from the other MPNs. Splenomegaly due to extramedullary hematopoiesis may be sufficiently massive to cause portal hypertension and variceal formation. In some patients, exuberant extramedullary hematopoiesis can dominate the clinical picture. An intriguing feature of PMF is the occurrence of autoimmune abnormalities such as immune complexes, antinuclear antibodies, rheumatoid factor, or a positive Coombs' test. Whether these represent a host reaction to the disorder or are involved in its pathogenesis is unknown. Cytogenetic analysis of the blood is useful both to exclude CML and for prognostic purposes because the development of complex karyotype abnormalities portends a poor prognosis in PMF. For unknown reasons, the number of circulating CD34+ cells is markedly increased in PMF ($>15,000/\mu\text{L}$) compared to the other MPNs, unless they too develop extramedullary hematopoiesis.

Importantly, 55% of PMF patients, like patients with its companion MPNs, express the *JAK2* V617F mutation, often as homozygotes. Such patients are usually older and have higher hematocrits than patients with *MPL* (4%) or *CALR* (36%) mutations; PMF patients expressing an *MPL* mutation tend to be more anemic and have lower leukocyte counts than *JAK2* V617F-positive patients. Somatic mutations (due to deletions [type 1] or insertions [type 2]) in exon 9 of *CALR* have been

TABLE 103-4 Three Current Scoring Systems for Estimating Prognosis in PMF Patients

RISK FACTOR	IPSS (2009) ^a	DIPSS (2010) ^b	DIPSS PLUS (2011) ^c
Anemia ($<10\text{ g/dL}$)	X	X	X
Leukocytosis ($>25,000/\mu\text{L}$)	X	X	X
Peripheral blood blasts ($\geq 1\%$)	X	X	X
Constitutional symptoms	X	X	X
Age (>65 years)	X	X	X
Unfavorable karyotype			X
Platelet count ($<100,000/\mu\text{L}$)			X
Transfusion dependence			X

^aBlood 113:2895, 2009. ^bBlood 115:1703, 2010. ^cJ Clin Oncol 29:392, 2011.

Note: The Dynamic International Prognostic Scoring System (DIPSS) was developed to determine if the International Prognostic Scoring System (IPSS) risk factors identified as important for survival at the time of primary myelofibrosis (PMF) diagnosis could also be used for risk stratification following their acquisition during the course of the disease. One point is assigned to each risk factor for IPSS scoring. For DIPSS, the same is true, but anemia is assigned 2 points. The DIPSS Plus scoring system represents recognition that the addition of unfavorable karyotype, thrombocytopenia, and transfusion dependence improved the DIPSS risk stratification system for which additional points are assigned (Table 103-5). More recent studies suggest that mutational analysis of the *ASXL1*, *EZH2*, *SRSF2*, and *IDH1/2* genes further improves risk stratification for survival and leukemic transformation (Leukemia 27:1861, 2013), as can cytogenetic abnormalities (Leukemia 32:1631, 2018). These prognostic scoring systems are not accurate for risk assessment in polycythemia vera or essential thrombocythemia patients who have developed myelofibrosis (Haematologica 99:e55, 2014).

found in a majority of patients with PMF who lack mutations in either *JAK2* or *MPL*. In some studies, type 1 mutations, the most common *CALR* mutation in PMF, had a survival advantage compared to *JAK2* or *MPL* mutations but not with respect to leukemic transformation. PMF patients who lack a known MPN driver mutation appear to have the worst prognosis.

■ COMPLICATIONS

Survival in PMF varies according to specific risk factors at diagnosis (Tables 103-4 and 103-5) but is shorter than in PV and ET patients. The natural history of PMF is one of increasing marrow failure with transfusion-dependent anemia and increasing organomegaly due to extramedullary hematopoiesis. As with CML, PMF can evolve from a chronic to an accelerated phase with constitutional symptoms and increasing marrow failure. About 10% of patients spontaneously transform to an aggressive form of acute leukemia for which therapy is usually ineffective. Additional important prognostic factors for disease acceleration during the course of PMF include the presence of complex cytogenetic abnormalities, thrombocytopenia, and transfusion-dependent anemia. Mutations in the *ASXL1*, *EZH2*, *SRSF2*, and *IDH1/2* genes have been identified as risk factors for early death or transformation to acute leukemia, as have complex cytogenetic abnormalities, and have proved to be more useful for PMF risk assessment than clinical scoring systems.

TABLE 103-5 IPSS and DIPSS Risk Stratification Systems

RISK CATEGORIES ^a	NUMBER OF RISK FACTORS		
	IPSS	DIPSS	DIPSS PLUS
Low	0	0	0
Intermediate-1	1	1–2	1
Intermediate-2	2	3–4	2–3
High	≥ 3	>4	4–6

^aThe corresponding survival curves for each risk category can be found in the references cited in the footnotes of Table 103-4.

Abbreviations: DIPSS, Dynamic International Prognostic Scoring System; IPSS, International Prognostic Scoring System.

TREATMENT

Primary Myelofibrosis

No specific therapy exists for PMF. The causes for anemia are multifarious and include ineffective erythropoiesis uncompensated by splenic extramedullary hematopoiesis, hemodilution due to splenomegaly, splenic sequestration, blood loss secondary to thrombocytopenia or portal hypertension, folic acid deficiency, systemic inflammation, and autoimmune hemolysis. Neither recombinant erythropoietin nor androgens such as danazol have proven to be consistently effective as therapy for anemia. Erythropoietin may worsen splenomegaly and will be ineffective if the serum erythropoietin level is >125 mU/L. Given the inflammatory milieu that characterizes PMF, glucocorticoids can ameliorate anemia as well as constitutional symptoms such as fever, chills, night sweats, anorexia, and weight loss, and combining these with low-dose thalidomide has proved effective as well. Thrombocytopenia can be due to impaired marrow function, splenic sequestration, or autoimmune destruction and may also respond to low-dose thalidomide and prednisone.

Splenomegaly is by far the most distressing and intractable problem for PMF patients, causing abdominal pain, portal hypertension, easy satiety, and cachexia, whereas surgical removal of a massive spleen is associated with significant postoperative complications including mesenteric venous thrombosis, hemorrhage, rebound leukocytosis and thrombocytosis, and hepatic extramedullary hematopoiesis with no amelioration of either anemia or thrombocytopenia when present. For unexplained reasons, splenectomy also increases the risk of blastic transformation.

Splenic irradiation is, at best, temporarily palliative and associated with a significant risk of neutropenia, infection, and subsequent operative hemorrhage if splenectomy is attempted. Allopurinol can control significant hyperuricemia, and bone pain can be alleviated by local irradiation. Pegylated IFN- α can ameliorate fibrosis in early PMF, but in advanced disease, it may exacerbate the bone marrow failure. The JAK2 inhibitor ruxolitinib has proved effective in reducing splenomegaly and alleviating constitutional symptoms in a majority of advanced PMF patients while possibly prolonging survival, although it usually does not significantly influence the JAK2 V617F neutrophil allele burden. Although anemia and thrombocytopenia are its major side effects, these are dose-dependent, and with time, anemia stabilizes and thrombocytopenia may improve. Fedratinib, a new tyrosine kinase inhibitor with anti-FLT3 activity, has proved useful in patients with disease refractory to ruxolitinib.

In some patients, hypomethylating agents such as azacytidine or decitabine in combination with high-dose ruxolitinib have been used to control the disease or prepare patients for bone marrow transplantation. Transformation to acute leukemia in PMF, like PV or ET, is usually refractory to treatment.

Allogeneic bone marrow transplantation is the only curative treatment for PMF and should be considered in younger patients and older patients with high-risk disease; nonmyeloablative conditioning regimens permit hematopoietic cell transplantation to be extended to older individuals.

ESSENTIAL THROMBOCYTOSIS

ET (other designations include *essential thrombocythemia*, *idiopathic thrombocytosis*, *primary thrombocytosis*, and *hemorrhagic thrombocythemia*) is a clonal hematopoietic stem cell disorder associated with mutations in *JAK2* (V617F), *MPL*, or *CALR* and manifested clinically by overproduction of platelets without a definable cause. ET has an incidence of 1–2/100,000 and a distinct female predominance. Canonical MPN driver mutations distinguish 90% of ET patients from the more common nonclonal, reactive forms of thrombocytosis (Table 103-6); mutation-negative ET patients may have either uncommon *MPL* mutations, *JAK2* V617F expression limited to the platelets, or a hereditary form of thrombocytosis. Once considered a disease of the elderly and responsible for significant morbidity due to hemorrhage

TABLE 103-6 Causes of Thrombocytosis

Tissue inflammation: collagen vascular disease, inflammatory bowel disease	Hemorrhage
Malignancy	Iron-deficiency anemia
Infection	Surgery
Myeloproliferative disorders: polycythemia vera, primary myelofibrosis, essential thrombocytosis, chronic myelogenous leukemia	Rebound: Correction of vitamin B ₁₂ or folate deficiency, post-ethanol abuse
Myelodysplastic disorders: 5q-syndrome, idiopathic refractory sideroblastic anemia	Hemolysis
Postsplenectomy or hyposplenism	Familial: Thrombopoietin overproduction, <i>JAK2</i> or <i>MPL</i> mutations

or thrombosis, it is now clear that ET can occur at any age in adults and often without symptoms or disturbances of hemostasis. There is an unexplained female predominance in contrast to PMF or the reactive forms of thrombocytosis where no sex difference exists. Because no specific clonal marker is available, clinical and laboratory criteria have been proposed to distinguish ET from other MPNs, which may also present with initially with isolated thrombocytosis but have differing prognoses and therapies (Table 103-6). These criteria are useful in identifying disorders such as CML, PV, PMF, or myelodysplasia, which can masquerade as ET. Furthermore, as with “idiopathic” erythrocytosis, nonclonal benign forms of thrombocytosis exist (e.g., hereditary overproduction of thrombopoietin and those with noncanonical *JAK2* driver mutations) that are not widely recognized because we currently lack diagnostic assays. Approximately 50% of ET patients express *JAK2* V617F, 30% *CALR* (both type 1 and type 2), and 8% *MPL* mutations. ET patients lacking a canonical MPN driver mutation usually have a benign prognosis.

Etiology

Megakaryocytopoiesis and platelet production depend on thrombopoietin and its receptor MPL. As in the case of early erythroid and myeloid progenitor cells, early megakaryocytic progenitors require the presence of interleukin 3 (IL-3) and stem cell factor for optimal proliferation in addition to thrombopoietin. Their subsequent terminal development is also enhanced by the chemokine stromal cell-derived factor 1 (SDF-1). Interestingly, terminal megakaryocyte maturation and platelet production do not require thrombopoietin.

Megakaryocytes are unique among hematopoietic progenitor cells because reduplication of their genome is endomitotic rather than mitotic and promoted by thrombopoietin. Unlike erythropoietin, thrombopoietin is produced primarily in the liver but has important functions in the bone marrow where it functions to maintain hematopoietic stem cells quiescent in their endosteal niches; once released from their niches, thrombopoietin promotes the proliferation of these cells in the sinusoidal niche. Like plasma erythropoietin and its target erythroblasts, an inverse correlation exists between the platelet count and plasma thrombopoietin. However, unlike erythropoietin, thrombopoietin is only constitutively produced and the plasma thrombopoietin level is controlled by the size of the platelet and megakaryocyte progenitor cell pools. Also, in contrast to erythropoietin, but like its myeloid counterparts, granulocyte and granulocyte-macrophage colony-stimulating factors, thrombopoietin not only enhances the proliferation of its target cells but also enhances the reactivity of their end-stage product, the platelet. Paradoxically, in the three MPNs, expression of the thrombopoietin receptor, MPL, is impaired and plasma thrombopoietin is increased despite the increased number of megakaryocytes and platelets.

The clonal nature of ET was established by analysis of glucose-6-phosphate dehydrogenase isoenzyme expression in patients hemizygous for this gene. Although thrombocytosis is its principal manifestation,

like the other MPNs, a hematopoietic stem cell is involved in ET. Furthermore, a number of families have been described in which ET was inherited, in one instance as an autosomal dominant trait. In addition to ET, PMF and PV have also been observed in such kindreds.

■ CLINICAL FEATURES

Clinically, ET is most often identified incidentally when a platelet count is obtained during the course of a routine medical evaluation. Occasionally, review of previous blood counts will reveal that an elevated platelet count was present but overlooked for many years. No symptoms or signs are specific for ET, but these patients can have hemorrhagic and thrombotic tendencies expressed as easy bruising for the former and microvascular occlusive events for the latter such as erythromelalgia, ocular migraine, or a TIA. Physical examination is generally unremarkable. Splenomegaly is indicative of another MPN, in particular PV, PMF, or CML.

Anemia is unusual, but a mild neutrophilic leukocytosis is not. The blood smear is most remarkable for the number of platelets present, some of which may be very large. The large mass of circulating platelets may prevent the accurate measurement of serum potassium due to release of platelet potassium upon blood clotting. This type of hyperkalemia is a test tube artifact and not associated with electrocardiographic abnormalities. Similarly, arterial oxygen measurements can be inaccurate unless thrombocytemic blood is collected on ice. The prothrombin and partial thromboplastin times are normal, whereas abnormalities of platelet function such as a prolonged bleeding time and impaired platelet aggregation can be present. However, despite much study, no platelet function abnormality is characteristic of ET, and no platelet function test predicts the risk of clinically significant bleeding or thrombosis.

The elevated platelet count may hinder marrow aspiration, but marrow biopsy usually reveals megakaryocyte hypertrophy and hyperplasia, as well as an overall increase in marrow cellularity. If marrow reticulin is increased, another diagnosis should be considered. The absence of stainable iron demands an explanation because iron deficiency alone can cause thrombocytosis, and absent marrow iron in the presence of marrow hypercellularity is a feature of PV.

Nonrandom cytogenetic abnormalities occur in ET but are uncommon, and no specific or consistent abnormality is notable, even those involving chromosomes 3 and 1, where the genes for thrombopoietin and its receptor, MPL, respectively, are located.

■ DIAGNOSIS

Thrombocytosis is encountered in a broad variety of clinical disorders (Table 103-6), in many of which inflammatory cytokine production is increased. The absolute level of the platelet count is not a useful diagnostic aid for distinguishing between benign and clonal causes of thrombocytosis. About 50% of ET patients express the *JAK2* V617F mutation. When *JAK2* V617F is absent, cytogenetic evaluation is mandatory to determine if the thrombocytosis is due to CML or a myelodysplastic disorder such as the 5q- syndrome or sideroblastic anemia. Because the *BCR-ABL* translocation can be present in the absence of the Ph chromosome, and because the *BCR-ABL* reverse transcriptase polymerase chain reaction is associated with false-positive results, fluorescence in situ hybridization (FISH) analysis for *BCR-ABL* is the preferred assay in patients with thrombocytosis in whom a cytogenetic study for the Ph chromosome is negative. *CALR* mutations (type 1 or type 2) are present in 30% and *MPL* mutations are present in 8% of ET patients who do not have a *JAK2* mutation. Anemia and ringed sideroblasts are not features of ET, but they are features of idiopathic refractory sideroblastic anemia, and in some of these patients, the thrombocytosis occurs in association with expression of *JAK2* V617F, *CALR*, or an *MPL* mutation. Significant splenomegaly should suggest the presence of another MPN, and in this setting, a red cell mass determination should be performed because splenomegaly can mask the presence of erythrocytosis. Importantly, what appears to be ET can evolve into PV (usually in women with *JAK2* V617F) or PMF (usually in men with type 1 *CALR* mutations) after a period of many years due to clonal evolution or succession. There is sufficient overlap of the

JAK2 V617F neutrophil allele burden between ET and PV that this cannot be used as a distinguishing diagnostic feature with the exception that, in ET, the quantitative *JAK2* V617F neutrophil allele is never greater than 50%; only a red cell mass and plasma volume determination can distinguish PV from ET, and importantly in this regard, 64% of *JAK2* V617F-positive ET patients in one study actually were found to have PV when red cell mass and plasma volume determinations were performed. Claims that ET and PV form a biological continuum are unfounded as these disorders have different gene expression profiles and different natural histories.

■ COMPLICATIONS

Perhaps no other condition in clinical medicine has caused otherwise astute physicians to intervene inappropriately more often than thrombocytosis, particularly if the platelet count is $>1 \times 10^9/\mu\text{L}$. It is commonly believed that a high platelet count causes thrombosis; however, no controlled clinical study has ever established this association, and in patients younger than age 60 years, the incidence of thrombosis was not greater in patients with thrombocytosis than in age-matched controls, and tobacco use appears to be the most important risk factor for thrombosis in ET patients.

To the contrary, very high platelet counts are associated primarily with hemorrhage due to acquired von Willebrand's disease. This is not meant to imply that an elevated platelet count cannot cause symptoms in an ET patient, but rather that the focus should be on the patient, not the platelet count. For example, some of the most dramatic neurologic problems in ET are migraine-related and respond only to lowering of the platelet count, whereas other symptoms such as erythromelalgia respond simply to platelet cyclooxygenase-1 inhibitors such as aspirin or ibuprofen, without a reduction in platelet number. Still others may represent an interaction between an atherosclerotic vascular system and a high platelet count, and others may have no relationship to the platelet count whatsoever. Recognition that PV can present with thrombocytosis alone as well as the discovery of previously unrecognized causes of hypercoagulability (Chaps. 116 and 117) make the older literature on the complications of thrombocytosis unreliable.

TREATMENT

Essential Thrombocythosis

Survival of ET patients is not different than the general population regardless of their driver mutation. An elevated platelet count in an asymptomatic patient without cardiovascular risk factors or tobacco use requires no therapy. Indeed, before any therapy is initiated in a patient with thrombocytosis, the cause of symptoms must be clearly identified as due to the elevated platelet count. When the platelet count rises above $1 \times 10^9/\mu\text{L}$, a substantial quantity of high-molecular-weight von Willebrand multimers are removed from the circulation and destroyed by the enlarged platelet mass, resulting in an acquired form of von Willebrand's disease. This can be identified by a reduction in ristocetin cofactor activity. In this situation, aspirin could promote hemorrhage. Bleeding in this situation is rarely spontaneous and usually responds to tranexamic acid or ϵ -aminocaproic acid, which can be given prophylactically before and after elective surgery.

Plateletpheresis is at best a temporary and inefficient remedy that is rarely required. Importantly, ET patients treated with ^{32}P or alkylating agents are at risk of developing acute leukemia without any proof of benefit; combining either therapy with hydroxyurea increases this risk. If platelet reduction is deemed necessary on the basis of symptoms refractory to salicylates alone, pegylated IFN- α , the quinazoline derivative anagrelide, or hydroxyurea can be used to reduce the platelet count, but none of these is uniformly effective or without significant side effects. Hydroxyurea and aspirin were more effective than anagrelide and aspirin for prevention of TIA because hydroxyurea is a nitric oxide donor, but they were not more effective for the prevention of other types of arterial thrombosis and actually less effective for venous thrombosis. The risk of

gastrointestinal bleeding is also higher when aspirin is combined with anagrelide. Normalizing the platelet count does not prevent either arterial or venous thrombosis. Pegylated interferon can produce a complete molecular remission in some ET patients, but a role for it or ruxolitinib in ET management has not yet been established.

As more clinical experience is acquired, ET appears more benign than previously thought. Evolution to acute leukemia is more likely to be a consequence of therapy than of the disease itself. In managing patients with thrombocytosis, the physician's first obligation is to do no harm.

FURTHER READING

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104

Acute Myeloid Leukemia

William Blum



INCIDENCE

Acute myeloid leukemia (AML) is a neoplasm characterized by infiltration of the blood, bone marrow, and other tissues by proliferative, clonal, poorly differentiated cells of the hematopoietic system. These leukemias comprise a spectrum of malignancies that untreated are uniformly fatal. In 2020, the estimated number of new AML cases in the United States was 19,940. AML is the diagnosis in 1.3% of all cancer cases and 31% of all new acute leukemias but causes 62% of leukemic deaths. AML is the most common acute leukemia in older patients, with a median age at diagnosis of 67 years. Long-term survival is infrequent; U.S. registry data report that only 27% of patients survive 5 years.

ETIOLOGY

Most cases of AML are idiopathic. Genetic predisposition, radiation, chemical/other occupational exposures, and drugs have been implicated in the development of AML, but AML cases with established etiology are relatively rare. No direct evidence suggests a viral etiology. Genome sequencing studies suggest that most cases of AML arise from a limited number of mutations that accumulate with advancing age. Indeed, genome sequencing is providing paradigm-shifting advances in our understanding of leukemogenesis. The Cancer Genome Atlas (TCGA) and other databases demonstrate that blood cells from up to 5–6% of normal individuals aged >70 years contain potentially “premalignant” mutations that are associated with clonal expansion.

Use of the term *premalignant* to describe these lesions is not precisely accurate; rather, these mutations represent clonal hematopoiesis of *indeterminate* potential (CHIP; sometimes called age-related clonal hematopoiesis). The genes most commonly altered include the epigenetic regulators *DNMT3A*, *TET2*, and *ASXL1*.

Study of CHIP is important because CHIP has relevance not just to blood cancer evolution but also other medical conditions. Clonal expansion driven by the acquisition of new mutations is associated with a 10-fold increase in risk for developing a hematologic malignancy (compared to matched patients without CHIP), but it is clear that additional “hits” must occur to drive toward leukemia. We do

not yet fully understand why or how these secondary lesions occur. Patients with CHIP also have increased risk of cardiovascular mortality that is not fully explained. The link between these two seemingly unrelated issues (cardiovascular and hematologic malignancy) may lie in understanding the interactions between circulating clonally expanded blood cells and vascular endothelium. A “proinflammatory” state caused by clonal, infiltrating monocytes leads to accelerated atherosclerotic plaque development and altered cardiac remodeling. Similar phenomena may occur in the marrow and blood—altered relationships between hematopoietic stem cells with the marrow microenvironment along with altered immune surveillance. Both increase the likelihood that a clone may survive, acquire additional mutations, and then further expand eventually to leukemia. Whether early identification of CHIP in patients will provide therapeutic opportunities for patients remains to be seen. Certainly, modifying cardiovascular risk in patients with CHIP seems prudent, but development of mutation-directed therapies to eliminate problematic clones to prevent leukemia is likely to be more elusive.

Genetic Predisposition Myeloid neoplasms typically occur sporadically in adults; inherited predisposition is rare. Yet, it is clear that myeloid neoplasms with germline predisposition represent an important and growing subset of disease. Germline mutations associated with increased risk of developing a myeloid neoplasm include *CEBPA*, *DDX41*, *RUNX1*, *ANKRD26*, *ETV6*, and *GATA2* (Table 104-1). Likewise, myeloid neoplasms with germline predisposition are a feature of several well-described clinical syndromes, including bone marrow failure disorders (e.g., Fanconi anemia, Shwachman-Diamond syndrome, Diamond-Blackfan anemia) and telomere biology disorders (e.g., dyskeratosis congenita). As new mutations and associations are added to a rapidly growing list, it is increasingly clear that genetic predisposition plays a larger role than has been previously understood.

Several genetic syndromes with somatic cell chromosome aneuploidy, such as Down syndrome with trisomy 21, are associated with an increased incidence of AML. Down syndrome-associated AML in young children (<4 years) is typically of the acute megakaryocytic subtype and is associated with mutation in the *GATA1* gene. Such

TABLE 104-1 WHO 2016 Classification of Myeloid Neoplasms with Germline Predisposition

CLASSIFICATION^a

Myeloid neoplasms with germline predisposition without a preexisting disorder or organ dysfunction
Acute myeloid leukemia with germline <i>CEBPA</i> mutation
Myeloid neoplasms with germline <i>DDX41</i> mutation ^b
Myeloid neoplasms with germline predisposition and preexisting platelet disorders
Myeloid neoplasms with germline <i>RUNX1</i> mutation ^b
Myeloid neoplasms with germline <i>ANKRD26</i> mutation ^b
Myeloid neoplasms with germline <i>ETV6</i> mutation ^b
Myeloid neoplasms with germline predisposition and other organ dysfunction
Myeloid neoplasms with germline <i>GATA2</i> mutation
Myeloid neoplasms associated with bone marrow failure syndromes
Myeloid neoplasms associated with telomere biology disorders
Myeloid neoplasms associated with Noonan syndrome
Myeloid neoplasms associated with Down syndrome ^b

^aRecognition of familial myeloid neoplasms requires that physicians take a thorough patient and family history to assess for typical signs and symptoms of known syndromes, including data on malignancies and previous bleeding episodes.

Molecular genetic diagnostics is guided by a detailed patient and family history. Diagnostics should be performed in close collaboration with a genetic counselor; patients with a suspected heritable myeloid neoplasm, who test negative for known predisposition genes, should ideally be entered on a research study to facilitate new syndrome discovery. ^bLymphoid neoplasms also reported.

Source: Reproduced with permission from L Peterson et al: Myeloid neoplasms with germline predisposition, in *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th revised ed. Geneva, Switzerland: World Health Organization, 2017.

patients have excellent clinical outcomes but require dose modification of chemotherapy due to high treatment-related toxicities. Inherited diseases with defective DNA repair (e.g., Fanconi anemia, Bloom syndrome, and ataxia-telangiectasia) are also associated with AML. Each syndrome is associated with unique clinical features and atypical toxicities with chemotherapy, requiring expert care. Congenital neutropenia (Kostmann syndrome), due to mutations in the genes encoding the granulocyte colony-stimulating factor receptor and neutrophil elastase, is another disorder that may evolve into AML.

Chemical, Radiation, and Other Exposures Anticancer drugs are the leading cause of therapy-associated AML. Alkylating agent-associated leukemias occur on average 4–6 years after exposure, and affected individuals often have multilineage dysplasia and monosomy/aberrations in chromosomes 5 and 7. Topoisomerase II inhibitor-associated leukemias occur 1–3 years after exposure, and affected individuals often have AML with monocytic features and aberrations involving chromosome 11q23. Exposure to ionizing radiation, benzene, chloramphenicol, phenylbutazone, and other drugs can uncommonly result in bone marrow failure that may evolve into AML.

CLASSIFICATION

The current categorization of AML uses the World Health Organization (WHO) classification (Table 104-2), which defines biologically distinct groups based on cytogenetic and molecular abnormalities in addition to clinical features and light microscope morphology. Myeloid neoplasms with germline predisposition, as introduced above, are included as a new and important feature of this classification (Table 104-1).

TABLE 104-2 WHO 2016 Classification of Acute Myeloid Leukemia and Related Neoplasms

Acute myeloid leukemia (AML) with recurrent genetic abnormalities
AML with t(8;21)(q22;q22); <i>RUNX1-RUNX1T1</i>
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i>
Acute promyelocytic leukemia with <i>PML-RARA</i>
AML with t(9;11)(p21.3;q23.3); <i>MLLT3-KMT2A</i>
AML with t(6;9)(p23;q34.1); <i>DEK-NUP214</i>
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); <i>GATA2, MECOM</i>
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); <i>RBM15-MKL1</i>
Provisional entity: AML with <i>BCR-ABL1</i>
AML with mutated <i>NPM1</i>
AML with biallelic mutations of <i>CEBPA</i>
Provisional entity: AML with mutated <i>RUNX1</i>
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, not otherwise specified (NOS)
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome

Note: Marrow blast count of $\geq 20\%$ is required, except for AML with the recurrent genetic abnormalities t(15;17), t(8;21), inv(16), or t(16;16).

Source: Adapted from DA Arber et al: Acute myeloid leukaemia (AML) with recurrent genetic abnormalities, in *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th revised ed. Geneva, Switzerland: World Health Organization; 2017.

The WHO classification enables the identification of subsets of disease that may be treated differently (now or in the future) and enhances recognition of the molecular basis of disease from the time of diagnosis. Marrow (or blood) blast count of $\geq 20\%$ is required to establish the diagnosis of AML, except for AML with the recurrent genetic abnormalities t(15;17), t(8;21), inv(16), or t(16;16).

Clinical Features Even with advances in molecular biology, recognizing clinical features remains important in understanding AML. For example, therapy-related AML is a distinct entity that develops following prior chemotherapy (e.g., alkylating agents, topoisomerase II inhibitors) or ionizing radiation. AML with myelodysplasia-related changes is recognized in part on morphology but also on a medical history of an antecedent myelodysplastic syndrome (MDS) or myelodysplastic/myeloproliferative neoplasm. These clinical features contribute to AML prognosis and are therefore in the WHO classification.



Genetic Findings Subtypes of AML are recognized due to the presence or absence of specific, recurrent cytogenetic, and/or genetic abnormalities. For example, the diagnosis of *acute promyelocytic leukemia* (APL) is based on the presence of either the t(15;17)(q22;q12) cytogenetic rearrangement or the *PML-RARA* fusion product of the translocation. Similarly, core binding factor (CBF) AML is designated based on the presence of t(8;21)(q22;q22), inv(16)(p13.1q22), or t(16;16)(p13.1;q22) or the respective fusion products *RUNX1-RUNX1T1* and *CBFB-MYH11*. Each of these three groups identifies patients with favorable clinical outcomes when appropriately treated.

Several cytogenetic or genetic AML subtypes often associate with a specific morphologic appearance, such as a complex karyotype (and/or mutation of *TP53*) and AML with myelodysplasia-related changes. Patients with such changes typically fare poorly with standard treatments. However, only one abnormality is invariably associated with specific morphologic features: t(15;17)(q22;q12) or the molecular fusion *PML-RARA* with APL. Other cytogenetic and genetic findings may be commonly, but not always, associated with a morphologic description, highlighting the necessity of genetic and cytogenetic testing for precise diagnosis. Several chromosomal abnormalities often associate primarily with one morphologic/immunophenotypic group. Examples include inv(16)(p13.1q22) with AML with abnormal bone marrow eosinophils; t(8;21)(q22;q22) with slender Auer rods, expression of CD19, and increased normal eosinophils; and t(9;11)(p22;q23) and other translocations involving 11q23 with monocytic features. Mutation of nucleophosmin (nucleolar phosphoprotein B23, numatrin, *NPM1*), especially when co-occurring with mutation of fms-related tyrosine kinase 3 (*FLT3*), often presents with “cup-shaped” nuclear morphology. Recurring chromosomal abnormalities in AML may also be loosely associated with specific clinical characteristics. More commonly associated with younger age are t(8;21) and t(15;17), and with older age, del(5q), del(7q), and mutated *TP53*. Myeloid sarcomas are associated with t(8;21); disseminated intravascular coagulation (DIC) is associated with t(15;17). 11q23 aberrations and monocytic leukemia are associated with extramedullary sites of involvement at presentation, especially gingival hypertrophy. High leukocyte count is commonly observed with *NPM1* or *FLT3* mutation.

The WHO classification also incorporates molecular abnormalities by recognizing fusion genes or specific genetic mutations with a role in leukemogenesis. As a classic example, t(15;17) results in the fusion gene *PML-RARA* that encodes a chimeric protein, promyelocytic leukemia (Pml)-retinoic acid receptor α (Rara), which is formed by the fusion of the retinoic acid receptor α (*RARA*) gene from chromosome 17 and the promyelocytic leukemia (*PML*) gene from chromosome 15. Unique clinical therapy with retinoic acid and arsenic trioxide has revolutionized the care of APL patients (see “Treatment of Acute Promyelocytic Leukemia” section). Similar examples of molecular subtypes included in the category of AML with recurrent genetic abnormalities are those characterized by the leukemogenic fusion genes *RUNX1-RUNX1T1* and *CBFB-MYH11* and the so-called CBF AML subtypes noted cytogenetically as t(8;21), inv(16), or t(16;16). Additional fusions

are *MLLT3-KMT2A* and *DEK-NUP214*, resulting from t(9;11) and t(6;9)(p23;q34), respectively, among others.

The WHO classification of AML continues to expand as knowledge of specific genetic or cytogenetic aberrations grows. Several AML subtypes are defined by the presence of genetic mutations rather than chromosomal aberrations. For example, *AML with mutated NPM1* and *AML with biallelic mutated CEBPA*, respectively, are associated with more favorable clinical outcome, though the presence of coexisting mutation in *FLT3* affects *NPM1* prognostic impact. Activating mutations of *FLT3* are present in ~30% of adult AML patients, primarily due to internal tandem duplications (ITDs) in the juxtamembrane domain that have negative prognostic impact. In contrast, point mutations of the activating loop of the kinase (called tyrosine kinase domain [TKD] mutations) have uncertain prognostic impact. Aberrant activation of the *FLT3*-encoded protein provides increased proliferation and antiapoptotic signals to the myeloid progenitor cell. *FLT3*-ITD, the more common of the *FLT3* mutations, occurs preferentially in patients with cytogenetically normal AML (CN-AML). The importance of identifying *FLT3*-ITD at diagnosis relates to the fact that it is not only useful as a prognosticator but also may predict response to specific treatment such as a tyrosine kinase inhibitor (TKI). Several TKIs targeting *FLT3* are either approved for AML (e.g., midostaurin, only in first-line therapy in combination with chemotherapy; gilteritinib, in relapse as monotherapy) or currently in clinical investigation (e.g., quizartinib, crenolanib, sorafenib, and others). The *FLT3* allelic ratio (of the number of mutated alleles to wild-type alleles) provides information beyond the mere presence or absence of the mutation. Several mutational scenarios, such as one mutated gene and one wild-type gene or one mutated gene with no (deleted) wild-type gene, and the ratio of malignant to nonmalignant cells in the sample affect the ratio. The allelic ratio affects the prognostic impact of the *FLT3*-ITD mutation; patients with *FLT3*-ITD “low” allelic ratio (<0.5) fare better. Accordingly, mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} is viewed as favorable risk by the European LeukemiaNet (ELN) risk stratification schema (Table 104-3). Conversely, *FLT3*-ITD^{high} has an adverse prognostic impact; patients with both mutated *NPM1* and *FLT3*-ITD with an allelic ratio >0.5 are intermediate risk by ELN stratification. Involving a different tyrosine kinase, AML with *BCR-ABL1* fusion is a new WHO provisional entity to recognize rare cases that may benefit from *BCR-ABL* TKI therapy (Table 104-2).

Immunophenotypic Findings The immunophenotype of human leukemia cells can be studied by multiparameter flow cytometry after the cells are labeled with monoclonal antibodies to cell-surface antigens. This can be important in quickly distinguishing AML from acute lymphoblastic leukemia and for identifying some subtypes of AML. For example, AML with minimal differentiation, characterized by immature morphology and no lineage-specific cytochemical reactions, may be diagnosed by flow-cytometric demonstration of the myeloid-specific antigens cluster designation (CD) 13 and/or 117. Similarly, acute megakaryoblastic leukemia can often be diagnosed only by expression of the platelet-specific antigens CD41 and/or CD61. Although flow cytometry is widely used, and in some cases essential for the diagnosis of AML, it has only a supportive role in establishing the different subtypes of AML through the WHO classification. Increasingly, multiparameter flow cytometry is used for the measurement of measurable residual disease (MRD) after remission is achieved.

■ PROGNOSTIC FACTORS

Several factors predict outcome of AML patients treated with chemotherapy; they should be used for risk stratification and treatment guidance.

Chromosome and molecular investigations performed at diagnosis currently provide the most important prognostic information. WHO has categorized patients as having favorable, intermediate, or adverse risk based on the presence of structural and/or numerical chromosomal or genetic aberrations. Patients with t(15;17) have a very good prognosis (~85% cured), and those with t(8;21) and inv(16) have a good prognosis (~55% cured), whereas those with no cytogenetic

TABLE 104-3 2017 European LeukemiaNet Risk Stratification by Genetics for Acute Myeloid Leukemia (AML)^a

RISK CATEGORY ^b	GENETIC ABNORMALITY
Favorable	t(8;21)(q22;q22); <i>RUNX1-RUNX1T1</i> inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i> Mutated <i>NPM1</i> without <i>FLT3</i> -ITD or with <i>FLT3</i> -ITD ^{low(c)} Biallelic mutated <i>CEBPA</i>
Intermediate	Mutated <i>NPM1</i> and <i>FLT3</i> -ITD ^{high(c)} Wild-type <i>NPM1</i> without <i>FLT3</i> -ITD or with <i>FLT3</i> -ITD ^{low(c)} (w/o adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); <i>MLLT3-KMT2A</i> ^d Oncogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); <i>DEK-NUP214</i> t(v;11q23.3); <i>KMT2A</i> rearranged t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i> inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2); <i>GATA2</i> , <i>MECOM</i> (<i>EVI1</i>) –5 or del(5q); –7; –17(abn17p) Complex karyotype, ^e monosomal karyotype ^f Wild type <i>NPM1</i> and <i>FLT3</i> -ITD ^{high(c)} Mutated <i>RUNX1</i> ^g Mutated <i>ASXL1</i> ^g Mutated <i>TP53</i> ^g

^aThis table excludes acute promyelocytic leukemia. Frequencies, response rates, and outcome measures should be reported by risk category and, if sufficient numbers are available, by specific genetic lesions indicated. ^bPrognostic impact of a marker is treatment-dependent and may change with new therapies. ^cLow, low allelic ratio (<0.5); high, high allelic ratio (≥ 0.5); semiquantitative assessment of *FLT3*-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve (AUC) “*FLT3*-ITD” divided by AUC “*FLT3*-wild type”; recent studies indicate that acute myeloid leukemia with *NPM1* mutation and *FLT3*-ITD low allelic ratio may also have a more favorable prognosis and patients should not routinely be assigned to allogeneic hematopoietic cell transplantation. ^dThe presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations. ^eThree or more unrelated chromosome abnormalities in the absence of one of the World Health Organization-designated recurring translocations or inversions, i.e., t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3), or t(3;3); AML with *BCR-ABL1*. ^fDefined by the presence of one single monosomy (excluding loss of X or Y) in association with at least one additional monosomy or structural chromosome abnormality (excluding core binding factor AML). ^gThese markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes. ^h*TP53* mutations are significantly associated with AML with complex and monosomal karyotype.

Source: Republished with permission of American Society of Hematology, from Diagnosis and management of acute myeloid leukemia in adults: 2017 recommendations from an international expert panel, Döhner H et al. 129:424, 2017; permission conveyed through Copyright Clearance Center, Inc.

abnormality have an intermediate outcome risk (~40% cured). Patients with a *TP53* mutation, complex karyotype, t(6;9), inv(3), or –7 have a very poor prognosis. Another cytogenetic subgroup, the monosomal karyotype, has been suggested to adversely influence the outcome of AML patients other than those with t(15;17), t(8;21), or inv(16) or t(16;16). The monosomal karyotype subgroup is defined by the presence of at least two autosomal monosomies (loss of chromosomes other than Y or X) or a single autosomal monosomy with additional structural abnormalities.

For patients lacking prognostic cytogenetic abnormalities, i.e., those with CN-AML, testing for several mutated genes can help to risk-stratify. In addition to *NPM1* mutation and *FLT3*-ITD as described above, biallelic *CEBPA* mutations have prognostic value. Such mutations predict favorable outcome. Given the proven prognostic importance of *NPM1*, *CEBPA*, and *FLT3*, molecular assessment of these genes at diagnosis has been incorporated into AML management guidelines by the National Comprehensive Cancer Network (NCCN) and the ELN. The same markers help to define genetic groups in the ELN standardized reporting system, which is based on both cytogenetic and molecular abnormalities and is used for comparing clinical features/treatment response among subsets of patients reported across different clinical studies (Table 104-3). These genetic groups should be used for risk stratification and treatment guidance.

TABLE 104-4 Molecular Prognostic Markers in AML^a

GENE SYMBOL	GENE LOCATION	PROGNOSTIC IMPACT
Genes Included in the WHO Classification and ELN Reporting System		
<i>NPM1</i> mutations	5q35.1	Favorable
<i>CEBPA</i> mutations	19q13.1	Favorable
<i>FLT3</i> -ITD	13q12	Depends on allelic ratio and <i>NPM1</i> mutational status
Genes Encoding Receptor Tyrosine Kinases		
<i>KIT</i> mutation	4q12	Adverse
<i>FLT3</i> -TKD	13q12	Unclear
Genes Encoding Transcription Factors		
<i>RUNX1</i> mutations	21q22.12	Adverse
<i>WT1</i> mutations	11p13	Adverse
Genes Encoding Epigenetic Modifiers		
<i>ASXL1</i> mutations	20q11.21	Adverse
<i>DNMT3A</i> mutations	2p23.3	Adverse
<i>IDH</i> mutations (<i>IDH1</i> and <i>IDH2</i>)	2q34 & 15q26.1	Adverse
<i>KMT2A</i> -PTD	11q23	Adverse
<i>TET2</i> mutations	4q24	Adverse
Deregulated Genes		
<i>BAALC</i> overexpression	8q22.3	Adverse
<i>ERG</i> overexpression	21q22.3	Adverse
<i>MN1</i> overexpression	22q12.1	Adverse
<i>EVI1</i> overexpression	3q26.2	Adverse
Deregulated MicroRNAs		
<i>miR-155</i> overexpression	21q21.3	Adverse
<i>miR-3151</i> overexpression	8q22.3	Adverse
<i>miR-181a</i> overexpression	1q32.1 and 9q33.3	Favorable

^aThis table excludes acute promyelocytic leukemia.

Abbreviations: AML, acute myeloid leukemia; ELN, European LeukemiaNet; ITD, internal tandem duplication; PTD, partial tandem duplication; TKD, tyrosine kinase domain; WHO, World Health Organization.

In addition to *NPM1*, *CEBPA*, *FLT3*, and *TP53* mutations, molecular aberrations in other genes may be routinely used for prognostication (Table 104-4). Among these mutated genes are those encoding receptor tyrosine kinases (*KIT*), transcription factors (*RUNX1* and *WT1*), and epigenetic modifiers (*ASXL1*, *DNMT3A*, isocitrate dehydrogenase 1 [*IDH1*], *IDH2*, *KMT2A* [also known as *MLL*], and *TET2*). Although *KIT* mutations are almost exclusively present in CBF AML and impact adversely the outcome, the remaining markers have been reported primarily in CN-AML. Mutations of *ASXL1* and *RUNX1* are associated with adverse outcome, independent of other prognostic factors. However, for some of these mutations, data remain unclear on the prognostic impact due to conflicting reports (e.g., *TET2*, *IDH1*, *IDH2*). Increasingly, novel drugs that inhibit/modulate aberrant pathways activated by some of these genes (especially *FLT3*, *IDH1*, and *IDH2*) have been remarkably effective in subsets of disease, leading to U.S. Food and Drug Administration approvals (see section on treatment of AML).

In addition to gene mutations, deregulation of the expression levels of coding genes and of short noncoding RNAs (microRNAs) also provides prognostic information (Table 104-4). Overexpression of genes such as *BAALC*, *ERG*, *MN1*, and *MDS1* and *EVI1* complex locus (*MECOM*; also known as *EVI1*) predict poor outcome, especially in CN-AML. Similarly, deregulated expression levels of microRNAs, naturally occurring noncoding RNAs that regulate the expression of proteins via degradation or translational inhibition of their target coding RNAs, have also been associated with prognosis in AML. Overexpression of *miR-155* and *miR-3151* predicts unfavorable outcome in CN-AML, whereas overexpression of *miR-181a* predicts favorable outcome both in CN-AML and cytogenetically abnormal AML.

Because prognostic molecular markers in AML are not mutually exclusive and often occur concurrently (>80% patients have at least two or more prognostic gene mutations), the likelihood that distinct marker combinations may be more informative than single markers is increasingly clear.

Epigenetic changes (e.g., DNA methylation and/or posttranslational histone modification) and microRNAs are often involved in deregulation of genes involved in hematopoiesis, contribute to leukemogenesis, and may associate with the previously discussed prognostic gene mutations. These changes have been shown to provide biologic insights into leukemogenic mechanisms and provide independent prognostic information. Therapeutic progress based on advances in understanding the role of epigenetic changes in AML is currently unfolding. For example, in patients with mutations of *IDH1* or *IDH2*, novel active enzymes produced from these respective mutations hijack the citric acid cycle, leading to production of a novel “oncometabolite,” 2-hydroxyglutarate, which disrupts a myriad of epigenetic processes. Pharmacologic inhibition of these aberrant enzymes can reverse these leukemogenic activities.

In addition to cytogenetic and molecular aberrations, several other factors are associated with outcome in AML. Age at diagnosis is one of the most important risk factors. Advancing age is associated with a poor prognosis for two reasons: (1) its influence on the ability to survive induction therapy due to coexisting medical comorbidities, and (2) with each successive decade of age, a greater proportion of patients have intrinsically more resistant disease. A prolonged symptomatic interval with cytopenias preceding AML diagnosis or a history of antecedent hematologic disorders including MDS or myeloproliferative neoplasms is often found in older patients. Cytopenia is a clinical feature associated with a lower complete remission (CR) rate and shorter survival time. The CR rate is lower in patients who have had anemia, leukopenia, and/or thrombocytopenia for >3 months before the diagnosis of AML, when compared to those without such a history. Responsiveness to chemotherapy declines as the duration of the antecedent disorder increases. Likewise, AML developing after treatment with cytotoxic agents for other malignancies is usually difficult to treat successfully. In addition, older patients less frequently harbor favorable cytogenetic abnormalities (i.e., t[8;21], inv[16], and t[16;16]) and more frequently harbor adverse cytogenetic (e.g., complex and monosomal karyotypes) and/or molecular (e.g., *ASXL1*, *TP53*) abnormalities.

Other factors independently associated with worse outcome are a poor performance status that influences ability to survive induction therapy and a high presenting leukocyte count that in some series is an adverse prognostic factor for attaining a CR. Among patients with hyperleukocytosis (>100,000/ μ L), early central nervous system bleeding and pulmonary leukostasis contribute to poor outcomes.

Following administration of therapy, achievement of CR is associated with better outcome and longer survival. CR is defined after examination of both blood and bone marrow and essentially represents eradication of detectable leukemia and restoration of normal hematopoiesis. The blood neutrophil count must be $\geq 1000/\mu$ L and the platelet count $\geq 100,000/\mu$ L. Hemoglobin concentration is not considered in determining CR. Circulating blasts should be absent. Although rare blasts may be detected in the blood during marrow regeneration, they should disappear on successive studies. At CR, the bone marrow should contain <5% blasts, and Auer rods should be absent. Extramedullary leukemia should not be present.

CLINICAL PRESENTATION

Symptoms Patients with AML usually present with nonspecific symptoms that begin gradually, or abruptly, and are the consequence of anemia, leukocytosis, leukopenia/leukocyte dysfunction, or thrombocytopenia. Nearly half have symptoms for ≤ 3 months before the leukemia is diagnosed.

Fatigue is a frequent first symptom among AML patients. Anorexia and weight loss are common. Fever with or without an identifiable infection is the initial symptom in 10% of patients. Signs of abnormal hemostasis (bleeding, easy bruising) are common. Bone pain,

lymphadenopathy, nonspecific cough, headache, or diaphoresis may also occur.

Rarely, patients may present with symptoms from a myeloid sarcoma (a tumor mass consisting of myeloid blasts occurring at anatomic sites other than bone marrow). Sites involved are most commonly the skin, lymph node, gastrointestinal tract, soft tissue, and testis. This rare presentation, often characterized by chromosome aberrations (e.g., monosomy 7, trisomy 8, 11q23 rearrangement, inv[16], trisomy 4, t[8;21]), may precede or coincide with blood and/or marrow involvement by AML. Patients who present with isolated myeloid sarcoma typically develop blood and/or marrow involvement quickly thereafter and cannot be cured with local therapy (radiation or surgery) alone.

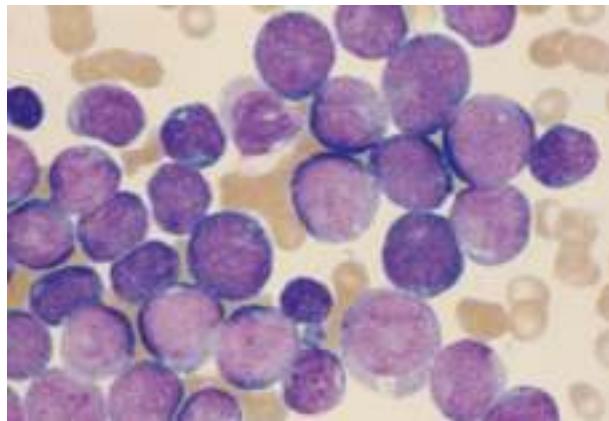
Physical Findings Fever, infection, and hemorrhage are often found at the time of diagnosis; splenomegaly, hepatomegaly, lymphadenopathy, and “bone pain” may also be present less commonly. Hemorrhagic complications are most commonly and, classically, found in APL. APL patients often present with DIC-associated minor hemorrhage but may have significant gastrointestinal bleeding, intrapulmonary hemorrhage, or intracranial hemorrhage. Likewise, thrombosis is another less frequent but well recognized clinical feature of DIC in APL. Bleeding associated with coagulopathy may also occur in monocytic AML and with extreme degrees of leukocytosis or thrombocytopenia in other morphologic subtypes. Retinal hemorrhages are detected in 15% of patients. Infiltration of the gingiva, skin, soft tissues, or meninges with leukemic blasts at diagnosis is characteristic of the monocytic subtypes and those with 11q23 chromosomal abnormalities.

Hematologic Findings Anemia is usually present at diagnosis, although it is not typically severe. The anemia is usually normocytic normochromic. Decreased erythropoiesis in the setting of AML often results in a reduced reticulocyte count, and red blood cell (RBC) survival is decreased by accelerated destruction. Active blood loss may rarely contribute to the anemia.

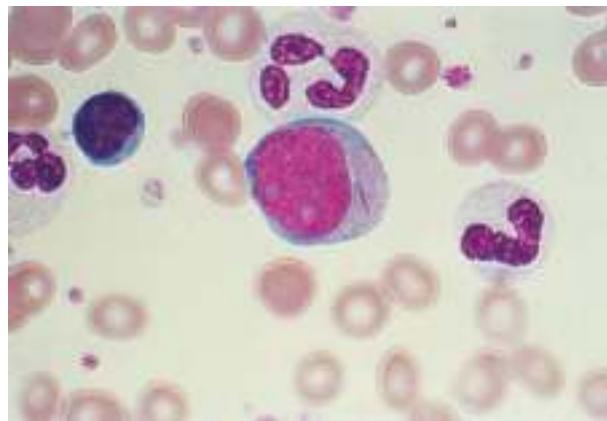
The median presenting leukocyte count is 15,000/ μ L. Lower presenting leukocyte counts are more typical of older patients and those with antecedent hematologic disorders. Between 25 and 40% of patients have counts <5000/ μ L, and 20% have counts >100,000/ μ L. Fewer than 5% have no detectable leukemic cells in the blood. In AML, the cytoplasm often contains primary (nonspecific) granules, and the nucleus shows fine, lacy chromatin with one or more nucleoli characteristic of immature cells. Abnormal rod-shaped granules called Auer rods are not uniformly present, but when they are, AML is virtually certain (Fig. 104-1).

Platelet counts <100,000/ μ L are found at diagnosis in 75% of patients, and 25% have counts <25,000/ μ L. Both morphologic and functional platelet abnormalities can be observed, including large and bizarre shapes with abnormal granulation and inability of platelets to aggregate or adhere normally to one another.

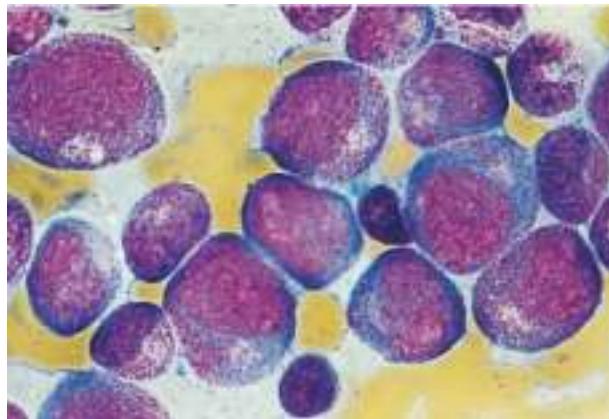
Pretreatment Evaluation Once the diagnosis of AML is suspected, thorough evaluation and initiation of appropriate therapy should follow. In addition to clarifying the subtype of leukemia, initial studies should evaluate the overall functional integrity of the major organ systems, including the cardiovascular, pulmonary, hepatic, and



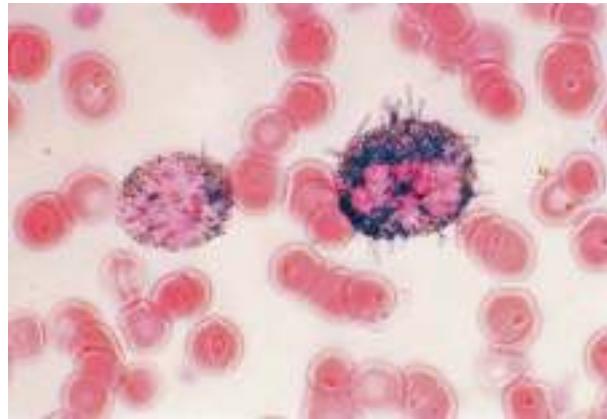
A



B



C



D

FIGURE 104-1 Morphology of acute myeloid leukemia (AML) cells. A. Uniform population of primitive myeloblasts with immature chromatin, nucleoli in some cells, and primary cytoplasmic granules. B. Leukemic myeloblast containing an Auer rod. C. Promyelocytic leukemia cells with prominent cytoplasmic primary granules. D. Peroxidase stain shows dark blue color characteristic of peroxidase in granules in AML.

TABLE 104-5 Initial Diagnostic Evaluation and Management of Adult Patients with AML**History**

- Increasing fatigue or decreased exercise tolerance (anemia)
- Excess bleeding or bleeding from unusual sites (DIC, thrombocytopenia)
- Fever or recurrent infections (neutropenia)
- Headache, vision changes, nonfocal neurologic abnormalities (CNS leukemia or bleed)
- Early satiety (splenomegaly)
- Family history of AML (Fanconi, Bloom, or Kostmann syndromes or ataxia-telangiectasia)
- History of cancer (exposure to alkylating agents, radiation, topoisomerase II inhibitors)
- Occupational exposures (radiation, benzene, petroleum products, paint, smoking, pesticides)

Physical Examination

- Performance status (prognostic factor)
- Echymosis and oozing from IV sites (DIC, possible acute promyelocytic leukemia)
- Fever and tachycardia (signs of infection)
- Papilledema, retinal infiltrates, cranial nerve abnormalities (CNS leukemia)
- Poor dentition, dental abscesses
- Gum hypertrophy (leukemic infiltration, most common in monocytic leukemia)
- Skin infiltration or nodules (leukemia infiltration, most common in monocytic leukemia)
- Lymphadenopathy, splenomegaly, hepatomegaly
- Back pain, lower extremity weakness (spinal granulocytic sarcoma, most likely in t[8;21] patients)

Laboratory and Radiologic Studies

- CBC with manual differential cell count
- Chemistry tests (electrolytes, creatinine, BUN, calcium, phosphorus, uric acid, hepatic enzymes, bilirubin, LDH, amylase, lipase)
- Clotting studies (prothrombin time, partial thromboplastin time, fibrinogen, D-dimer)
- Viral serologies (CMV, HSV-1, varicella-zoster)
- RBC type and screen
- HLA typing for potential allogeneic HCT
- Bone marrow aspirate and biopsy (morphology, cytogenetics, flow cytometry, molecular studies for *NPM1* and *CEBPA* mutations and *FLT3*-ITD)
- Cryopreservation of viable leukemia cells
- Myocardial function (echocardiogram or MUGA scan)
- PA and lateral chest radiograph
- Placement of central venous access device

Interventions for Specific Patients

- Dental evaluation (for those with poor dentition)
 - Lumbar puncture (for those with symptoms of CNS involvement)
 - Screening spine MRI (for patients with back pain, lower extremity weakness, paresthesias)
 - Social work referral for patient and family psychosocial support
- Counseling for All Patients**
- Provide patients with information regarding their disease and genetic risks, sperm banking or menstrual suppression, financial counseling, and support group contact

Abbreviations: AML, acute myeloid leukemia; BUN, blood urea nitrogen; CBC, complete blood count; CMV, cytomegalovirus; CNS, central nervous system; DIC, disseminated intravascular coagulation; HLA, human leukocyte antigen; HCT, hematopoietic stem cell transplantation; HSV, herpes simplex virus; IV, intravenous; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; MUGA, multigated acquisition; PA, posteroanterior; RBC, red blood (cell) count.

renal systems (**Table 104-5**). Factors that have prognostic significance, either for achieving CR or for CR duration, should also be assessed before initiating treatment including cytogenetics and molecular markers. Leukemic cells should be obtained from all patients and

cryopreserved for future investigational testing as well as potential future use as new diagnostics and therapeutics become available. All patients should be evaluated for infection. During the ongoing global pandemic, testing for the presence of the novel coronavirus, SARS-CoV2, is recommended before initiation of chemotherapy.

Most patients are anemic and thrombocytopenic at presentation. Replacement of the appropriate blood components, if necessary, should begin promptly. Because qualitative platelet dysfunction or the presence of an infection may increase the likelihood of bleeding, evidence of hemorrhage justifies the immediate use of platelet transfusion, even if the platelet count is only moderately decreased.

About 50% of patients have a mild to moderate elevation of serum uric acid at presentation. Only 10% have marked elevations, but renal precipitation of uric acid and the nephropathy that may result is a serious but uncommon complication. The initiation of chemotherapy may aggravate hyperuricemia, and patients are usually started immediately on allopurinol and hydration at diagnosis. Rasburicase (recombinant uric oxidase) is also useful for treating uric acid nephropathy and often can normalize the serum uric acid level within hours with a single dose of treatment, although its expense suggests that limiting its use to patients with severe hyperuricemia and/or kidney injury may be prudent. The presence of high concentrations of lysozyme, a marker for monocytic differentiation, may be etiologic in renal tubular dysfunction for a minority of patients.

TREATMENT**Acute Myeloid Leukemia**

Treatment of the newly diagnosed patient with AML is usually divided into two phases, induction and postremission management (consolidation) (**Fig. 104-2**). The initial goal is to induce CR. Once CR is obtained, further therapy must be given to prolong survival and achieve cure. The initial induction treatment and subsequent postremission therapy are chosen based on the patient's age, overall fitness, and cytogenetic/molecular risk. Intensive therapy with cytarabine and anthracycline in younger patients (<60 years) increases the cure rate of AML. In older patients, the benefit of intensive therapy is controversial in all but favorable-risk patients; novel approaches for selecting patients predicted to be responsive to treatment and new therapies are being pursued. Additional options for therapy have emerged for older AML patients such as the addition of the BCL2 antagonist venetoclax to one of several low-intensity chemotherapies. Likewise, novel oral drugs targeting IDH1 or IDH2, alone or in combination with low-intensity chemotherapy, may be considered as initial therapy for older patients who have mutations in those respective pathways.

INDUCTION CHEMOTHERAPY

The most commonly used induction regimens (for patients other than those with APL) consist of combination chemotherapy with cytarabine and an anthracycline (e.g., daunorubicin, idarubicin). Cytarabine is a cell cycle S-phase-specific antimetabolite that becomes phosphorylated intracellularly to an active triphosphate form that interferes with DNA synthesis. Anthracyclines are DNA intercalators. Their primary mode of action is thought to be inhibition of topoisomerase II, leading to DNA breaks.

In adults, cytarabine used at standard dose (100–200 mg/m²) is administered as a continuous intravenous infusion for 7 days. With cytarabine, anthracycline therapy generally consists of daunorubicin (60–90 mg/m²) or idarubicin (12 mg/m²) intravenously on days 1, 2, and 3 (the 7 and 3 regimen). Other agents can be added (e.g., gemtuzumab ozogamicin) when 60 mg/m² of daunorubicin is used. With the 7 and 3 regimen, it is now clearly established that 45 mg/m² dosing of daunorubicin results in inferior outcomes; patients should receive higher doses as described. Patients failing remission after one induction are offered reinduction with the same (or slightly modified) therapy. The CD33-targeting immunoconjugate gemtuzumab ozogamicin may be added to induction therapy for subsets of patients, especially those with CBF AML.

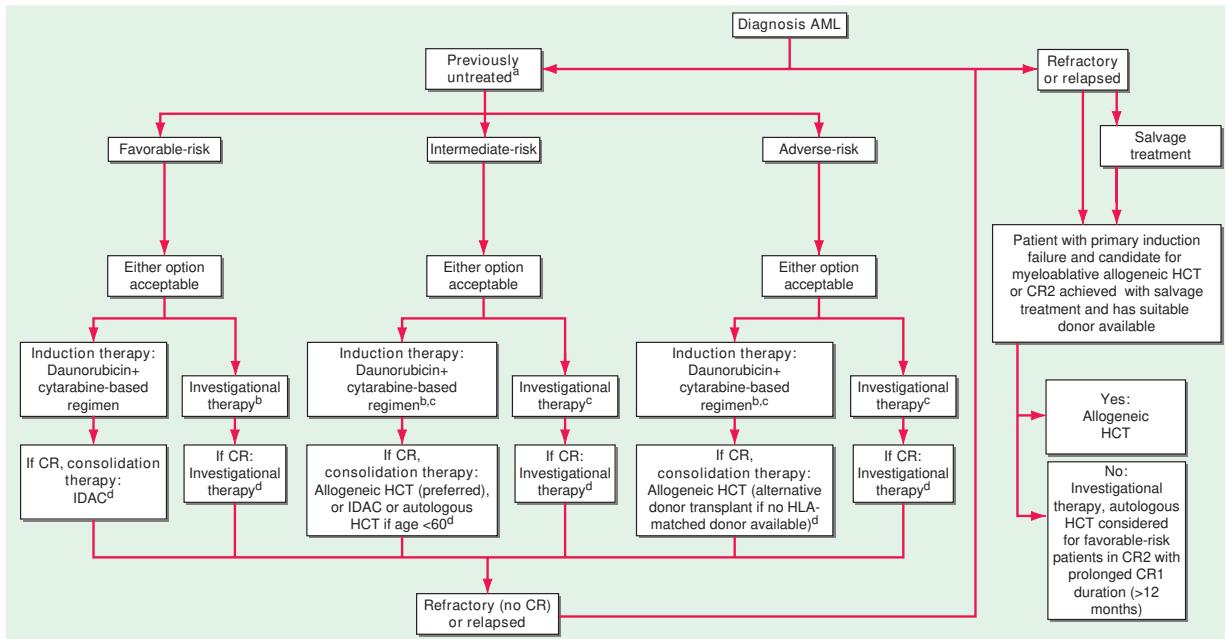


FIGURE 104-2 Algorithm for the therapy of newly diagnosed acute myeloid leukemia (AML). ^aRisk stratification according to the European LeukemiaNet (see Table 104-3). ^bYounger patients (<60–65 years) should routinely be offered investigational therapy on a backbone of standard chemotherapy for induction and consolidation. ^cOlder patients, especially those >65 years or with adverse risk disease, or those who are unfit for intensive anthracycline + cytarabine regimens, may be considered for investigational therapy alone or in combination with lower intensity chemotherapy (azacitidine, decitabine, cytarabine), or lower intensity chemotherapy in combination with venetoclax. ^dInvestigational therapy as maintenance should be considered if available (after consolidation for younger patients and older patients with favorable-risk disease, and for all other older patients after induction).

Allogeneic hematopoietic cell transplantation (HCT) is a consideration for all eligible patients in first complete remission (CR) with non-favorable-risk disease and highly recommended for older patients (60–75 years) and those with adverse risk.

For all forms of AML in fit patients, except acute promyelocytic leukemia (APL), standard induction therapy includes a regimen based on a 7-day continuous infusion of cytarabine (100–200 mg/m²/d) and a 3-day course of daunorubicin (60–90 mg/m²/d) with or without additional drugs. Idarubicin (12 mg/m²/d) can be used in place of daunorubicin (not shown). The value of postremission/consolidation therapy for older patients (>60 years) who do not have favorable-risk disease is uncertain. Patients who achieve CR undergo postremission consolidation therapy, including sequential courses of intermediate-dose cytarabine, allogeneic HCT, autologous HCT, or novel therapies, based on their predicted risk of relapse (i.e., risk-stratified therapy). Patients receiving induction of lower intensity chemotherapy with venetoclax (or investigational therapy) typically receive repetitive cycles of same on an attenuated schedule, if necessary due to myelotoxicity, after achieving remission. Patients with APL (see text for treatment) usually receive tretinoin and arsenic trioxide-based regimens with or without anthracycline-based chemotherapy and possibly maintenance with tretinoin. HLA, human leukocyte antigen; IDAC, intermediate-dose cytarabine.

In older patients (age ≥60–65 years), the outcome with conventional intensive therapy is generally poor due to a higher frequency of resistant disease and increased rate of treatment-related mortality. This is especially true in patients with prior hematologic disorders (MDS or myeloproliferative neoplasms), therapy-related AML, or cytogenetic and genetic abnormalities that adversely influence clinical outcome. Patients still fare far better with treatment than with supportive care only. Conventional therapy for fit older patients is similar to that for younger patients: the 7 and 3 regimen with standard-dose cytarabine and idarubicin (12 mg/m²), or daunorubicin (60 mg/m²). For patients aged >65 years, high-dose daunorubicin (90 mg/m²) has increased toxicity and is not recommended. A novel liposomal preparation of cytarabine and daunorubicin in a fixed molar ratio may instead be administered to fit patients with AML with myelodysplasia-related changes or arising from MDS. Older patients and those unable to receive intensive therapy due to medical comorbidity may receive repetitive cycles of lower intensity therapy with a hypomethylating agent (HMA; decitabine or azacitidine) or low-dose cytarabine, in combination with daily venetoclax. As noted, targeted IDH1- or IDH2-directed therapy is another consideration. All patients should be considered for clinical trials. Investigational therapy remains the best option for many older patients but especially those with adverse-risk features. (Table 104-6).

With the 7 and 3 regimen, 60–80% of younger and 33–60% of older patients (among those who are candidates for intensive

therapy) with primary AML achieve CR. Response rates around 60% have been similarly reported with the combination of HMA plus venetoclax in older or infirm patient groups. Of patients who do not achieve CR, most have drug-resistant leukemia. Induction death is more frequent with advancing age and medical comorbidity. Patients with refractory disease after induction should be considered for salvage treatments, preferably on clinical trials. Planning for the possibility of allogeneic hematopoietic stem cell transplantation (HCT) for all eligible patients under age 75 years is part of optimal initial AML care. Typically, allogeneic HCT is performed for patients in CR but at risk for relapse, but fit younger patients with primary refractory disease (not in remission after initial induction) have 15–20% cure rates with allogeneic HCT (after myeloablative conditioning). For this reason, early planning for possible future allogeneic HCT (including human leukocyte antigen [HLA] typing, donor search, etc.) should be part of the initial approach for most AML patients.

POSTREMISSEN THERAPY

Induction of a durable first CR (CR1) is critical to long-term survival in AML. However, without further therapy, virtually all CR patients will eventually relapse. Thus, postremission therapy is designed to eradicate residual (typically undetectable) leukemic cells to prevent relapse and prolong survival. As with induction, the type of postremission therapy in AML is selected for each individual patient based on age, fitness, and cytogenetic/molecular risk.

TABLE 104-6 Novel Therapies in Clinical Development in Acute Myeloid Leukemia (AML)

Protein kinase inhibitors	<ul style="list-style-type: none"> FLT3 inhibitors (midostaurin, quizartinib, gilteritinib, crenolanib, sorafenib) KIT inhibitors PI3K/AKT/mTOR inhibitors Aurora and polo-like kinase inhibitors, CDK4/6 inhibitors, CHK1, WEE1, and MPS1 inhibitors SRC and HCK inhibitors Syk inhibitors
Epigenetic modulators	<ul style="list-style-type: none"> New DNA methyltransferase inhibitors (SGI-110) Histone deacetylase (HDAC) inhibitors IDH1 and IDH2 inhibitors DOT1L inhibitors BET-bromodomain inhibitors
Chemotherapeutic agents	<ul style="list-style-type: none"> CPX-351 (liposomal cytarabine and daunorubicin, especially in secondary AML) Vosaroxin Nucleoside analogues
Mitochondrial inhibitors	<ul style="list-style-type: none"> Bcl-2, Bcl-xL, and Mcl-1 inhibitors Caseinolytic protease inhibitors
Therapies targeting oncogenic proteins	<ul style="list-style-type: none"> Fusion transcript targeting EVI1 targeting NPM1 targeting Hedgehog inhibitors (glasdegib)
Antibodies and immunotherapies	<ul style="list-style-type: none"> Monoclonal antibodies against CD33, CD44, CD47, CD123, CLEC12A Immunoconjugates (e.g., gemtuzumab ozogamicin, SGN33A) Bispecific T-cell engagers (BiTEs) and dual affinity retargeting molecules (DARTs) Chimeric antigen receptor (CAR) T cells or genetically engineered T-cell receptor (TCR) T cells Immune checkpoint inhibitors (PD-1/PD-L1, CTLA-4) Vaccines (e.g., WT1)
Therapies targeting AML environment	<ul style="list-style-type: none"> CXCR4 and CXCL12 antagonists Antiangiogenic therapies

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The choice between consolidation with chemotherapy or with transplantation is complex and based on age, risk, and practical considerations. In younger patients receiving chemotherapy, postremission therapy with intermediate- or high-dose cytarabine for two to four cycles is standard practice. Higher doses of cytarabine during postremission therapy appear more effective than standard doses (such as are used in induction) for those who do not have adverse-risk genetics. Recent studies suggest that the long-standing practice of high-dose cytarabine ($3 \text{ g}/\text{m}^2$, every 12 h on days 1, 3, and 5) may not improve survival over intermediate-dose cytarabine (IDAC; $1\text{--}1.5 \text{ g}/\text{m}^2$) for such patients. Thus, the ELN has recommended IDAC at $1\text{--}1.5 \text{ g}/\text{m}^2$, every 12 h, on days 1–3, as the optimal postremission chemotherapy approach for favorable- and intermediate-risk younger patients, for two to four cycles. While high-dose cytarabine may not be necessary, it is important to note that younger, favorable-risk patients have worse outcomes when doses $<1 \text{ g}/\text{m}^2$ are used. In contrast to favorable-risk patients, intermediate- or adverse-risk patients should proceed with allogeneic HCT in CR1 when feasible (see transplant discussion below). Because older patients have increased toxicities with higher doses of cytarabine, ELN recommends relatively attenuated cytarabine doses ($0.5\text{--}1 \text{ g}/\text{m}^2$, every 12 h, on days 1–3) in favorable-risk older patients. There is no

clear value for intensive postremission therapy in non-favorable-risk older patients; allogeneic HCT in CR1 (up to age 75 years) or investigational postremission therapy is recommended. Indeed, postremission therapy is an appropriate setting for introduction of new agents in both older and younger patients (Table 104-6).

For patients treated initially with lower intensity regimens that include venetoclax, the current practice is to continue repetitive cycles of the same combination of agents after remission until disease progression. Therapy often must be abbreviated over time due to cumulative myelotoxicity.

Allogeneic HCT is the best relapse-prevention strategy currently available for AML. Allogeneic HCT is probably best understood as an opportunity for immunotherapy; residual leukemia cells potentially elicit an immunologic response from donor immune cells, the so-called graft-versus-leukemia (GVL) effect. The benefit of GVL in relapse risk reduction, unfortunately, is offset somewhat by increased morbidity and mortality from complications of allogeneic HCT including graft-versus-host disease (GVHD). Given that relapsed AML is typically resistant to chemotherapy, allogeneic HCT in CR1 (e.g., before relapse ever occurs) is a favored strategy. We have often explained to patients that transplant can effectively “eliminate the needle in a haystack, but not a stack of needles.” Transplant is recommended for patients age <75 years who do not have favorable-risk disease and who have an HLA-matched donor (related or unrelated). We also recommend allogeneic HCT in CR1 for patients with intermediate-risk disease (Table 104-3). However, considerable debate exists regarding whether allogeneic HCT in CR1 is a requirement for younger patients with intermediate-risk AML, as one large series from the Medical Research Council reported that such patients have similar outcomes if transplanted only after relapse (and achievement of CR2), sparing some the long-term morbidity of transplantation. That said, allogeneic HCT is generally recommended as soon as possible after CR1 is achieved unless the patient is in a favorable-risk group. Increasingly, patients without HLA-matched donors are considered for alternative donor transplants (e.g., HLA-mismatched unrelated, haploidentical related, and umbilical cord blood) even in CR1. More effective and safe methods of *in vivo* T-cell depletion (i.e., posttransplant cyclophosphamide following mismatched transplantation) have broadened the availability of potential allogeneic HCT donors. Now, virtually any patient with a healthy parent or child (i.e., haploidentical) has an available donor suitable for allogeneic HCT if desired. Long-term outcomes with conventional chemotherapy for older patients are dismal; transplantation for such patients is expanding. Even for older patients, nonrandomized data demonstrate curative potential for older patients in CR1 treated with reduced-intensity conditioning regimens and allogeneic HCT.

Trials comparing allogeneic HCT with intensive chemotherapy or autologous HCT have shown improved duration of remission with allogeneic HCT. However, the relapse risk reduction observed with allogeneic HCT is partially offset by the increase in fatal treatment-related toxicity (GVHD, organ toxicity). Despite this, there is no debate that patients with adverse-risk AML have improved long-term survival with early allogeneic HCT. Alternatively, high-dose chemotherapy with autologous HCT rescue is another postremission approach in non-adverse-risk subsets. Autologous HCT patients receive their own stem cells (collected during remission and cryopreserved), following administration of myeloablative chemotherapy. The toxicity is relatively low with autologous HCT (5% mortality rate), but the relapse rate is higher than with allogeneic HCT due to the absence of the GVL effect. Favorable- and intermediate-risk patients may benefit from autologous HCT more so than adverse-risk patients. Practically speaking, however, autologous HCT in AML patients is less frequently employed currently due to enhanced relapse risk reduction seen with allogeneic HCT and the growing availability of HLA-mismatched donors (in novel transplantation approaches).

Prognostic factors help to select the appropriate postremission therapy in patients in CR1. Our approach includes allogeneic HCT

in CR1 for patients without favorable cytogenetics or genotype. Patients with adverse-risk disease should proceed to allogeneic HCT at CR1 if possible. The decision for allogeneic HCT for younger intermediate-risk patients is complex and individualized as described above; we recommend it when an HLA-matched donor is available. Subsets of patients may benefit from targeted therapy given during remission; emerging data demonstrate survival benefit from incorporation of the FLT3 inhibitor midostaurin, for example, into induction and postremission therapies for patients with *FLT3*-mutated AML. Allogeneic transplantation in CR1 is still recommended for these patients.

For patients in morphologic CR, measurement of MRD remains a very important and challenging research area. Cytogenetics are a mainstay of disease assessment, and persistence of abnormal karyotype (in spite of morphologic CR) is clearly associated with poor clinical outcomes. Immunophenotyping to detect minute populations of blasts or sensitive molecular assays (e.g., reverse transcriptase polymerase chain reaction [RT-PCR]) to detect AML-associated molecular abnormalities (e.g., *NPM1*, *RUNX1/RUNX1T1* and *CBFB/MYH11* transcripts, *PML/RARA*) can be performed to assess whether MRD is present at sequential time points during or after treatment. Whether emerging next-generation sequencing or serial quantitative assessment using flow or RT-PCR, performed during remission, can effectively direct successful subsequent therapy and improve clinical outcome remains to be determined. Currently, no consensus exists for the optimal MRD measurement technique or its application, although it is increasingly employed in clinical practice. Data suggest that MRD measurement can in some settings be a reliable discriminator between patients who will continue in CR or relapse, but whether subsequent therapy (i.e., allogeneic HCT or additional therapy) can effectively eradicate disease in such patients is not yet clear. However, in the subset of patients with APL, serial RT-PCR (for the *PML/RARA* transcript) is a very useful and reliable tool to detect early relapse and to direct initiation of reinduction therapy prior to onset of overt relapse. Critical in the general understanding of MRD in all disease subsets is the recognition that even patients with undetectable levels of MRD remain at risk for leukemic relapse.

SUPPORTIVE CARE

Measures geared to supporting patients through several weeks of neutropenia and thrombocytopenia are critical to successful AML therapy. Patients with AML should be treated in centers expert in providing supportive care. Multi-lumen central venous catheters should be inserted as soon as newly diagnosed AML patients have been stabilized. They should be used thereafter for administration of intravenous medications/chemotherapy and transfusions, as well as for blood drawing instead of venipuncture during prolonged periods of myelosuppression.

Adequate and prompt blood bank support is critical to therapy of AML. Platelet transfusions should be given as needed to maintain a platelet count $\geq 10,000/\mu\text{L}$. The platelet count should be kept at higher levels in febrile patients and during episodes of active bleeding or DIC. Patients with poor posttransfusion platelet count increments may benefit from administration of ABO-matched platelets or platelets from HLA-matched donors. RBC transfusions should be considered to keep the hemoglobin level $>70-80 \text{ g/L}$ ($7-8 \text{ g/dL}$) in the absence of active bleeding, DIC, or congestive heart failure, which require higher hemoglobin levels. Blood products leukodepleted by filtration should be used to avert or delay alloimmunization as well as febrile reactions. Blood products may also be irradiated to prevent transfusion-associated GVHD. Cytomegalovirus (CMV)-negative blood products should be used for CMV-seronegative patients who are potential candidates for allogeneic HCT; fortunately, white blood cell filtration is quite effective at reducing CMV exposure as well.

Neutropenia (neutrophils $<500/\mu\text{L}$ or $<1000/\mu\text{L}$ and predicted to decline to $<500/\mu\text{L}$ over the next 48 h) can be part of the initial presentation and/or a side effect of the chemotherapy treatment in

AML patients. Thus, infectious complications remain the major cause of morbidity and death during induction and postremission chemotherapy for AML. Antibacterial (i.e., quinolones) and antifungal (i.e., posaconazole) prophylaxis, especially in conjunction with regimens that cause mucositis, is beneficial. For patients who are herpes simplex virus or varicella-zoster seropositive, antiviral prophylaxis should be initiated (e.g., acyclovir, valacyclovir).

Fever develops in most patients with AML, but infections are documented in only half of febrile patients. Early initiation of empirical broad-spectrum antibacterial and antifungal antibiotics has significantly reduced the number of patients dying of infectious complications (Chap. 74). An antibiotic regimen adequate to treat gram-negative organisms should be instituted at the onset of fever in a neutropenic patient after clinical evaluation, including a detailed physical examination with inspection of the indwelling catheter exit site and a perirectal examination (for perirectal abscess), as well as procurement of cultures and radiographs aimed at documenting the source of fever. Specific antibiotic regimens should be based on institutional antibiotic sensitivity data obtained from where the patient is being treated. Acceptable regimens for empiric antibiotic therapy include monotherapy with imipenem-cilastatin, meropenem, piperacillin/tazobactam, or an extended-spectrum antipseudomonal cephalosporin (cefepime or ceftazidime). The combination of an aminoglycoside with an antipseudomonal penicillin (e.g., piperacillin) or an aminoglycoside in combination with an extended-spectrum antipseudomonal cephalosporin should be considered in complicated or resistant cases. Aminoglycosides should be avoided, if possible, in patients with renal insufficiency. Empirical vancomycin should be added in neutropenic patients with catheter-related infections, blood cultures positive for gram-positive bacteria before final identification and susceptibility testing, hypotension or shock, or known colonization with penicillin/cephalosporin-resistant pneumococci or methicillin-resistant *Staphylococcus aureus*. In special situations where decreased susceptibility to vancomycin, vancomycin-resistant organisms, or vancomycin toxicity is documented, other options including linezolid and daptomycin need to be considered.

Caspofungin (or a similar echinocandin), voriconazole, isavuconazonium, or liposomal amphotericin B should be considered for antifungal treatment if fever persists for 4–7 days following initiation of empiric antibiotic therapy. Although liposomal formulations of amphotericin B have improved the toxicity profile of this agent, use has been limited to situations with high risk of or documented mold infections, especially in those in whom an azole fails. Caspofungin has been approved for empiric antifungal treatment. Voriconazole has also been shown to be equivalent in efficacy and less toxic than amphotericin B; isavuconazonium may also be effective with fewer drug-drug interactions. Antibacterial and antifungal antibiotics should be continued until patients are no longer neutropenic, regardless of whether a specific source has been found for the fever. Unfortunately, this practice likely contributes to development of resistance and increased incidence of nosocomial infections such as *Clostridium difficile* colitis, so great care should be taken preferably in hospital-wide antibiotic surveillance and isolation strategies to reduce these complications. Recombinant hematopoietic growth factors have a limited role in AML; myeloid growth factors may be useful in the postremission setting but are not recommended in induction or for “palliative” care for patients not in remission.

TREATMENT FOR REFRACTORY OR RELAPSED AML

In patients who relapse after achieving CR, the length of first CR is predictive of response to salvage chemotherapy treatment; patients with longer first CR (>12 months) generally relapse with drug-sensitive disease and have a higher chance of attaining a CR, even with the same chemotherapeutic agents used for first remission induction. Patients with short prior CR duration are at high risk for treatment failure. Similar to patients with refractory disease, patients with relapsed disease are rarely cured by salvage chemotherapy treatments alone. Therefore, patients who eventually

achieve a second CR and are eligible for allogeneic HCT should be transplanted. For patients who relapse after allogeneic HCT, no consensus for best therapy exists; outcomes in this setting are very poor.

Because achievement of a second CR with routine salvage therapies is relatively uncommon, especially in patients who relapse rapidly after achievement of first CR (<12 months), these patients and those lacking HLA-compatible donors or who are not candidates for allogeneic HCT should be considered for innovative approaches on clinical trials. Many new agents are in current testing (Table 104-6). The discovery of novel gene mutations and mechanisms of leukemogenesis that might represent actionable therapeutic targets has prompted the development of many new targeting agents. In addition to kinase inhibitors for *FLT3*-mutated AML, other compounds targeting the aberrant activity of mutant proteins (e.g., IDH1/2 inhibitors) and numerous other biologic mechanisms are being tested in clinical trials. Inhibitors of *FLT3* (gilteritinib), IDH1 (ivosidenib), or IDH2 (enasidenib) are monotherapies for relapsed AML patients who have targetable mutations. Furthermore, approaches with antibodies targeting markers commonly expressed on leukemia blasts (e.g., CD33) or leukemia-initiating cells (e.g., CD123) are also under investigation. Once these compounds have demonstrated safety and activity as single agents, investigation of combinations with other molecular targeting compounds and/or chemotherapy should be pursued.

TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA

APL is a highly curable AML subtype, and 85% of these patients achieve long-term survival with current approaches. APL has long been shown to be responsive to cytarabine and daunorubicin, but in the past, patients who were treated with these drugs alone frequently died from DIC induced by the release of granule components by the chemotherapy-treated leukemia cells. However, the prognosis of APL patients has changed dramatically with the introduction of tretinoin (*all-trans*-retinoic acid [ATRA]), an oral drug that induces the differentiation of leukemic cells bearing the t(15;17), where disruption of the *RARA* gene encoding a retinoid acid receptor occurs. ATRA decreases the frequency of DIC but often produces another complication called the APL (differentiation) syndrome. Occurring within the first 3 weeks of treatment, it is characterized by fever, fluid retention, dyspnea, chest pain, pulmonary infiltrates, pleural and pericardial effusions, and hypoxemia. The syndrome is related to adhesion of differentiated neoplastic cells to the pulmonary vasculature endothelium. Glucocorticoids, chemotherapy for cytoreduction, and/or supportive measures can be effective for management of the APL syndrome. Temporary discontinuation of ATRA is necessary in cases of severe APL syndrome (i.e., patients developing renal failure or requiring admission to the intensive care unit due to respiratory distress). The mortality rate of this syndrome is 10%. APL syndrome may also occur, less commonly, with arsenic trioxide (ATO) in APL.

In adults with low-risk APL (low leukocyte count at presentation), ATRA (45 mg/m²/d) plus ATO (0.15 mg/kg/d) was recently compared to ATRA plus concurrent idarubicin chemotherapy. ATRA/ATO was superior and is the new standard of care for such patients. CR rates in low-risk disease approach 100%, with excellent long-term survival. Notably, patients with high-risk APL (defined as leukocyte count >10,000/ μ L) must be uniquely treated, as they require immediate cytoreduction with chemotherapy due to life-threatening APL syndrome and rapidly rising leukocyte count after initiation of ATRA. High-risk patients are at increased risk for induction death due to this syndrome as well as increased frequency of hemorrhagic complications (related to DIC).

Assessment of residual disease by RT-PCR amplification of the t(15;17) chimeric gene product *PML-RARA* following the final cycle of treatment is important. Disappearance of the signal is associated with long-term disease-free survival; its persistence or reemergence invariably predicts relapse. Sequential monitoring of RT-PCR for *PML-RARA* is now considered standard for postremission monitoring of APL, at least in high-risk patients.

Patients in molecular, cytogenetic, or clinical relapse should be salvaged with ATO with or without ATRA; in patients who were treated with ATRA plus chemotherapy in the front-line setting, ATO-based therapy at relapse produces meaningful responses in up to 85% of patients. Although experience with relapsed APL in patients who received ATO during initial induction is limited (given that few relapses occur in low-risk patients and widespread use of ATO during first-line therapy is relatively new), ATO remains the preferred reinduction therapy for patients who relapse, although the duration of prior remission should be a factor in this choice. Achievement of CR2 should be followed by consolidation with autologous HCT (for patients who achieve RT-PCR-negative status). In the minority who do not achieve negative RT-PCR or who relapse again, allogeneic HCT may still be potentially curative.

A

Clara Bloomfield, an important contributor to the field and to this chapter in past editions, passed away since the publication of the 20th edition. Material from prior versions of this chapter on which she was an author have been retained here.

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Chronic Myeloid Leukemia

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Chronic myeloid leukemia (CML) is a clonal hematopoietic myeloproliferative stem cell neoplasm. The disease is driven by the *BCR/ABL1* chimeric gene that codes for a constitutively active tyrosine kinase, resulting from a reciprocal balanced translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34.1;q11.2), known as the

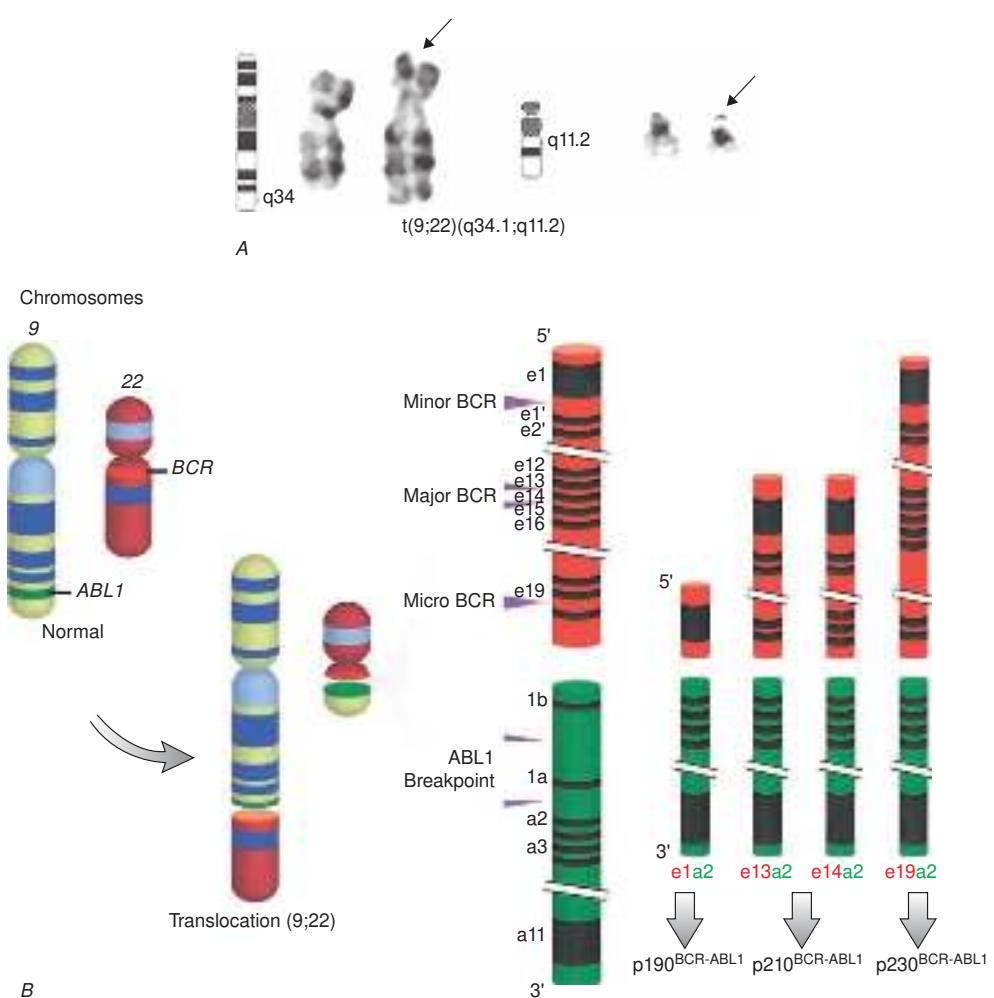


FIGURE 105-1 *A*, The Philadelphia (Ph) chromosome cytogenetic abnormality. *B*, Breakpoints in the long arms of chromosome 9 (ABL1 locus) and chromosome 22 (BCR regions) result in at least three different BCR-ABL1 oncogene messages, p210^{BCR-ABL1} (most common message in chronic myeloid leukemia [CML]), p190^{BCR-ABL1} (present in two-thirds of patients with Ph-positive acute lymphoblastic leukemia; rare in CML), and p230^{BCR-ABL1} (rare in CML and associated with an indolent course). Other rearrangements (e.g., e14a3, e14a2) are less common. (© 2013 The University of Texas MD Anderson Cancer Center.)

Philadelphia chromosome (Ph) (Fig. 105-1). Untreated, the course of CML is typically biphasic or triphasic, with an early indolent or chronic phase, followed often by an accelerated phase and a terminal blastic phase. Before the era of BCR-ABL1 tyrosine kinase inhibitors (TKIs), the median survival in CML was 3–7 years, and the 10-year survival rate was 30% or less. Introduced into standard CML therapy in 2000, TKIs have revolutionized the treatment, natural history, and prognosis of CML. Today, the estimated 10-year survival rate with imatinib mesylate, the first BCR-ABL1 TKI approved, is greater than 85% and approaches that of the general population. Allogeneic stem cell transplantation (SCT), a curative approach but one that involves more risks, is now offered as second- or third-line therapy after failure of TKIs.

■ INCIDENCE AND EPIDEMIOLOGY

CML accounts for 15% of all cases of leukemia. There is a slight male predominance (male-to-female ratio 1.6:1). The median age at diagnosis is 55–65 years. It is uncommon in children; only 3% of patients with CML are younger than 20 years, although in recent years, a higher proportion of young patients are diagnosed. The incidence of CML increases gradually with age, with a steeper increase after the age of 40–50 years. The annual incidence of CML is 1.6 cases per 100,000 individuals. In the United States, this translates into about 8500–9000 new cases per year. The incidence of CML has not changed

over several decades. By extrapolation, the worldwide annual incidence of CML is about 200,000 cases. With a median survival of 3–6 years before 2000, the disease prevalence in the United States was ~30,000 cases. With TKI therapy, the annual mortality has been reduced from 10–20% to about 2%. Therefore, the prevalence of CML is expected to continue to increase. Based on an estimated annual mortality of 2% and an incidence of 8500 cases per year, the plateau prevalence of CML is estimated to be reached at ~425,000 in the United States (8500 × 100/2) by about 2040, with full TKI optimal treatment penetration. The worldwide prevalence will depend on the treatment penetration of TKIs and their effect on reduction of worldwide annual mortality. Ideally, with full TKI treatment penetration, the worldwide prevalence should plateau at 35 times the incidence, or ~9–10 million patients. These estimates are all based on extrapolations from the incidence and prevalence of CML in the United States, as well as an estimated annual mortality of 2% with modern TKI therapy; they could vary considerably if the estimates were to change.

■ ETIOLOGY

There are no familial associations in CML. The risk of developing CML is not increased in monozygotic twins or in relatives of patients with CML. No etiologic agents are incriminated, and no associations exist with exposures to benzene or other toxins, fertilizers, insecticides, or

viruses. CML is not a frequent secondary leukemia following therapy of other cancers with alkylating agents and/or radiation. Exposure to ionizing radiation (e.g., nuclear accidents, radiation treatment for ankylosing spondylitis or cervical cancer) has increased the risk of CML, which peaks at 5–10 years after exposure and is dose-related. The median time to development of CML among atomic bomb survivors was 6.3 years. Following the Chernobyl accident, no increase in the incidence of CML was reported, suggesting that larger dose exposures of radiation are required to cause CML. Because of adequate protection, the risk of CML has not increased among individuals working in the nuclear industry or among radiologists.

■ PATHOPHYSIOLOGY

The t(9;22)(q34.1;q11.2) is present in >90% of classical CML cases. It results from a balanced reciprocal translocation between the long arms of chromosomes 9 and 22. It is present in hematopoietic cells (myeloid, erythroid, megakaryocytes, and monocytes; less often mature B lymphocytes; rarely mature T lymphocytes, but not stromal cells), but not in other cells in the human body. As a result of the genetic translocation, DNA sequences from the cellular oncogene *ABL1* are juxtaposed to the major breakpoint cluster region (*BCR*) gene on chromosome 22, generating a hybrid oncogene, *BCR/ABL1*. Depending on the breakpoint site in the major *BCR* region on chromosome 22 (e13 or e14), two main messenger RNA transcripts occur, e13a2 (previously b2a2) and e14a2 (previously b3a2). Both of them encode for a novel oncoprotein of molecular weight 210 kDa, referred to as p210^{BCR-ABL1} (Fig. 105-1B). This oncoprotein exhibits constitutive kinase activity that leads to excessive proliferation and reduced apoptosis of CML cells, endowing them with a growth advantage over their normal counterparts. Over time, normal hematopoiesis is suppressed, but normal stem cells can persist and reemerge following effective therapy, for example with TKIs. In most instances of Ph-positive acute lymphoblastic leukemia (ALL) and in rare cases of CML, the breakpoint in *BCR* is more centromeric, in a region called the minor *BCR* region (*mBCR*). As a result, a shorter sequence of *BCR* is fused to *ABL1*, with a consequent e1a2 transcript and a smaller BCR-ABL1 oncoprotein, p190^{BCR-ABL1}. When occurring in Ph-positive CML, this translocation is associated with a worse outcome. A rarer breakpoint in *BCR* occurs telomeric to the major *BCR* region in the *micro-BCR* (μ -BCR) region. It juxtaposes a larger fragment of the *BCR* gene to *ABL1* and produces an e19a2 transcript and a larger p230^{BCR-ABL1} oncoprotein (associated with a more indolent CML course). Other rearrangements (based on different breakpoints in the *ABL* region), such as e13a3 or e14a3 (also resulting in a p210^{BCR-ABL1} oncoprotein), occur much less frequently. These are not readily identifiable nor quantifiable with the routine polymerase chain reaction (PCR) probes, thus producing falsely negative PCR levels on follow-up studies if not tested at diagnosis.

The constitutive activation of *BCR/ABL1* results in autophosphorylation and activation of multiple downstream pathways that affect gene transcription, apoptosis, stromal adherence, skeletal organization, and degradation of inhibitory proteins. These transduction pathways involve RAS, mitogen-activated protein (MAP) kinases, signal transducers and activators of transcription (STAT), phosphatidylinositol-3-kinase (PI3k), MYC, and others. These interactions are mostly mediated through tyrosine phosphorylation and require binding of BCR-ABL1 to adapter proteins such as GRB-2, CRK, CRK-like (CRK-L) protein, and Src homology containing proteins (SHC). Most BCR-ABL1 TKIs bind to the BCR-ABL1 ATP-binding domain, inhibiting its kinase activity, preventing the activation of transformation pathways, and inhibiting downstream signaling. As a result, proliferation of CML cells is inhibited and apoptosis induced, allowing the reemergence of normal hematopoiesis. An additional layer of complexity is related to differences in signal transduction between CML-differentiated cells and early progenitors. Beta-catenin, Wnt1, Foxo3a, transforming growth factor β , interleukin-6, PP2A, SIRT1, and others have been implicated in CML stem cell survival. *ABL1* also has a myristoyl site that functions as a negative regulator of its kinase activity. This site and its negative regulatory activity are lost upon fusion with *BCR*. Novel *ABL1* inhibitors (e.g., asciminib) bind this myristoyl site

and restore the lost inhibitory activity. Mutations in other cancer-associated genes may also occur at diagnosis, most frequently in *ASXL1*, *IKZF1*, and *RUNX1*; their presence is associated with worse response to therapy and a higher risk of transformation to blastic phase.

Experimental models have established the causal relationship between the *BCR/ABL1* rearrangement and the development of CML. In animal models, expression of *BCR/ABL1* in normal hematopoietic cells produced CML-like disorders or lymphoid leukemia, demonstrating the leukemogenic potential of *BCR/ABL1* as a single oncogenic abnormality. Other models, however, suggest the need for a “second hit.”

The cause of the *BCR/ABL1* rearrangement is unknown. Molecular techniques that detect *BCR/ABL1* at a level of 1 in 10⁸ cells identify this molecular abnormality in the blood of up to 25% of normal adults and 5% of infants, but 0% of cord blood samples. This suggests that *BCR/ABL1* is not sufficient to cause overt CML in the overwhelming majority of individuals in whom it occurs. Because CML develops in only 1.6 of 100,000 individuals annually, additional molecular events or poor immune recognition of the rearranged cells may contribute to overt CML.

CML is defined by the presence of the *BCR/ABL1* fusion gene in a patient with a myeloproliferative neoplasm. In some patients with a typical morphologic picture of CML, the Ph chromosome is not detectable by standard G-banding karyotype, but fluorescence in situ hybridization (FISH) and/or molecular studies (PCR) detect *BCR/ABL1*. These patients have a course similar to patients with Ph-positive CML and respond to TKI therapy. Many of the remaining patients have atypical morphologic or clinical features and have other diseases, such as atypical CML, chronic myelomonocytic leukemia, and myelodysplastic/myeloproliferative neoplasms (MDS/MPN). These individuals do not respond to TKI therapy and usually have a poor prognosis with a median survival of about 2–3 years. Detection of mutations in the granulocyte colony-stimulating factor receptor (*CSF3R*) in chronic neutrophilic leukemia (80% of cases) and in some cases of atypical CML (5–10% of cases), mutations in *SETBP1* in atypical CML (25% of cases), and mutations in *SF3B1* in MDS/MPN with ringed sideroblasts and marked thrombocytosis (MDS/MPN-RS-T; 50–70% of cases, associated with longer median survival of 7 years vs 3.3 years with wild-type *SF3B1*) supports the notion that these are distinct molecular and biologic entities. Patients with chronic neutrophilic leukemia or atypical CML whose disease is associated with *CSF3R* mutation may respond well to ruxolitinib (a JAK2 inhibitor) therapy (complete response in 50–60% of such patients).

The events associated with the transition of CML from a chronic to accelerated-blastic phase are poorly understood. Characteristic chromosomal abnormalities such as a double Ph, trisomy 8, isochromosome 17 or deletion of 17p (loss of *TP53*), 20q-, translocations involving 3q26, and others may be seen with disease acceleration. Molecular events associated with transformation include mutations in *TP53*, retinoblastoma 1 (*RBL1*), myeloid transcription factors like *RUNX1*, and cell cycle regulators like *p16*. A plethora of other mutations or functional abnormalities have been implicated in blastic transformation, but no unifying theme has emerged other than the fact that *BCR/ABL1* itself induces genetic instability that favors the acquisition of additional molecular defects and eventually results in blastic transformation. One critical effect of TKIs is to stabilize the CML genome, leading to a reduced transformation rate. In particular, the previously observed sudden blastic transformations (i.e., abrupt transformation to blastic phase in a patient who had been in cytogenetic response) have become uncommon, occurring rarely in younger patients in the first 1–2 years of TKI therapy (usually sudden lymphoid blastic transformations). Sudden transformations beyond the third year of TKI therapy are rare in patients who continue on TKI therapy. Moreover, the course of CML is now frequently more indolent in patients treated with TKI, even without cytogenetic response, compared to previous experience with hydroxyurea/busulfan, suggesting a definite clinical benefit of continued inhibition of the kinase activity.

Among patients developing resistance to TKIs, several resistance mechanisms have been observed. The most clinically relevant one is the development of *ABL1* kinase domain mutations that may prevent the

binding of TKIs to the catalytic site (ATP-binding site) of the kinase or maintain the kinase activity despite the presence of a TKI. More than 100 *ABL1* kinase domain mutations have now been described, many of which confer relative or absolute resistance to imatinib. Consequently, second-generation (i.e., dasatinib, nilotinib, bosutinib) and third-generation (ponatinib) TKIs were developed, the latter with significant efficacy against T315I, a “gatekeeper” mutation that prevents binding of and causes resistance to all other currently available TKIs. Asciminib, olveremabatib (HQPI351) and other novel TKIs under development are also active against the T315I mutation.

■ CLINICAL PRESENTATION

The presenting signs and symptoms in CML depend on the availability of and access to health care, including physical examinations and screening tests. In the United States, because of the wider access to health care screening and physical examinations, 50–60% of patients are diagnosed on routine blood tests and have minimal symptoms at presentation, such as fatigue. In geographic locations where access to health care is more limited, patients often present with high CML burden including splenomegaly, anemia, and related symptoms (abdominal pain, weight loss, fatigue), associated with a higher frequency of high-risk CML. Presenting findings in patients diagnosed in the United States are shown in **Table 105-1**.

Symptoms Most patients with CML (90%) present in the indolent or chronic phase. Depending on the timing of diagnosis, patients are often asymptomatic (if the diagnosis is discovered during health care screening tests). Common symptoms, when present, are manifestations of anemia and splenomegaly. These may include fatigue, malaise, weight loss (if high leukemia burden), or early satiety and left upper quadrant pain or masses (from splenomegaly). Less common presenting findings include thrombotic or hyperviscosity-related events from severe leukocytosis or thrombocytosis. These include priapism, cardiovascular complications, myocardial infarction, venous thrombosis, visual disturbances, dyspnea and pulmonary insufficiency, drowsiness, loss of coordination, confusion, or cerebrovascular accidents.

TABLE 105-1 Presenting Signs and Symptoms of Newly Diagnosed Philadelphia Chromosome–Positive Chronic Myeloid Leukemia in Chronic Phase

PARAMETER	PERCENTAGE
Age ≥60 years (median)	40–50 (55–65)
Female gender	35–45
Splenomegaly	30
Hepatomegaly	5–10
Lymphadenopathy	5
Other extramedullary disease	2
Hemoglobin <10 g/dL	10–15
Platelets	
>450 × 10 ⁹ cells/L	30–35
<100 × 10 ⁹ cells/L	3–5
White blood cells ≥50 × 10 ⁹ cells/L	35–40
Marrow	
≥5% blasts	5
≥5% basophils	10–15
Peripheral blood	
≥3% blasts	8–10
≥7% basophils	10
Cytogenetic clonal evolution other than the Philadelphia chromosome	4–5
Sokal risk	
Low	60–65
Intermediate	25–30
High	10

Manifestations of bleeding diatheses include retinal hemorrhages, gastrointestinal bleeding, and others. Patients who present with, or progress to, the accelerated or blastic phases frequently have additional symptoms including unexplained fever, significant weight loss, severe fatigue, bone and joint pain, bleeding and thrombotic events, and infections.

Physical Findings Splenomegaly is the most common physical finding, occurring in 20–70% of patients depending on health care screening frequency. Other less common findings include hepatomegaly (5–10%), lymphadenopathy (5–10%), and extramedullary disease (skin or subcutaneous lesions). The latter indicates CML transformation if a biopsy confirms predominance of blasts. Other physical findings are manifestations of complications of high tumor burden described earlier (e.g., cardiovascular, cerebrovascular, bleeding). High basophil counts may be associated with histamine overproduction causing pruritus, diarrhea, flushing, and even gastrointestinal ulcers.

Hematologic and Marrow Findings In untreated CML, leukocytosis ranging from 10–500 × 10⁹/L is common. The peripheral blood differential shows left-shifted hematopoiesis with predominance of neutrophils and the presence of bands, myelocytes, metamyelocytes, promyelocytes, and blasts (usually ≤5%). Basophils and/or eosinophils are frequently increased. Thrombocytosis is common, but thrombocytopenia is rare and, when present, suggests a worse prognosis, disease acceleration, or an unrelated etiology. Anemia is present in one-third of patients. Cyclic oscillations of counts are noted in 10–20% of patients without treatment. Biochemical abnormalities include a low leukocyte alkaline phosphatase score and high levels of vitamin B₁₂, uric acid, lactic dehydrogenase, and lysozyme. The presence of unexplained and sustained leukocytosis, with or without splenomegaly, should lead to a marrow examination and cytogenetic analysis.

The bone marrow is hypercellular with marked myeloid hyperplasia and a high myeloid-to-erythroid ratio of 15–20:1. Marrow blasts are typically 5% or less; when higher, they carry a worse prognosis or represent transformation to accelerated phase (if they are ≥15%). Increased reticulin fibrosis (detected with silver stain) is common, with 30–40% of patients demonstrating grade 3–4 reticulin fibrosis. This was considered adverse in the pre-TKI era. With TKI therapy, reticulin fibrosis resolves in most patients and is not an indicator of poor prognosis. Collagen fibrosis (Wright-Giemsa stain) is rare at diagnosis. Disease progression with a “spent phase” of myelofibrosis (myelophthisis, or burnt-out marrow) was a relatively common end-stage CML condition with busulfan therapy (20–30%); it is extremely rare now with TKI therapy.

Cytogenetic and Molecular Findings The diagnosis of CML is straightforward and depends on documenting the t(9;22) (q34.1;q11.2), which is identified by G-banding in 90% of cases. This is known as the Philadelphia chromosome (initially identified in Philadelphia as a minute chromosome, later identified to be chromosome 22) (Fig. 105-1). Some patients (~10%) may have complex translocations (complex variant Ph) involving three or more chromosomes including chromosomes 9 and 22 and one or more additional chromosomes. Others may have a “masked Ph,” involving translocations between chromosome 9 and a chromosome other than 22 (but molecularly showing the *BCR/ABL1* rearrangement; known as simple variant Ph). The prognosis of these patients and their response to TKI therapy are similar to those in patients with Ph. About 5–10% of patients may have additional chromosomal abnormalities (ACAs) in the Ph-positive cells at diagnosis. These usually involve trisomy 8, a double Ph, isochromosome 17 or 17p deletion, 20q-, or others. This is referred to as cytogenetic clonal evolution and was historically a sign of adverse prognosis, particularly when trisomy 8, double Ph, or chromosome 17 abnormalities were noted. A less common abnormality involving chromosome 3q26.2 occurs with disease progression and carries a poor prognosis.

Techniques such as FISH and PCR are now used to aid in the diagnosis of CML. They are more sensitive to estimate the CML burden in patients on TKI therapy. They can be done on peripheral blood and thus are more convenient to patients. Patients with CML at diagnosis

should have a FISH analysis to quantify the percentage of Ph-positive cells, if FISH is used to replace marrow cytogenetic analysis in monitoring response to therapy. FISH will not detect additional chromosomal abnormalities (clonal evolution); thus, a cytogenetic analysis is recommended at the time of diagnosis. In addition, 10–15% of patients may develop chromosomal abnormalities in Ph-negative metaphases after responding to TKIs. These abnormalities may carry a worse prognosis but are not detected by FISH unless already identified and FISH is used to follow them. Molecular studies at diagnosis are important to document the type and presence of *BCR-ABL1* transcripts to avoid spurious “undetectable” *BCR-ABL1* transcripts on follow-up studies, with the false impression of a complete molecular response. The presence of the Philadelphia chromosome with “negative” PCR with standard methodology should prompt investigation of atypical transcripts.

Both FISH and PCR studies can be falsely positive at low levels or falsely negative because of technical issues. Therefore, a diagnosis of CML must always rely on a marrow analysis with routine cytogenetics. The diagnostic bone marrow confirms the presence of the Ph chromosome, detects clonal evolution, and quantifies the percentage of marrow blasts and basophils. In 10% of patients, the percentage of marrow blasts and basophils can be significantly higher than in the peripheral blood, conferring poorer prognosis or even representing disease transformation.

Monitoring patients on TKI therapy by cytogenetics, FISH, and PCR has become an important standard practice to assess response to therapy, emphasize compliance, evaluate possible treatment resistance, identify the need to change TKI therapy, and determine the need to assess for kinase domain mutations. Because of the decreasing reliance of bone marrow aspirations to monitor response, equivalence has been established to correlate cytogenetic results with PCR values. These are not absolute correlations but provide adequate guidance. A partial cytogenetic response is defined as the presence of 35% or less Ph-positive metaphases by routine cytogenetic analysis. This is roughly equivalent to *BCR-ABL1* transcripts by the International Scale (IS) of 10% or less. A complete cytogenetic response refers to the absence of Ph-positive metaphases (0% Ph positivity). This is approximately equivalent to *BCR-ABL1* transcripts (IS) of 1% or less. A major molecular response (MMR or MR3) refers to *BCR-ABL1* transcripts (IS) $\leq 0.1\%$, or roughly a 3-log or greater reduction of *BCR-ABL1* transcripts from a standardized baseline. MR4 refers to *BCR-ABL1* transcripts (IS) $\leq 0.01\%$, and MR4.5 (deep molecular response) refers to *BCR-ABL1* transcripts (IS) $\leq 0.0032\%$, roughly equivalent to a 4.5-log reduction or greater of transcripts.

Findings in CML Transformation Progression of CML is usually associated with leukocytosis resistant to therapy, increasing anemia, fever and constitutional symptoms, and increased blasts and basophils in the peripheral blood or marrow. Criteria of accelerated-phase CML, historically associated with median survival of <1.5 years, include the presence of 15% or more peripheral blasts, 30% or more peripheral blasts plus promyelocytes, 20% or more peripheral basophils, cytogenetic clonal evolution (presence of chromosomal abnormalities in addition to Ph), and thrombocytopenia $<100 \times 10^9/L$ (unrelated to therapy). About 5–10% of patients present with de novo accelerated phase or blastic phase. The prognosis of de novo accelerated phase with TKI therapy has improved significantly, with an estimated 8-year survival rate of 75%. The median survival of accelerated phase evolving from chronic phase has also improved from a historical median survival of 18 months to an estimated 4-year survival rate of 70% on TKI therapy. Therefore, the criteria for accelerated-phase CML should be revisited because most clinical criteria defining accelerated phase have lost much of their prognostic significance. Blastic-phase CML is defined by the presence of 30% or more peripheral or marrow blasts or the presence of sheets of blasts in extramedullary disease (usually skin, soft tissues, or lytic bone lesions). Blastic-phase CML is commonly myeloid (60%) but can present uncommonly as erythroid, promyelocytic, monocytic, or megakaryocytic. Lymphoid blastic phase occurs in about 25% of patients. Lymphoblasts are terminal deoxynucleotide transferase positive and peroxidase negative (although occasionally

with low positivity up to 3–5%) and express lymphoid markers (CD10, CD19, CD20, CD22). However, they also often express myeloid markers (50–80%), resulting in diagnostic challenges. Proper immunophenotypic diagnosis is important because lymphoid blastic-phase CML is quite responsive to anti-ALL-type chemotherapy (e.g., hyper-CVAD [cyclophosphamide, vincristine, doxorubicin, and dexamethasone]) in combination with TKIs (complete response rate 70%; median survival 3 years; high rates of bridging to allogeneic SCT and possible cure).

■ PROGNOSIS AND CML COURSE

Before the imatinib era, the annual mortality in CML was 10% in the first 2 years and 15–20% thereafter. The median survival in CML was 3–7 years (with hydroxyurea-busulfan and interferon α). Without a curative option of allogeneic SCT, the course of CML was toward transformation to, and death from, accelerated or blastic phases for most patients as the rate of complete cytogenetic response with interferon was low. Even apparent disease stability was unpredictable, with some patients demonstrating sudden transformation to a blastic phase. With imatinib therapy, the annual mortality in CML has decreased to 1–2% in the first 20 years of observation. More than half of the deaths are from conditions other than CML, such as old age, comorbidities, accidents, suicides, other cancers, and other medical conditions (e.g., infections, surgical procedures). The estimated 10-year survival rate is 86%, or 92% if only CML-related deaths are considered (Fig. 105-2). The course of CML has also become quite predictable. In the first 2 years of TKI therapy, rare sudden transformations are still reported (1–2%), usually lymphoid blastic transformations that respond to combinations of chemotherapy and TKIs followed by allogeneic SCT. These may be explained by the intrinsic mechanisms of sudden transformation already existing in the CML clones before the start of therapy that were not amenable to TKI inhibition, in particular imatinib. Second-generation TKIs (nilotinib, dasatinib, bosutinib) used as frontline therapy have reduced the incidence of transformation in the first 2–3 years from 6–8% with imatinib to 2–5% with second-generation TKIs. Disease transformation to accelerated or blastic phase is rare with continued TKI therapy, estimated at <1% annually in years 4–10 of follow-up on the original imatinib trials. Patients usually develop resistance in the form of cytogenetic resistance or relapse, followed by hematologic relapse and subsequent transformation, rather than the previously feared sudden transformations without the warning signals of cytogenetic-hematologic relapse.

Before the imatinib era, several pretreatment prognostic factors predicted for worse outcome in CML and have been incorporated into prognostic models and staging systems. These have included older age, significant splenomegaly, anemia, thrombocytopenia or thrombocytosis, high percentages of blasts and basophils (and/or eosinophils), marrow fibrosis, interstitial deletions in the long arm of chromosome 9, clonal evolution, and others. Different risk models and staging systems, derived from multivariate analyses, were proposed to define different risk groups. As with the introduction of cisplatin into testicular cancer therapy, the introduction of TKIs into CML therapy has decreased or, in some instances, eliminated the prognostic impact of most of these prognostic factors and the significance of the CML models (e.g., Sokal, Hasford, European Treatment and Outcome Study [EUTOS]). Treatment-related prognostic factors have emerged as the most important prognostic factors in the era of imatinib therapy. Achievement of complete cytogenetic response has become the major therapeutic endpoint and is the only endpoint associated with improvement in survival. Achievement of MMR or MR3 is associated with decreased risk of events (relapse) and CML transformation but has not been associated with survival prolongation among patients with complete cytogenetic response. This may be due to the survival benefit conferred by the achievement of complete cytogenetic response, which approximates normal life expectancy, and to the efficacy of salvage TKI therapies, which are and should be implemented at the first evidence of cytogenetic relapse. Achievement of undetectable *BCR-ABL1* transcripts (complete molecular response [CMR]) or deep molecular response (DMR; defined as MR4 or MR4.5), particularly when sustained (>2–5 years), may offer the possibility of treatment-free remission and may

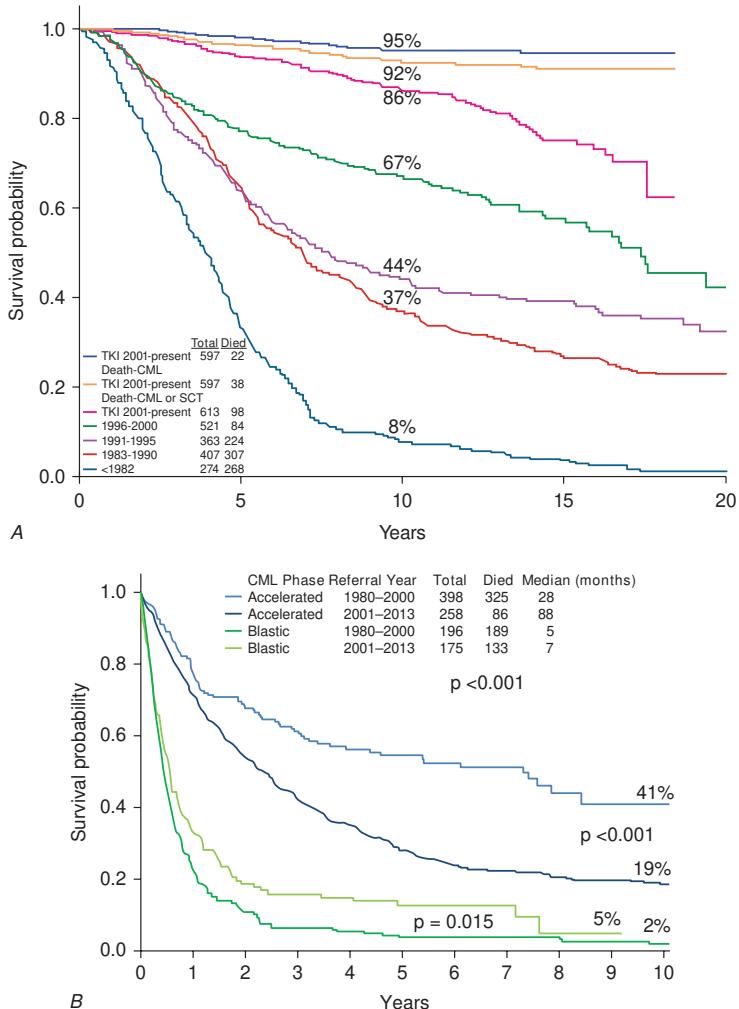


FIGURE 105-2 A. Survival in newly diagnosed chronic-phase chronic myeloid leukemia (CML) by era of therapy (MD Anderson Cancer Center experience from 1965 to present). Top blue curve is survival with tyrosine kinase inhibitors (TKIs), accounting for only CML-related deaths. The orange curve (second from top) accounts for deaths related to CML or CML treatment complications (e.g., deaths following allogeneic stem cell transplant [SCT]). The red curve (third from top) is survival including all deaths regardless of causality (old age, car accidents, suicide, gun shots, second cancers, complications of unrelated surgeries, infections, others). The difference in the denominators, 613 minus 597 cases, is because 16 deaths were from unknown/undocumented causes (outside MD Anderson and no good tracking for cause of death). B. Survival in patients with accelerated- and blastic-phase CML referred to MD Anderson Cancer Center by era of therapy, demonstrating the significant survival benefit in the TKI era in accelerated-phase CML but the modest benefit in blastic-phase CML. Referred cases included de novo and post-chronic-phase transformations.

allow temporary therapy interruption in women pursuing pregnancy. The lack of achievement of MMR or DMR should not be considered as “failure” of a particular TKI therapy and/or an indication to change the TKI or to consider allogeneic SCT.

Long-term updates of randomized trials suggest that second-generation TKIs and imatinib are similarly effective in lower-risk CML; second-generation TKIs may offer a therapeutic advantage among patients with high-risk CML.

TREATMENT

Chronic Myeloid Leukemia

Since 2001, six drugs have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of CML. These include five oral TKIs: imatinib (Gleevec, Glivec), nilotinib (Tasigna), dasatinib (Sprycel), bosutinib (Bosulif), and ponatinib (Iclusig). Dasatinib, nilotinib, and bosutinib are referred to as second-generation TKIs; ponatinib is referred to as a third-generation TKI. Nilotinib is

similar in structure to imatinib but 30 times more potent. Dasatinib and bosutinib inhibit the SRC family of kinases in addition to ABL1, with dasatinib reported to be 300 times more potent and bosutinib 30–50 times more potent than imatinib. In contrast to all other TKIs, bosutinib has no activity against c-Kit or platelet-derived growth factor receptor (PDGFR). Ponatinib is highly effective against wild-type and mutant *BCR/ABL1* clones. It is also the only available BCR-ABL1 TKI active against T315I, a gatekeeper mutation resistant to the other four ATP-competitive TKIs (Table 105-2). Ponatinib also inhibits vascular endothelial growth factor receptor (VEGFR), which may be at least partly responsible for the high incidence of hypertension observed with this agent (Table 105-2). Imatinib 400 mg orally daily, nilotinib 300 mg orally twice a day (on an empty stomach), dasatinib 100 mg orally daily, and bosutinib 400 mg orally daily are approved for frontline therapy of CML. Dasatinib 50 mg orally daily is as effective in frontline therapy as 100 mg daily, and significantly less toxic. All four are also approved for salvage therapy (nilotinib 400 mg twice daily; bosutinib 500 mg daily; others at the same dose as frontline therapy), in addition to ponatinib

TABLE 105-2 Medical Therapeutic Options in Chronic Myeloid Leukemia

AGENT (BRAND NAME)	APPROVED INDICATIONS	DOSE SCHEDULE	NOTABLE TOXICITIES
Imatinib mesylate (Gleevec)	All phases	400 mg daily	See text
Dasatinib (Sprycel)	All phases	First-line: 100 mg daily Salvage: 100 mg daily in chronic phase; 140 mg daily in transformation	Myelosuppression; pleural and pericardial effusions; pulmonary hypertension
Nilotinib (Tasigna)	All phases except blastic phase	First-line: 300 mg twice daily Salvage: 400 mg twice daily	Diabetes; arterio-occlusive disease; pancreatitis
Bosutinib (Bosulif)	All phases	First line: 400 mg daily Salvage: 500 mg daily	Diarrhea; liver toxicity; renal dysfunction
Ponatinib (Iclusig)	Optimal TKI if T315I mutation Failure of ≥2 tyrosine kinase inhibitors	45 mg daily (may consider lower starting doses in the future, e.g., 30 mg daily). (Lower the dose to 15 mg daily once a complete cytogenetic response is achieved).	Skin rashes (10–20%); pancreatitis (5%); arterio-occlusive disease (10–20%); systemic hypertension (10–15%)
Omacetaxine mepesuccinate (Synribo)	Failure ≥2 tyrosine kinase inhibitors	1.25 mg/m ² subcutaneously twice daily for 14 days of induction; 7 days of maintenance every month (may consider shorter dose schedules, 7 days of induction, 2–5 days of maintenance)	Myelosuppression

(45 mg daily). Ponatinib 45 mg daily may be associated with serious side effects: arterio-occlusive events, pancreatitis, hypertension, and skin rashes. A response-directed dose adjusted regimen, with a starting dose of 45 mg and reduction to 15 mg once a cytogenetic response is achieved, has resulted in a reduced incidence of arterio-occlusive events and has become standard. Imatinib, dasatinib (140 mg daily), bosutinib, and ponatinib are also approved for the treatment of CML in transformation (accelerated and blastic phase), whereas nilotinib is only approved for chronic and accelerated phase. The sixth approved drug is omacetaxine (Synribo), a protein synthesis inhibitor with presumed more selective inhibition of the synthesis of the BCR-ABL1 oncprotein. It is approved for the treatment of chronic- and accelerated-phase CML after failure of two or more TKIs, at 1.25 mg/m² subcutaneously twice a day for 14 days for induction and for 7 days for consolidation-maintenance. The main adverse event of omacetaxine is prolonged myelosuppression; thus, many experts use shorter schedules (e.g., omacetaxine 5–7 days induction and 2–5 days maintenance), often combined with a TKI (Table 105-2).

Imatinib, dasatinib, bosutinib, and nilotinib are all acceptable frontline therapies in CML. The long-term results of imatinib are very favorable. The 10-year follow-up results show a cumulative complete cytogenetic response rate (occurring at least once) of 83%, with 60–65% of patients being in complete cytogenetic response at 5-year follow-up. The estimated 10-year survival rate is ~85%. Among patients continuing on imatinib, the annual rate of transformation to accelerated-blastic phase in years 4–8 is <1%. In three randomized studies, one comparing nilotinib 300 mg twice daily or 400 mg twice daily with imatinib (ENESTnd), another comparing dasatinib 100 mg daily with imatinib (DASISION), and a third comparing bosutinib 400 mg daily with imatinib (BFORE), the second-generation TKIs were associated with better outcomes in early surrogate endpoints, including higher rates of complete cytogenetic responses (85–87% vs 77–82%), MMRs (5-year rates 76–77% vs 60–64%), and MR4.5 (5-year rates 42–53% vs 31–33%), with lower rates of transformation to accelerated and blastic phase (2–5% vs 7%). However, no study has shown a survival benefit with second-generation TKIs. This may be because the rate of complete cytogenetic response is ultimately similarly high with imatinib versus second-generation TKIs, and also because sequential therapy with TKIs (following close observation and treatment change at progression) provides highly effective therapy for most patients; this ensures adequate long-term outcome despite relapse or intolerance after initial therapy.

Salvage therapy in chronic phase with dasatinib, nilotinib, bosutinib, or ponatinib is associated with complete cytogenetic response rates of 30–60%, depending on the salvage status (cytogenetic vs

hematologic relapse), prior response to other TKIs, number of prior TKIs used, and the mutations at the time of relapse. Complete cytogenetic responses are generally durable, particularly in the absence of clonal evolution. Ponatinib is the only TKI active in the setting of T315I mutation, with complete cytogenetic response rates of 50–70% among patients who have received two or more TKIs. The estimated 5-year survival rates with new TKIs as salvage are 70–75% (compared with <50% before their availability). For example, with dasatinib salvage after imatinib failure in chronic-phase CML, the estimated 7-year rate of major molecular was 46%, the estimated 7-year survival rate was 65%, and progression-free survival rate was 42%. Thus, TKIs in the salvage setting have already reduced the annual mortality from the historical rate of 10–15% to ~5%.

The goal of CML therapy is survival prolongation. The achievement of treatment-free remission (TFR) status has become a therapeutic goal of increased interest (sustained DMR or CMR after discontinuation of TKI therapy). In current practice, with the availability of appropriate TKI therapy and with compliance, monitoring, and changing of TKI therapy as indicated by response/resistance and side effects, patients can have a near-normal life expectancy, with a “relative” survival similar to that of the general population. Therefore, in standard practice, achievement and maintenance of a complete cytogenetic response are the aims of therapy, because complete cytogenetic response is the only outcome associated with survival prolongation. Lack of achievement of an MMR (protects against events; associated with longer event-free survival) or of DMR (offers the potential of treatment discontinuation and of TFR) should not be considered indications to change TKI therapy or to consider allogeneic SCT. A general practice rule is to continue the particular TKI chosen at the most tolerable dose schedule not associated with grade 3–4 side effects or with bothersome chronic side effects, for as long as possible, until either cytogenetic relapse or the persistence of unacceptable side effects. These two factors (i.e., cytogenetic relapse and intolerable side effects) are the indicators of “failure” of a particular TKI therapy. A second emerging general practice rule is that patients with CML should always receive daily TKI therapy throughout their lifetime (chronic, transformation), either alone (chronic) or in combinations (possibly for those in transformation, although combinations not formally approved), except perhaps in situations of “molecular cure” (TFR; elective discontinuation of TKI if DMR sustained for >2 to 5 years, followed by close monitoring) or after allogeneic SCT with undetectable disease.

Because of the increasing prevalence of CML (cost of TKI therapy) and the emerging evidence of possible organ toxicities with long-term use (e.g., renal with imatinib and bosutinib; arterio-occlusive with nilotinib, dasatinib, and ponatinib), a goal of therapy

of increasing interest in CML is to achieve eradication of the disease (molecular “cure” or TFR) that is prolonged and durable, with recovery of nonneoplastic, nonclonal hematopoiesis off TKI therapy. The first step toward this aim is to obtain the highest rates of DMR lasting for at least 2 or more years. This is currently achievable in about 25–30% of patients treated with imatinib and in 40–45% of patients treated with second-generation TKIs. Approximately 50–60% of those who meet these criteria and discontinue therapy remain free from therapy and in DMR-MMR. As a result, TFR rates are estimated to be about 15–20% after imatinib therapy and 25–30% after second-generation TKIs.

Recommendations provided by the National Comprehensive Cancer Network (NCCN) and by the European LeukemiaNet (ELN) propose optimal/expected, suboptimal/warning, and failure response scenarios at different time points of TKI treatment duration. Unfortunately, they may have been misinterpreted in current practice because oncologists often report that their aim of treatment is the achievement of MMR and disease eradication. Significantly, a substantial proportion of oncologists consider a change of TKI therapy in a patient in complete cytogenetic response if they note loss of MMR (increase of *BCR-ABL1* transcripts [IS] from $\leq 0.1\%$ to $> 0.1\%$). This perception may be the result of confusion regarding the aims of the NCCN and ELN guidelines, which have been updated often as a result of maturing data and have multiple treatment endpoint considerations. Although such endpoints may have been suggested as possible criteria for failure or suboptimal response, it is important to emphasize that no randomized study has yet shown that a change of TKI treatment in patients with complete cytogenetic response because of a loss of MMR, versus changing at the time of cytogenetic relapse, improves survival or other long-term outcomes. This is likely because of the high efficacy of salvage TKI therapy at the time of cytogenetic relapse.

Side effects of TKIs are generally mild to moderate, although with long-term TKI therapy, they could affect the patient’s quality of life. Serious side effects occur in <5–10% of patients. With imatinib therapy, common mild to moderate side effects include fluid retention, weight gain, nausea, diarrhea, skin rashes, periorbital edema, bone or muscle aches, fatigue, and others (rates of 10–20%). In general, second-generation TKIs are associated with lower rates of these bothersome adverse events. However, dasatinib 100 mg daily is associated with higher rates of myelosuppression (20–30%), particularly thrombocytopenia, with pleural (10–25%) or pericardial effusions ($\leq 5\%$), and with pulmonary hypertension (<5%). A lower dose of dasatinib (50 mg daily instead of 100 mg daily) used in frontline CML therapy has resulted in similar efficacy and a lower incidence of serious side effects (pleural effusions <5%, myelosuppression <10%). Nilotinib is associated with higher rates of hyperglycemia (10–20%), pruritus and skin rashes, hyperbilirubinemia (typically among patients with Gilbert’s syndrome and mostly of no clinical consequences), and headaches. Nilotinib is also associated with occasional instances of pancreatitis (<5%). Nilotinib 300–400 mg twice daily is associated with a 10-year cumulative incidence of cardiovascular complications of 15–25%. Bosutinib is associated with higher rates of liver toxicity, renal dysfunction, and early and self-limited gastrointestinal adverse events, particularly diarrhea (70–85%). Occasionally, the gastrointestinal symptoms mimic chronic severe enterocolitis, which reverses with treatment discontinuation. Ponatinib 45 mg daily is associated with higher rates of serious skin rashes (10–15%), pancreatitis (10%), elevations of amylase/lipase (10%), and systemic hypertension (50–60%; severe in 20%). Arterio-occlusive events (cardiovascular, cerebrovascular, and peripheral arterial) have been reported with most TKIs. The incidence appears to be highest with ponatinib, but both nilotinib and dasatinib are associated with these events at an incidence significantly higher than imatinib. Among the TKIs, bosutinib is associated with the lowest incidence of cardiovascular events. Nilotinib and dasatinib may cause prolongation of the QTc interval; therefore, they should be evaluated cautiously in patients with prolonged QTc

interval on electrocardiogram (>470–480 ms), and drugs given for other medical conditions should have relatively smaller or no effects on QTc. These side effects can often be dose-dependent and are generally reversible with treatment interruptions and dose reductions. Dose reductions can be individualized. However, the lowest estimated effective doses of TKIs (from different studies and treatment practices) are imatinib 100–200 mg daily; nilotinib 150 mg twice daily or 200 mg daily; dasatinib 20 mg daily; bosutinib 200–300 mg daily; and ponatinib 15 mg daily.

With long-term follow-up, rare but clinically relevant serious toxicities are emerging. Renal dysfunction and occasionally renal failure (creatinine elevations >2–3 mg/dL) are observed in 2–3% of patients, more frequently with imatinib and bosutinib than other TKIs, and usually reverse with TKI discontinuation and/or dose reduction. Rarely, patients may develop TKI-related peripheral neuropathy or even central neurotoxicities that are misdiagnosed as dementia or Alzheimer’s disease; they may reverse slowly after TKI discontinuation. Pulmonary hypertension has been reported with dasatinib (<1–2%) and should be considered in a patient with shortness of breath and a normal chest x-ray (echocardiogram with emphasis on measurement of pulmonary artery pressure). This may be reversible with dasatinib discontinuation and occasionally the use of sildenafil citrate. Systemic hypertension has been observed more often with ponatinib. Hyperglycemia and occasionally diabetes have been noted more frequently with nilotinib. Finally, mid- and small-vessel arterio-occlusive and vasospastic events have been reported at low but significant rates with nilotinib and ponatinib and should be considered possibly TKI-related and represent indications to interrupt or reduce the dose of the TKI. These events include angina, coronary artery disease, myocardial infarction, peripheral arterial occlusive disease, transient ischemic attacks, cerebral vascular accidents, Raynaud’s phenomenon, and accelerated atherosclerosis. Although these events are uncommon (<5%) (10-year cumulative rates of 15% with nilotinib 300 mg BID and 20–25% with 400 mg BID, compared with <5% with imatinib), they are clinically significant for the patient’s long-term prognosis and occur at significantly higher rates than in the general population, particularly among patients with other risk factors for such events. Serious arterio-occlusive and vasospastic events are more common with ponatinib 45 mg daily (5-year rates 20%).

Discontinuation of TKIs and Treatment-Free Remissions Several studies have confirmed that TKI discontinuation among patients who achieve DMR (MR4.5) for longer than 2–3 years can result in TFR rates of 40–60%. Discontinuation of TKI therapy after 5+ years of CMR is associated with TFR rates of 70–80% or greater. Since the incidence of durable MR4.5 (*BCR-ABL* transcripts [IS] $\leq 0.0032\%$) is 30–60%, ~15–30% of all patients with CML on TKI therapy may achieve TFR. This approach is ready for community practice provided it is done under optimal conditions. These include the following: patients must have low or intermediate Sokal risk CML in first chronic phase (no evidence or history of transformation), with history of quantifiable *BCR-ABL1* transcripts (e13a2, e14a2), on long-term TKI therapy (5–8+ years), with documented DMR for >2–3 years (assessed every 6 months during this time span and with a PCR with adequate sensitivity), and should be monitored at referral centers that offer rigorous testing of residual CML disease. Patients must also be compliant to frequent monitoring (PCR studies every 1–2 months for the first 6 months, then every 2 months until 2 years and every 3–6 months thereafter).

ALLOGENEIC STEM CELL TRANSPLANT

Allogeneic SCT, a curative modality in CML, is associated with long-term survival rates of 40–60% when implemented in chronic phase. It is associated with early (1-year) mortality rates of 5–30%. Although the 5- to 10-year survival rates were reported to be ~50–60% (and considered as cure rates), ~10–15% of patients die in the subsequent 1–2 decades from subtle long-term complications of the transplant (rather than from CML relapse). These are related

to chronic graft-versus-host disease (GVHD), organ dysfunction, development of second cancers, occasional late relapses, and hazard ratios for mortality higher than in the normal population. Other significant morbidities include infertility, chronic immune-mediated complications, cataracts, hip necrosis, and other morbidities affecting quality of life. The cure and early mortality rates in chronic-phase CML are also associated with several factors: patient age, duration of chronic phase, whether the donor is related or unrelated, degree of matching, preparative regimen, and others. In accelerated-phase CML, the cure rates with allogeneic SCT are 30–50%, depending on the definition of accelerated disease. Patients with clonal evolution as the only criterion have cure rates of up to 40–50%. Patients undergoing allogeneic SCT in second chronic phase have cure rates of 40–50%. The cure rates with allogeneic SCT in blastic-phase CML are $\leq 20\%$. Post-allogeneic SCT strategies are now implemented in the setting of molecular or cytogenetic relapse or in hematologic relapse/transformation. These include the use of TKIs for prevention or treatment of relapse, donor lymphocyte infusions, and second allogeneic SCTs, among others. TKIs appear to be highly successful at reinducing cytogenetic/molecular remissions in the setting of cytogenetic or molecular relapse after allogeneic SCT.

Choice and Timing of Allogeneic SCT Allogeneic SCT was considered first-line CML therapy before 2000. The maturing positive experience with TKIs has now relegated its use to after first-line TKI failures. An important question is the optimal timing and sequence of TKIs and allogeneic SCT (whether allogeneic SCT should be used as second- or third-line therapy). Among patients who present with or evolve to blastic phase, combinations of chemotherapy and TKIs should be used to induce remission, followed by allogeneic SCT as soon as possible. The same applies to patients who evolve from chronic to accelerated phase. Patients with de novo accelerated-phase CML may do well with long-term TKI therapy (estimated 8-year survival rate 75%); the timing of allogeneic SCT depends on their optimal response to TKI (achievement of complete cytogenetic response). Among patients who relapse in chronic phase, the treatment sequence depends on several factors: (1) patient age and availability of appropriate donors; (2) risk of allogeneic SCT; (3) presence or absence of clonal evolution and mutations; (4) patient's prior history and comorbidities; and (5) patient and physician preferences (Table 105-3). Patients with T315I mutations at relapse should be offered ponatinib and considered for allogeneic SCT particularly if in blastic phase and perhaps also in accelerated phase (because of the short follow-up with ponatinib). Patients with mutations involving Y253H, E255K/V, and F359V/C/I respond better to dasatinib or bosutinib. Patients with mutations involving V299L, T315A, and F317L/F/I/C respond better to nilotinib. Comorbidities such as diabetes, hypertension, pulmonary hypertension, chronic lung disease, cardiac conditions, and pancreatitis may influence the choice for or against a particular TKI. Patients with clonal evolution, unfavorable mutations, or lack of major/complete cytogenetic response within 1 year of salvage TKI therapy have short remission durations and should consider allogeneic SCT as more urgent in the setting of salvage. Patients without clonal evolution or mutations at relapse and who achieve a complete cytogenetic response with TKI salvage have long-lasting complete remissions and may delay the option of allogeneic SCT to third-line therapy. Finally, older patients (age 65–70 years or older) and those with high risk of mortality with allogeneic SCT may forgo this curative option for several years of disease control in chronic phase with or without cytogenetic response (Table 105-3). In emerging nations, where generic imatinib is now available at the annual price of \$400–3000, frontline imatinib is a cost-effective therapy. However, second-line therapy with allogeneic SCT, a one-time curative option with a cost of \$20,000–100,000, may be considered (in preference to second-generation TKIs—annual cost above \$40,000–100,000) as a more cost-effective national health

TABLE 105-3 General Suggestions Regarding the Use of Tyrosine Kinase Inhibitors (TKIs) and Allogeneic Stem Cell Transplantation (SCT) in Chronic Myeloid Leukemia (CML)

CML PHASE	USE OF TKI	CONSIDERATION OF ALLOGENEIC SCT
Accelerated or blastic	Interim therapy to achieve minimal CML burden	As soon as possible (exception: de novo accelerated phase)
T315I mutation	Ponatinib to achieve minimal CML burden	Depends on longer term follow-up results of ponatinib efficacy
Imatinib failure in chronic phase; no clonal evolution, no mutations, good initial response; no T315I	Second-line TKIs long term	Third-line after second-line TKI failures
Clonal evolution or mutations, or no cytogenetic response to second-line TKI	Interim therapy with alternative second-generation TKI or ponatinib to achieve minimal CML burden	Second-line
Older patients (≥ 65 –70 years) after imatinib failure in chronic phase	Salvage TKIs as longer-term therapy	May forgo allogeneic SCT in favor of good quality of life and survival in chronic phase
Imatinib failure; emerging nation	—	Second-line: curative, one-time cost \$20,000–100,000 (vs >\$40,000–100,000/year with TKI)

Note: Mutations involving Y253H, E255K/V, or F359V/C/I: prefer dasatinib or bosutinib. Mutations involving V299L, T315A, or F317L/F/I/C: prefer nilotinib.

care strategy in CML. Table 105-3 summarizes a general guidance to the choice of TKIs versus allogeneic SCT.

MONITORING THERAPY IN CML

Achievement of complete cytogenetic response by 12 months of imatinib therapy and its persistence later, the only consistent prognostic factor associated with prolonged survival, is now the main therapeutic endpoint in CML. Failure to achieve a complete cytogenetic response by 12 months or occurrence of later cytogenetic or hematologic relapse is considered as treatment failure and an indication to change therapy. Because salvage therapy with other TKIs may re-establish good outcome, it is important to ensure patient compliance to continued TKI therapy and change therapy when cytogenetic relapse is confirmed unless this is related to non-adherence. Patients on frontline imatinib therapy should be closely monitored until documentation of complete cytogenetic response, at which time they can be monitored every 6 months with peripheral blood PCR, or more frequently (e.g., every 3 months), if there are concerns about changes in *BCR-ABL1* transcripts. Cytogenetic relapse on imatinib is an indication of treatment failure and need to change TKI therapy. Mutational analysis in this instance helps in the selection of the next TKI and identifies mutations in 30–50% of patients. Mutational studies by standard Sanger sequencing (which is the technique currently available in most clinical laboratories) in patients in complete cytogenetic response (in whom there may be concerns of increasing *BCR-ABL1* transcripts) identify mutations in $\leq 5\%$ and are therefore not indicated. Earlier response has been identified as a prognostic factor for long-term outcome, including achievement of partial cytogenetic response (*BCR-ABL1* transcripts $\leq 10\%$) by 3–6 months of therapy. Failure to achieve such a response has been associated with significantly worse survival.

The use of second-generation TKIs (dasatinib, bosutinib, nilotinib) as frontline therapy changed the monitoring approach slightly. Patients are expected to achieve major cytogenetic response (or *BCR-ABL1* transcripts $\leq 10\%$) by 3–6 months of therapy. Failure to do so is associated with worse event-free survival, transformation

rates, and survival. However, the 3- to 5-year estimated survival among such patients is still high, ~80–90%, which is better than what would be anticipated if such patients were offered allogeneic SCT at that time. Changes of therapy for patients with “slow” response have not been proven to be of long-term benefit compared to changes when more obvious signs of resistance appear. Thus, slow response to therapy is considered a warning signal, but it is not known whether changing therapy to other TKIs at that time would improve longer-term outcome.

TREATMENT OF ACCELERATED AND BLASTIC PHASES

Patients in accelerated or blastic phase may receive therapy with TKIs, preferably second- or third-generation TKIs (dasatinib, nilotinib, bosutinib, ponatinib), alone or in combination with chemotherapy, to reduce the CML burden, before undergoing allogeneic SCT. Response rates (major hematologic) with single-agent TKIs range from 30 to 50% in accelerated phase and from 20 to 30% in blastic phase. Cytogenetic responses, particularly complete cytogenetic responses, are uncommon (10–30%) and transient in blastic phase. Studies of TKIs in combination with chemotherapy show that combined TKI-chemotherapy strategies increase the response rates and their durability and improve survival. This is particularly true in CML lymphoid blastic phase, where the combination of anti-ALL chemotherapy with TKIs results in complete response rates of 70% and median survival times of 3 years (compared with historical response rates of 40–50% and median survival times of 12–18 months). This allows many patients to undergo allogeneic SCT in a state of minimal CML burden or second chronic phase, which are associated with higher probability of long-term survival. In CML nonlymphoid blastic phase, anti-acute myeloid leukemia chemotherapy combined with TKIs results in CR rates of 30–50% and median survival times of 9–12 months (compared with historical response rates of 20–30% and median survival times of 3–5 months). In accelerated phase, response to single TKIs is significant in conditions where “softer” accelerated phase criteria are considered (e.g., clonal evolution alone, thrombocytosis alone, significant splenomegaly or resistance to hydroxyurea, but without evidence of high blast and basophil percentages). In accelerated phase, combinations frequently include TKIs with low-intensity chemotherapy such as low-dose cytarabine, decitabine, interferon α , hydroxyurea, or others.

OTHER TREATMENTS AND SPECIAL THERAPEUTIC CONSIDERATIONS

Interferon α Interferon α is considered in combination with TKIs (an investigational approach), sometimes after CML failure on TKIs, occasionally in patients during pregnancy, or as part of investigational strategies with TKIs to eradicate residual molecular disease.

Chemotherapeutic Agents Hydroxyurea remains a safe and effective agent (at daily doses of 0.5–10 g) to reduce initial CML burden, as a temporary measure in between definitive therapies, or in combination with TKIs to sustain complete hematologic or cytogenetic responses. Busulfan is often used in allogeneic SCT preparative regimens. Because of its side effects (delayed myelosuppression, Addison-like disease, pulmonary and cardiac fibrosis, myelofibrosis), it is now rarely used in the chronic management of CML. Low-dose cytarabine, decitabine, anthracyclines, 6-mercaptopurine, 6-thioguanine, thiotapec, anagrelide, and other agents are sometimes useful in different CML settings to control the disease burden.

Others Splenectomy is now seldom considered to alleviate symptoms of massive splenomegaly and/or hypersplenism. Splenic irradiation is rarely used, if at all, because of the postirradiation adhesions and complications. Leukapheresis is occasionally used in patients presenting with extreme leukocytosis and leukostatic complications. Single doses of high-dose cytarabine or high doses

of hydroxyurea, with tumor lysis management, may be as effective and less cumbersome.

Special Considerations Women with CML who become pregnant should discontinue TKI therapy immediately. Among 125 babies delivered to women with CML who discontinued imatinib therapy as soon as the pregnancy was known, three babies were born with neurologic, skeletal, and renal malformations, suggesting the teratogenicity of imatinib known from animal studies. A similar experience has been reported with dasatinib, where the incidence of malformations was reported to be higher, 10–12%. There are no or little data with other TKIs. Control of CML during pregnancy can be managed with leukapheresis for severe symptomatic leukocytosis in the first trimester and with hydroxyurea subsequently until delivery. There are reports of successful pregnancies and deliveries of normal babies with interferon α therapy and registry studies in essential thrombocythemia of its safety, but interferon α has side effects that may be troublesome during pregnancy, can be antiangiogenic, and may increase the risk of spontaneous abortions.

Approximately 10–15% of patients on TKI therapy may develop chromosomal abnormalities in the Ph-negative cells. These may involve loss of chromosome Y, trisomy 8, 20q-, chromosome 5 or 7 abnormalities, and others. Most chromosomal abnormalities disappear spontaneously and may be indicative of the genetic instability of the hematopoietic stem cells that predisposes the patient to develop CML in the first place. Rarely (in <1% of instances), abnormalities involving chromosomes 5 or 7 may be truly clonal and evolve into myelodysplastic syndrome or acute myeloid leukemia. This is thought to be part of the natural course of patients in whom CML was suppressed and who live long enough to develop other hematologic malignancies.

■ GLOBAL ASPECTS OF CML

Routine physical examinations and blood tests in the United States and advanced countries result in early detection of CML in most patients. About 50–70% of patients with CML are diagnosed incidentally, and high-risk CML as defined by prognostic models (e.g., Sokal risk groups) is found in only 10% of patients. This is not the same situation in emerging nations where most patients are diagnosed following evaluation for symptoms and many present with high tumor burden, such as massive splenomegaly, and advanced phases of CML (high-risk CML documented in 20–30%). Therefore, the prognosis of such patients on TKI therapy may be worse than the published experience.

The high cost of TKI therapies (annual costs of \$90,000–140,000 in the United States; lower but variable in the rest of the world) makes the general affordability of such treatments difficult. Although TKI treatment penetration is high in nations where cost of therapy is not an issue (e.g., Sweden, European Union), it may be less so in other nations, even in advanced ones like the United States, where out-of-pocket expenses may be prohibitive to a subset of patients. Although the estimated 10-year survival in CML is >85% in single-institution studies (e.g., MD Anderson Cancer Center), in national studies in countries with TKI affordability (Sweden) (Figs. 105-2 and 105-3) or in clinical trials (where all patients have access to TKIs throughout their care), the estimated 10-year survival worldwide, even 16 years after the introduction of TKI therapies, is likely to be <50%. The Surveillance, Epidemiology, and End Results (SEER) data from the United States report an estimated 5-year survival rate of 60% in the era of TKIs. It appears that the treatment penetration of imatinib and other TKIs into CML therapy worldwide is still not optimal.

The current high cost of TKI therapies poses two additional considerations. The first are the treatment pathways and guidelines in nations where TKIs may not be affordable by patients or the health care system. In these conditions, there are trends of pathways advocating allogeneic SCT as frontline or second-line therapy (i.e., after imatinib failure; as a one-time cost of \$20,000–100,000) despite the associated mortality and morbidities. The second is the choice of frontline TKI therapy. Imatinib is now available in generic forms at affordable costs (\$400–10,000 per

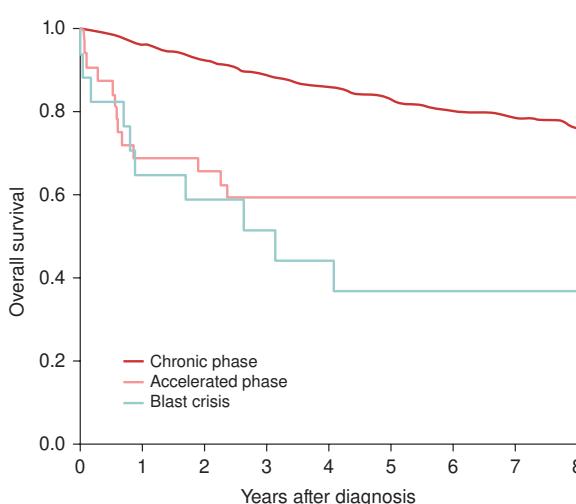


FIGURE 105-3 Survival in chronic (CP), accelerated (AP), and blastic crisis (BC) phases of chronic myeloid leukemia (CML) in the population-based Swedish national registry study. The accelerated- and blastic-phase cases are de novo presentations. The favorable outcome with de novo blastic phase may be due to use of 20% blasts or more to define blastic phase. (With permission from Dr. Martin Hoglund, Swedish CML Registry, 2013.)

year). Dasatinib is available in generic forms in many geographies. Safe and effective generic TKIs may become preferred frontline and salvage therapies in CML, precluding the necessity of an allogeneic SCT in first salvage in poorer nations.

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106 Acute Lymphoid Leukemia

Dieter Hoelzer



In acute lymphoblastic leukemia (ALL), the malignant clone arises from hematopoietic progenitors in the bone marrow or lymphatic system resulting in an increase of immature nonfunctioning leukemic cells. Infiltration of bone marrow leads to anemia, granulocytopenia, and thrombocytopenia with the clinical manifestations of fatigue, weakness, infection, and hemorrhage. These symptoms are more often the reason a patient first seeks medical advice rather than consequences of tumor bulk, such as lymph node enlargement, hepatosplenomegaly caused by leukemic infiltration, or symptoms of the central nervous system (meningeosis leukemica).

INCIDENCE AND AGE

ALL is the most frequent neoplastic disease in children with an early peak at the age of 3–4 years. The incidence in adults ranges from 0.7 to 1.8/100 000 per year, being somewhat higher in adolescents and young adults (AYAs), decreasing in adults, but increasing again in elderly people. Thus, Philadelphia chromosome-positive ALL (Ph+ ALL; *BCR/ABL* translocation) is observed in half of elderly B-lineage patients. The frequency of immunologic, cytogenetic, and genetic subtypes changes substantially with age.

ETIOLOGY

The etiology of acute leukemias is unknown. Internal and external factors influence the incidence of leukemia. Exposure to ionizing radiation or to chemicals, including prior chemotherapy, is associated with an increased risk of developing leukemia, more often observed in acute myeloid leukemia (AML). However, increasingly, secondary ALLs have been observed, particularly after cytostatic treatment with alkylating agents and topoisomerase inhibitors as treatment for primary tumors, most often for AML, myelodysplastic syndromes, or breast cancer.

CONGENITAL DISORDERS

Patients with some rare congenital chromosomal abnormalities have a higher risk of development of acute leukemia (e.g., Klinefelter's syndrome, Fanconi's anemia, Bloom's syndrome, ataxia-telangiectasia, and neurofibromatosis). Those with Down's syndrome have a twentyfold increased incidence of leukemia; ALL is increased in childhood and AML at an older age.

INFECTIOUS AGENTS

No direct evidence implicates viruses as a major cause of human acute leukemia. However, viruses are involved in the pathogenesis of two lymphoid neoplasias. In the endemic African type of Burkitt's lymphoma, the Epstein-Barr virus, a DNA virus of the herpes family, has been implicated as a potential causative agent (see Chap. 194). Endemic infection with human T-cell leukemia virus I in Japan and the Caribbean has been shown to be an etiologic agent for rare cases of adult T-cell leukemia/lymphoma (see Chap. 201).

DIAGNOSIS AND CLASSIFICATION

The diagnosis of acute leukemia is first made by examination of the peripheral blood and bone marrow. For further classification of the leukemic blast cells, cytochemical stains, immunologic markers, and cytogenetic and molecular analysis are required. The immunologic markers are still the major criteria to subdivide into B-cell lineage or T-cell lineage ALL leukemias.

PERIPHERAL BLOOD

Peripheral blood counts and a differential count from a Wright-Giemsa-stained blood smear are essential at the time of presentation. The white blood cell (WBC) count in ~40% of ALL patients is reduced or normal (Table 106-1). Only 16% of patients have a WBC above

TABLE 106-1 Laboratory Values at Diagnosis of Acute Lymphoblastic Leukemia (ALL)		
NO.		ALL
Initial white blood cell count ($\times 10^9/L$)	<10 10–50 >50–100 >100	41% 31% 28% 16%
Neutrophils ($\times 10^9/L$)	<50–100 <100,000	12% 16%
Platelets ($\times 10^9/L$)	<20 21–40 41–100 >100	22% 22% 29% 27%
Hemoglobin (g/dL)	<7 7–9 >9	20% 33% 47%
Leukemic blasts in peripheral blood	0% 25–75% >75%	8% 34% 36%
Leukemic blasts in bone marrow	<50% 51–90% >90%	4% 25% 71%

Source: Data from three consecutive German Multicenter Trials for Adult ALL (GMALL).

$100 \times 10^9/L$. It is noteworthy that in 8% of ALL patients, no circulating leukemic blast cells were observed. Thus, in the frequently used automatic blood cell counting, the diagnosis may not be detected.

Peripheral blood characteristically shows anemia, thrombocytopenia, and neutropenia. Nearly one-third of patients have hemoglobin levels $<7–8 \text{ g/dL}$. A platelet count below the critical number of $20 \times 10^9/L$ and neutropenia (neutrophils $<0.5 \times 10^9/L$), which is associated with a higher risk of infection, are each noted in one-fifth of adults with ALL.

BONE MARROW EXAMINATION

Bone marrow aspirates/biopsies are important to assess immunologic, cytogenetic, and genomic markers. Direct smears from the bone marrow are essential to confirm the diagnosis of acute leukemia and to distinguish between AML and ALL. The bone marrow is usually heavily packed with leukemic blast cells with $>90\%$ in ~70% of patients, and thus, the normal hemopoietic elements are greatly reduced or absent. A biopsy of the bone marrow will further demonstrate marked hypercellularity with replacement of fat spaces, normal elements, and occasionally increased fibrosis.

LUMBAR PUNCTURE

The examination of the cerebrospinal fluid is an essential routine diagnostic measure for ALL. Central nervous system (CNS) leukemia is diagnosed if $\geq 5 \text{ cells}/\mu\text{L}$ or leukemic blast cells were observed by morphology in cerebrospinal fluid. Opinions differ as to when the first lumbar puncture should be done—i.e., either delay lumbar puncture until remission is achieved to avoid seeding of the CNS with leukemic blast cells from the peripheral blood during the spinal tap, or perform the lumbar puncture before treatment starts, since early recognition of CNS disease will lead to immediate CNS-specific therapy. Lumbar puncture is restricted to patients with an adequate platelet count ($>20 \times 10^9/L$) and without manifest clinical hemorrhages. To eliminate potentially transferred blast cells, patients should receive intrathecal methotrexate at the first lumbar puncture.

MORPHOLOGIC SUBTYPES IN ALL

The French-American-British (FAB) classification distinguished three subgroups. L1 and L2 morphology has no clinical consequences. Only the L3 morphology, observed in up to 5% of adult patients, is indicative for mature B-cell lineage ALL (B-ALL) (see Chap. 62).

IMMUNOLOGIC SUBTYPES

A series of monoclonal antibodies is employed to identify antigens expressed on the surface of leukemic cells, corresponding to the pathways of normal B-cell differentiation (see Fig. 108-2). The aim of the immunologic classification is to subdivide ALLs according to the presence or absence of B-cell or T-cell markers. A marker is considered positive if $>20\%$ of the cells are stained with the monoclonal antibody.

There are different immunologic classifications, such as that of the European Group for the Immunological Characterization of Leukemias (EGIL), with clear therapeutic implications. Table 106-2 gives a simplified correlation of immunologic subtypes, cytogenetics and molecular aberrations, and clinical characteristics.

B-Cell Lineage ALL (B-ALL) More than 70% of adult ALLs are of B-cell origin, and the most frequent immunologic subtype, common ALL, is characterized by the presence of the ALL antigen CD10 without markers of relatively mature B cells such as cytoplasmic or surface membrane immunoglobulins. Pre-B-ALL (early B-ALL) is characterized by the expression of cytoplasmic immunoglobulin, which is negative in common ALL, but otherwise is identical with respect to all other cell markers. Pro-B-ALL corresponds to early B-cell differentiation and was formerly termed non-T-, non-B-ALL or null ALL because neither T-cell nor B-cell features could be demonstrated. This subtype is HLA-DR, terminal deoxynucleotidyl transferase, and CD19 positive and composes ~12% of adult ALLs. Mature B-ALL is seen in 3–4% of adults and is also known as Burkitt's leukemia. In mature B-ALL, blast cells express surface antigens of mature B cells, including the IgM.

T-Cell Lineage ALL (T-ALL) Approximately 25% of adult ALLs are of T-cell lineage. All cases express the T-cell antigen CD7 and cytoplasmic CD3 (CyCD3) or surface CD3. According to their phase of T-cell differentiation, they may express other T-cell antigens (e.g., the E-rosette receptor CD2 and/or the cortical thymocyte antigen CD1a). Early pro/pre-T-ALL (also termed early T precursor ALL [ETP-ALL]), cortical or thymic T-ALL, and mature T-ALL can be distinguished with these markers. ETP-ALL is characterized by lack of CD1a and CD8, weak CD5 expression, and at least one myeloid/stem cell marker.

Biphenotypic or Mixed Leukemias Biphenotypic leukemias are defined as those expressing markers of both lymphoid and myeloid lineages on the same leukemic cells. Bilineage leukemias are those with two populations of blast cells with either lymphoid or myeloid antigens. It is not clear whether these patients should receive an ALL or AML treatment protocol. In pediatric studies, starting with a pediatric ALL protocol seemed preferable, which was then followed by AML consolidation elements.

CYTOGENETIC AND MOLECULAR ANALYSIS

Cytogenetic and molecular analyses should be performed in all cases in ALL. They are important to define ALL subtypes, can identify independent prognostic markers of disease-free survival, and may determine specific targeted therapies.

The diagnostic techniques for ALL are standard cytogenetics, fluorescence in situ hybridization, and reverse transcriptase polymerase chain reaction. These methods allow the detection of Ph+ ALL, with the chromosomal translocation t(9;22)(q34;q11) and the detection of the corresponding *BCR-ABL1* gene rearrangement. Further ALL entities that have been identified are t(4;11)(q21;q23)/*MLL-AF4*, abn11q23/*MLL*, and t(1;19)(q23;p13)/*PBX-E2A*.

Gene expression profiling, single nucleotide polymorphism array analysis, array-comparative genomic hybridization, and next-generation sequencing recognize the newly defined ALL entities: ETP-ALL and Ph-like ALL.

Ph-like ALL, also known as *BCR-ABL1*-like ALL, is characterized by genetic lesions similar to Ph+ ALL, associated with *IKZF1* (Ikaros) gene deletion, *CLRF2* (gene for cytokine-like receptor-2) overexpression, and tyrosine kinase activating rearrangements involving *ABL1*, *JAK2*, *PDGFRB*, and several other genes; however, it is *BCR-ABL1* negative. The frequency is 10% in children and 25–30% in young adults but does not increase further with age like Ph+ ALL. Treatment based on the underlying genetic lesion with *BCR-ABL* inhibitors

TABLE 106-2 Immunologic, Cytogenetic, Molecular, and Clinical Characteristics of Adult Acute Lymphoblastic Leukemia (ALL)

Subtypes	Marker	Incidence	Frequent Cytogenetic Aberrations	Genetic Aberrations and Fusion Transcripts	Clinical Characteristics	Relapse Kinetics and Localization
B-lineage ALL (B-ALL)	HLA-DR+, TdT+, CD19+, and/or CD79a+, and/or CD22+	76%				
Pro B-ALL	No additional differentiation markers Frequent myeloid coexpression (>50%) CD10-	12%	t(4;11) (q21;q23)	70% <i>ALL1-AF4</i> (20% Flt3 in MLL+)	High WBC (>100,000/ μ L) (26%)	Mainly BM (>90%)
Common ALL	CD10+	49%	t(9;22)(q34;q11) del(6q)	33% <i>BCR-ABL</i> with 54% <i>IKZF1</i> del >25% <i>CDKN2A/B</i>	Higher age >50 years (24%)	Mainly BM (>90%) Prolonged relapse kinetics (up to 5–7 years)
Pre-B-ALL	CD10 \pm , cIgM+	11%	t(9;22)(q34;q11) t(1;19)(q23;p13)	4% t(1;19)/ <i>PBX-E2A</i>		
Mature B-ALL	CD10 \pm , sIgM+	4%	t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)		Higher age >55 years (27%) Frequent organ involvement (32%) and CNS involvement (13%)	Frequent CNS (10%) Short relapse kinetics (up to 1–1.5 years)
T-lineage ALL (T-ALL)	cyCD3 or sCD3	24%	t(10;14)(q24;q11) t(11;14)(p13;q11)	50% <i>NOTCH1B</i> 33% <i>HOX11b</i> 5% <i>HOX11L2b</i> 4% <i>NUP213-ABL1</i>	Younger age (90% <50 years) Frequent mediastinal tumors (60%) Frequent CNS involvement (8%) High WBC (>50/ μ L) (46%)	Frequent CNS (up to 10%) Extramedullary (6%) Intermediate relapse kinetics (up to 3–4 years) When relapsed, fast progression
Early Pro/ Pre T-ALL	No additional differentiation markers, mostly CD2– CD1a+, sCD3 \pm sCD3+, CD1a–	6%				
Cortical T-ALL Mature T-ALL		12% 6%				

Abbreviations: BM, bone marrow; CNS, central nervous system; WBC, white blood cells.

(e.g., dasatinib) or JAK2 inhibitors (e.g., ruxolitinib) has so far had limited success in adults.

■ MINIMAL RESIDUAL DISEASE

Minimal residual disease (MRD) is the detection of residual leukemic cells that are not recognizable by light microscopy. Methods for determining MRD are based on the detection of leukemia-specific aberrant immunophenotypes by flow cytometry, the evaluation of leukemia-specific rearranged immunoglobulin or T-cell receptor sequences by real-time quantitative polymerase chain reaction, or the detection of fusion genes associated with chromosomal abnormalities (e.g., *BCR-ABL*, *MLL-AF4*). The detection limit with these methods is 10^{-3} – 10^{-5} (0.1–0.001%). With new techniques such as next-generation sequencing (NGS) or digital droplet polymerase chain reaction (ddPCR), the sensitivity may increase to 10^{-5} – 10^{-6} . The phenotypic aberrations are unique to each patient with ALL and can be detected in up to 95% of individuals. Collection of bone marrow at diagnosis for identification of patients' individual markers is essential for follow-up of MRD.

■ MOLECULAR RESPONSE AFTER INDUCTION THERAPY AND IMPACT ON OUTCOME

Achievement of molecular complete response/molecular remission is the most relevant independent prognostic factor for disease-free survival and overall survival in pediatric and adult ALL (Table 106-3). Patients with molecular complete remission after induction therapy had significantly superior outcomes in several studies, with a disease-free survival rate of ~70% compared to <40% for MRD-positive patients. Patients with molecular failure after induction should proceed to a targeted therapy to reduce the tumor load, followed by allogeneic stem cell transplantation (SCT), if possible.

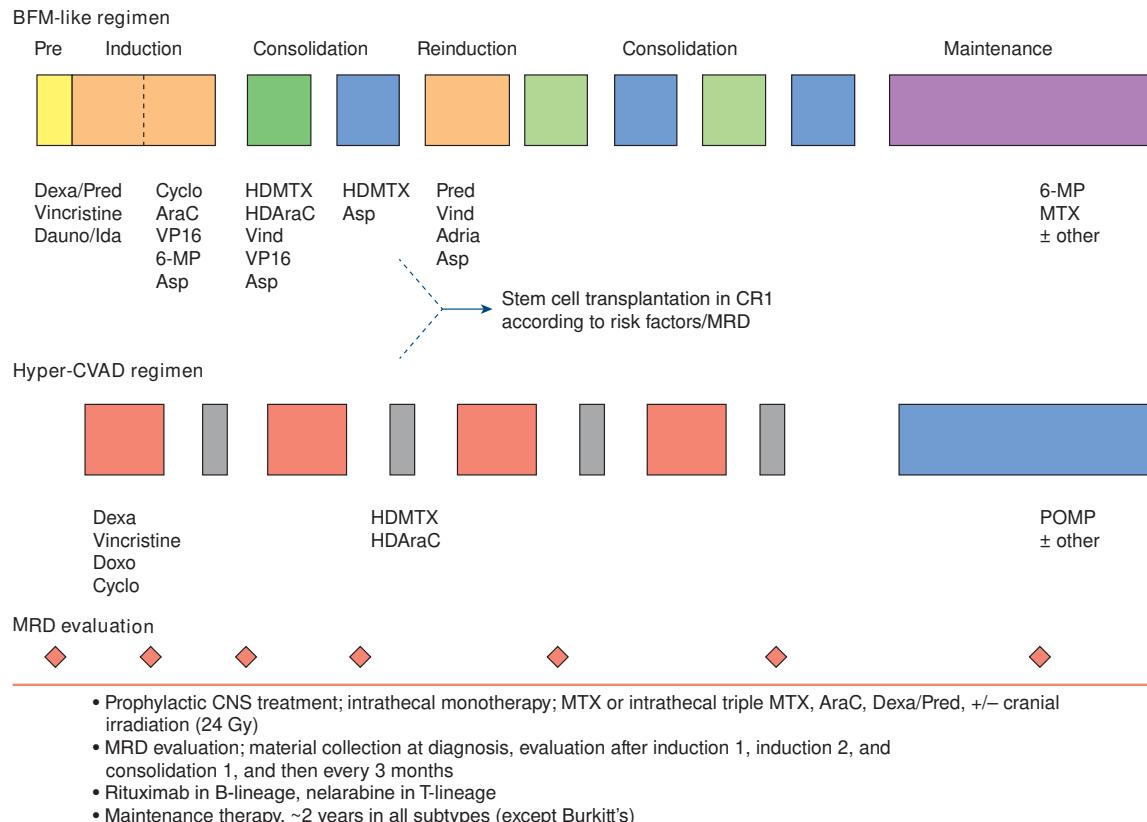
■ PROGNOSTIC FACTORS, RISK STRATIFICATION, AND MRD

The aim of identification of prognostic parameters at diagnosis, which include age, white blood cell count, immunophenotype, and cytogenetic and genetic aberrations, is to stratify patients into risk groups: standard-risk patients are patients without any risk factor, and high-risk patients are those with one or more risk factors. High-risk patients are most often candidates for SCT in first complete remission (CR). MRD is thus the most important prognostic factor during therapy (Fig. 106-1); 20–30% of adult ALL patients who are MRD negative after induction will relapse. Potential reasons include loss of sensitivity, evolution of leukemic subclones, and extramedullary origin of disease. If the MRD status of a patient is not available, risk stratification should rely on clinical and laboratory risk factors evaluated at diagnosis.

TABLE 106-3 Response Parameters According to Minimal Residual Disease (MRD)

Terminology	Definition
Complete hematologic remission (CHR)	Leukemic cells not detectable by light microscopy (<5% blast cells in bone marrow [BM])
Complete molecular remission/MRD negativity	Patient in complete remission, MRD not detectable, $\leq 0.01\% = \leq 1$ leukemia cell in 10,000 BM cells
Molecular failure/MRD positivity	Patient in complete hematologic remission, but not in molecular complete remission $>0.01\%$
Molecular relapse/MRD positivity	Patient still in complete remission, had prior molecular complete remission, leukemic blast cells in BM not detectable (<5%)
Hematologic relapse	>5% blast cells in BM/blood

Frequent chemotherapy regimens in adult ALL



- Prophylactic CNS treatment; intrathecal monotherapy; MTX or intrathecal triple MTX, AraC, Dexa/Pred, +/- cranial irradiation (24 Gy)
- MRD evaluation; material collection at diagnosis, evaluation after induction 1, induction 2, and consolidation 1, and then every 3 months
- Rituximab in B-lineage, nelarabine in T-lineage
- Maintenance therapy, ~2 years in all subtypes (except Burkitt's)

FIGURE 106-1 A schematic treatment algorithm in acute lymphoblastic leukemia (ALL). 6-MP, 6-mercaptopurine; Adria, Adriamycin (doxorubicin); AraC, cytarabine; Asp, asparaginase; BRM, Berlin-Frankfurt-Münster; CNS, central nervous system; CR1, first complete remission; Cyclo, cyclophosphamide; Dauno, daunorubicin; Dexa, dexamethasone; Doxo, doxorubicin; HD, high-dose; Hyper-CVAD, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; Ida, idarubicin; MRD, minimal residual disease; MTX, methotrexate; POMP, mercaptopurine, vincristine, methotrexate, and prednisolone; Pred, prednisolone; Vind, vindesine; VP16, etoposide.

TREATMENT PRINCIPLES

Treatment of ALL consists usually of pre-phase therapy, induction therapy, consolidation cycles, and maintenance treatment. Treatment should start immediately when the diagnosis of ALL is established.

Pre-Phase Therapy Pre-phase therapy consisting of glucocorticoids (prednisone 20–60 mg/d or dexamethasone 6–16 mg/d, both IV or PO) alone or in combination with another drug (e.g., vincristine, cyclophosphamide) is usually given for ~5–7 days. It allows safe tumor reduction to avoid tumor lysis syndrome, to initiate supportive therapy, such as substitution of platelets/erythrocytes, or to treat infections. The time required for pre-phase therapy will also allow time to obtain results of the diagnostic workup (e.g., cytogenetics, molecular genetics).

Induction Therapy The goal of induction therapy is the achievement of a CR or, even better, a molecular CR. With current regimens, the CR rate has increased to 80–90% and is higher for standard-risk patients (>90%) and lower for high-risk patients (~60%).

Induction regimens are centered around vincristine, glucocorticoids, and anthracyclines with or without cyclophosphamide or cytarabine. L-Asparaginase is the only ALL-specific drug and is now more intensively used in adults. Pegylated asparaginase has the advantage of a significantly longer period of asparagine depletion. Dexamethasone is often preferred to prednisone because it penetrates the blood-brain barrier and also acts on resting leukemic blast cells.

Two chemotherapy regimens are widespread (Fig. 106-1). One is patterned after the pediatric BFM (Berlin-Frankfurt-Münster) protocol, which is mostly used in European adult ALL trials. Another

approach is to repeat two different alternating intensive chemotherapy cycles, identical for induction and consolidation, for eight cycles, such as Hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) protocol, which is preferentially used in the United States but also in many other parts of the world.

Postremission Consolidation Usual protocols use six to eight courses and often contain systemic high-dose (HD) therapy to reach sufficient drug levels in sanctuary sites such as the CNS. Most often HD methotrexate ($1-1.5 \text{ g/m}^2$ and up to $3-5 \text{ g/m}^2$) and/or HD cytarabine (4–12 doses at $1-3 \text{ g/m}^2$) are administered.

Maintenance Therapy Maintenance therapy, a strategy transferred from childhood ALL, is mandatory. It consists of 6-mercaptopurine and methotrexate plus intrathecal therapy. The potential effect of further intensification cycles during maintenance remains unclear. The duration of maintenance therapy for T-ALL and B-ALL is 2–2.5 years, except for Burkitt's leukemia, for which it is not required. In Ph+ ALL, patients also require maintenance therapy that should include a tyrosine kinase inhibitor (TKI), most likely the TKI that has been used during induction and consolidation therapy. It is also standard to give a TKI after allogeneic SCT. The duration of maintenance therapy with a TKI is also 2–2.5 years and should be guided by MRD evaluation. TKI use is often interrupted or switched to another TKI if toxicity occurs.

TREATMENT OF ALL PATIENTS ACCORDING TO AGE

The outcome of ALL is strictly related to the age of a patient, with cure rates of ~90% in children, decreasing to <10% in elderly or frail

TABLE 106-4 Best Results in Recent Studies for Adult Acute Lymphoblastic Leukemia (ALL)

SUBTYPE	TREATMENT	OVERALL SURVIVAL
Burkitt's leukemia	Short intensive chemotherapy + rituximab; no SCT; no maintenance	80–90%
B-lineage ALL, Ph-		
AYA 15–35/45 years	Pediatric inspired, few/no SCT	≥70–80%
Adults 45–55 years	Intensive chemotherapy +/- SCT	50–60%
Elderly 55–70 years	Less intensive chemotherapy + immunotherapy	~30%
Frail >70/75 years	Various	≤10%
B-lineage ALL, Ph+		
Ph BCR-ABL	Intensive chemotherapy + TKI +/- SCT	60–70%
Ph-like ALL	Chemotherapy + dasatinib/JAK inhibitors	≤50%
T-lineage ALL		
Early (ETP)	Intensive chemotherapy + nelarabine + SCT	40–50%
Cortical/thymic	Intensive chemotherapy + nelarabine, no SCT	70%
Mature	Intensive chemotherapy + nelarabine + SCT	30–50%

Abbreviations: AYA, adolescent and young adult; ETP, early T precursor; Ph, Philadelphia chromosome; SCT, stem cell transplantation; TKI, tyrosine kinase inhibitor.

patients. Thus, age-adapted protocols have emerged, where the age limits are directed by the hematologic and nonhematologic toxicities. Table 106-4 provides a summary of the best results obtained in adult ALL according to ALL subtype, age, and treatment. Molecular CRs are often durable. The major risk of relapse is in the first 2 years; thereafter, relapse is much less likely.

PROPHYLAXIS AND TREATMENT OF CENTRAL NERVOUS SYSTEM LEUKEMIA

Prophylactic CNS therapy in ALL is essential in order to prevent CNS leukemia and to avoid spread of leukemic cells from the CNS back to the periphery. Treatment options include intrathecal therapy, systemic HD chemotherapy, and cranial radiation therapy (CRT). Intrathecal therapy mostly consists of methotrexate as a single drug or in combination with cytosine arabinoside (AC) with or without glucocorticoids. The route of intrathecal therapy application is generally lumbar puncture. Systemic HD chemotherapy may comprise HDAC or HD methotrexate since both drugs reach cytotoxic drug levels in the CSF and show effectiveness in overt CNS leukemia. CRT (18–24 Gy in 12 fractions over 16 days) is also effective as preventive treatment of CNS leukemia. Using combined modalities for CNS prophylaxis, the CNS relapse rate has decreased to 2–5%.

Particular attention to CNS prophylaxis is required for targeted therapies. In Ph+ ALL, not all TKIs cross the blood-brain barrier equally. Dasatinib and probably ponatinib do cross the blood-brain barrier, whereas imatinib and nilotinib do not. In addition to immunotherapy, intrathecal therapy is required because most antibodies do not enter the CNS.

CNS involvement at diagnosis is observed in 5–10% of adult patients and is higher in mature B-ALL (up to 10–15%) and T-ALL (up to 10%). Treatment consists of the standard chemotherapy with additional intrathecal applications 3–5 times per week until blast cells are cleared in the spinal fluid. Patients with initial CNS involvement have a similar overall survival as CNS-negative patients.

Relapse in CNS is usually accompanied by bone marrow involvement, and if blast cells are not seen morphologically, MRD as a sign of discrete infiltration is positive in nearly all cases. CNS relapse requires local as well as systemic therapy. The outcome after CNS relapse is

dismal, and salvage chemotherapy followed by allogeneic SCT is the most effective option. Chimeric antigen receptor (CAR) T cells (most often targeting CD19) can cross the blood-brain barrier and achieve CRs in patients with CNS relapse.

STEM CELL TRANSPLANTATION

SCT is an essential part of the treatment strategy for adult ALL. Peripheral blood cells are increasingly being used as a stem cell source, instead of bone marrow. In addition, a shift from sibling stem cell donors to matched unrelated donors or haploidentical transplants from relatives has occurred. Indications for SCT in first CR are controversial. However, in most studies, SCT is recommended for high-risk patients defined either by conventional prognostic factors or by MRD positivity. High-risk patients transplanted in first CR have a survival rate of 50% or greater; decreasing transplant-related mortality from 20–30% to 10–15% has contributed substantially to better outcomes. For standard-risk patients with sustained molecular remission, allogeneic SCT in first CR is not recommended. Autologous SCT should be restricted to MRD-negative patients, BCR-ABL-negative patients, Ph+ patients, and older patients because it is less toxic but associated with a substantially higher relapse rate. For all relapsed adult ALL patients, an allogeneic SCT is thus far the only curative option.

PEDIATRIC INSPIRED THERAPIES FOR ADOLESCENTS AND YOUNG ADULTS

The principle of pediatric-inspired therapies is to have higher doses and more applications of ALL-specific drugs such as glucocorticoids, vincristine, and L-asparaginase and fewer myeloablative anthracyclines or alkylating agents, with strict adherence to time-dose intensity, thereby reducing the role of SCT. The overall survival rates for AYAs are 70–80%.

ADULT ALL

The treatment results for adult ALL patients have greatly improved with more intensive chemotherapy, optimized SCT, and better supportive care. In several recent multicenter prospective trials, the overall survival rate for standard-risk patients was >70% with chemotherapy alone, and for high-risk patients, the overall survival rate has increased from 20–30% to >50%.

ELDERLY ALL

Palliative treatment regimens for elderly patients have failed, with CR rates of ~40%, a high early death rate of 24%, and a poor overall survival of only a few months. Intensive chemotherapy has also failed, with a higher CR rate of 56%, but still an early death rate of 23%, and only moderate improvement of overall survival to 14 months. Specific elderly ALL protocols with less intensive therapy based on glucocorticoids, vincristine, and asparaginase, largely avoiding anthracyclines and alkylating agents, have improved outcomes. The early treatment-related death rate decreased to <10%, CR rates improved to ~90%, and overall survival of ~30 months was noted.

Frail patients above the age of 70–75 years have very poor survival of <10%. Hopefully, this will improve with ongoing targeted therapies with either TKIs in Ph+ ALL or immunotherapies.

TARGETED THERAPIES

Substantial progress in adult ALL has been made in the past decade by the introduction of new targeted therapies, including TKIs and immunotherapeutic approaches (Table 106-5).

TYROSINE KINASE INHIBITORS IN PHILADELPHIA POSITIVE ALL

Patients with Ph+ ALL constitute ~25% of adult B-ALL patients, with the frequency increasing to ~50% among elderly patients. In the imatinib era, CR rates were 60–70%; survival with chemotherapy was ~10%, and after allogeneic SCT, it was ~30%. With the first-generation TKI imatinib, CR rates increased to 80–90%, the rate of BCR-ABL negativity increased from 5 to 50%, and the 5- to 10-year overall survival improved to 50–70%.

Faster and deeper molecular responses are achieved with second-generation TKIs (dasatinib, nilotinib), and these responses apparently

TABLE 106-5 Targeted Therapies in Adult Acute Lymphoblastic Leukemia (ALL)**Tyrosine Kinase Inhibitors (TKIs)****Ph/BCR-ABL+ ALL****TKIs**

Imatinib, dasatinib, nilotinib, bosutinib, ponatinib

Ph/BCR-ABL-like ALL

ABL1, ABL2: dasatinib; JAK2: ruxolitinib

Immunologic Approaches**Antibodies directed leukemia surface antigens****Monovalent antibodies**

Bivalent antibodies against the tumor and CD3 (e.g., blinatumomab)

Adoptive cellular therapy

T cells engineered to kill leukemic cells

Checkpoint Inhibitors

translate into a survival benefit. The third-generation TKI ponatinib is also effective in tumors bearing mutations (particularly T315I) that convey resistance to earlier-generation TKIs.

Treating adult Ph+ ALL with an allogeneic SCT in first CR is still a good treatment option for adult patients, with a 5-year overall survival of 60–70%. In elderly patients, when low-intensity chemotherapy was combined with dasatinib, the CR rate was >90%. In a next step, by combining mini-chemotherapy with a TKI and adding immunotherapy with inotuzumab (an anti-CD22 antibody), the CR rate was >90% and the overall survival improved further. A pilot experience with a chemotherapy-free regimen composed of dexamethasone, the TKI dasatinib, and the bispecific antibody blinatumomab (anti-CD19 and anti-CD3) demonstrated a CR rate of 98% and 2-year overall and disease-free survival rates of 95% and 88%, respectively. Blinatumomab eliminates Ph+ leukemic cells with resistant mutations.

■ IMMUNOTHERAPEUTIC APPROACHES

Treatments involving monoclonal antibodies or activated T cells are currently changing the treatment paradigm of ALL. The prerequisite is that B-lineage blast cells express a variety of specific antigens, such as CD19, CD20, and CD22 (**Table 106-6**) that are targetable with a wide variety of monoclonal antibodies. A new treatment principle is the activation of the patient's T cells to destroy their CD19+ leukemic blasts.

Anti-CD20 The anti-CD20 monoclonal antibody rituximab has improved the outcome of patients with de novo Burkitt's leukemia/

lymphoma. With repeated short cycles of intensive chemotherapy combined with rituximab, the overall survival increased to >80%. Rituximab is now included in most B-ALL regimens and is given at the usual dose of 375 mg/m² on day –1 before chemotherapy for at least eight or more cycles. This leads to a significant increase in MRD negativity and improved survival.

Anti-CD22 Monoclonal antibodies directed against CD22 are linked to cytotoxic agents, such as calicheamicin (inotuzumab ozogamicin), or to plant or bacterial toxins (epratuzumab). In a randomized trial of relapsed or refractory ALL patients, the CR rate was 66% and significantly superior to the CR rate with standard chemotherapy. Inotuzumab is now included in first-line therapy for Ph+ and Ph- patients.

Anti-CD19 Targeting CD19 is of great interest because this antigen is highly expressed in all B-lineage cells, most likely including early lymphoid precursor cells. A new promising approach is the bispecific antibody blinatumomab, which combines single-chain antibodies to CD19 and CD3, such that T cells lyse the CD19-bearing B cells.

Blinatumomab is particularly effective in MRD-positive patients, with a 70–80% conversion to MRD negativity, translating into improved overall survival; ~25% of MRD-negative patients survived without any further treatment. Blinatumomab has also moved to frontline therapy.

CAR-T Cells The adoptive transfer of CAR-modified T cells directed against CD19 is a promising approach for the treatment of CD19+ childhood or adult ALL. In the first three larger studies in adults with relapsed or refractory ALL, the CR rate ranged from 67 to 91% with MRD negativity in 60–81% of the patients who achieved CR. Overall survival is 50% or more at ≥2 years, which is remarkable for these heavily pretreated patients. CAR-T cells are also effective in CNS leukemia and in other extramedullary sites. CAR-T cell therapy in relapsed or refractory ALL was first considered as a bridge to allogeneic SCT, applied in 10–50% of patients, but the necessity for an allogeneic SCT after CAR-T cells is unclear. CAR-T cell therapies are also moving to the frontline. CD19-negative relapses after CAR-T cell therapy or blinatumomab due to downregulation of CD19 expression are a relevant obstacle.

Toxicities of Immunotherapies The anti-CD22 agent inotuzumab ozogamicin is associated with hepatotoxicity, including veno-occlusive disease, particularly after allogeneic SCT, but can be managed by reduced dosing and limitation of cycles. For anti-CD19 therapies, cytokine release syndrome and severe neurotoxicity are the most prominent toxicities and often require intensive care unit care (more so after CAR-T cells than blinatumomab). Management of these complications has improved with early recognition. Because toxic death after immunotherapies is very low compared to intensive chemotherapy or allogeneic SCT, immunotherapies are now increasingly included in frontline therapy.

■ TREATMENT OF T ALL

Immunotherapy for T-ALL is still not available and intensive chemotherapy is still the mainstay in combination with the T cell-specific drug nelarabine. Currently, γ-secretase targeting NOTCH1, checkpoint inhibitors such as bortezomib and venetoclax, and HDAC inhibitors are being explored.

■ CONCLUSION AND FUTURE DIRECTIONS

Cytogenetic and molecular analysis at diagnosis allows identification of ALL subentities, requiring different treatment options. Evaluation of MRD is the most important parameter for treatment decisions. The greatest progress has been achieved by targeted therapies, such as TKIs for Ph+ ALL and new immunotherapeutic approaches. This will lead to further improved outcome of adult ALL patients, 50% of whom are already surviving 5–10 years and are most likely cured. New options and advances, such as low-intensity chemotherapy, reduction of SCT, incorporation of targeted therapies, and reduction of toxicities, will improve the quality of life of patients and lead to individualized approaches for each patient.

TABLE 106-6 Expression of Antigens in B-Cell Lineage Acute Lymphoblastic Leukemia (ALL) for Potential Antibody Therapy

SURFACE ANTIGEN	ALL SUBTYPES	EXPRESSION ON LBC ^a	MONOCLONAL ANTIBODY
CD20	Burkitt's lymphoma/leukemia B-precursor	86–100%	Rituximab
		30–40%	Ofatumumab
CD22	B-precursor	93–98%	Inotuzumab
	Mature B-ALL	~100%	Epratuzumab Moxetumomab pasudotox
CD19	B-precursor	95–<100%	T cell-activating therapies
	Mature B-ALL	94–<100%	Blinatumomab Bispecific CD3/CD19 Chimeric antigen receptor modified T cells (CAR T cells)

^aDefined as ≥20% positive blast cells.

Abbreviation: LBC, leukemic blast count.

Source: Republished with permission of American Society of Hematology, from D Hoelzer: Novel antibody-based therapies for acute lymphoblastic leukemia. 2011: 243, 2011; permission conveyed through Copyright Clearance Center, Inc.

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Chronic Lymphocytic Leukemia

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Chronic lymphocytic leukemia (CLL) is a monoclonal proliferation of mature B lymphocytes defined by an absolute number of malignant cells in the blood ($5 \times 10^9/\text{mL}$). The presence of malignant B cells under this count in the blood without nodal, spleen, or liver involvement and absent cytopenias is a precursor of this disease called *monoclonal B cell lymphocytosis* (MBL) with ~1–2% chance per year of progressing to overt CLL. CLL is a heterogeneous disease in terms of natural history, with some patients presenting asymptotically and never requiring therapy, whereas others present with symptomatic disease, require multiple lines of therapy, and eventually die of their disease. Over the past 10–15 years, the understanding of CLL origin and biology has grown exponentially, leading first to more refined disease definition, prognostic markers, and, subsequently, introduction of novel therapies that have significantly changed the natural history of this disease. In this chapter, we review the epidemiology, biology, and management of CLL, with a focus on new knowledge that is currently changing standards of care.

EPIDEMIOLOGY

CLL is primarily a disease of older adults, with a median age at diagnosis of 71 and an age-adjusted incidence of 4.5/100,000 people in the United States. The prevalence of CLL has increased over the past decades due to improvements in therapy for this disease and also survival of older patients from other medical ailments. In 1980, the 5-year overall survival of patients was 69%, and this increased to 87.9% in 2007 and is likely even higher today. The male-to-female ratio is 2:1; however, as patients age, the ratio becomes more even, and over the age of 80, the incidence is equal between men and women. The disease is most common in Caucasians, less common in Hispanic and African Americans, and rare in the Asian population.

Unlike many other malignancies, there have been no definitive links between CLL and exposures. Indeed, CLL is one of the only types of leukemia not linked to radiation exposure. Agent Orange exposure has been implicated, and CLL is thus a service-connected condition for those who were exposed to Agent Orange in the Vietnam conflict.

CLL is one of the most familial-associated malignancies, and the first-degree relative of a CLL patient has an 8.5-fold elevated risk of developing CLL than the general population. MBL is also more common in families with two first-degree relatives having CLL, further supporting a genetic predisposition of this disease. Despite this, specific genes conferring risk in the familial setting outside of specific families have been difficult to identify. In genome-wide association studies (GWAS), ~30 single nucleotide polymorphisms have been identified, which is estimated to account for 19% of the familial risk of CLL. Genes involved in apoptosis, telomere function, B-cell receptor (BCR) activation, and B-cell differentiation have all been implicated in GWAS. Variants in shelterin complex proteins involved in telomere maintenance such as POT1 have been identified in a small number of families.

BIOLOGY AND PATHOPHYSIOLOGY

CELL OF ORIGIN

The cell of origin in CLL has not definitively been established. The morphology, immunophenotype, and gene expression pattern of CLL cells are that of a mature B cell (Fig. 107-1), and so it has been presumed that the initiating cell is a mature lymphocyte, perhaps memory B cells. However, many facets of CLL biology do not support this idea, including antigen-binding characteristics of CLL cells and the presence of stereotyped BCRs. Other possibilities include a stepwise process including a series of transforming events at various stages of B-cell development, potentially including de-differentiation of more mature cells. The self-renewing, multipotent hematopoietic stem cell (HSC) might also be the originating cell of CLL, postulated based on transplant studies in mice showing clonal leukemic cell development with different characteristics from donor leukemia after transplantation of HSCs. More work will be required to elucidate the origins of CLL.

B CELL RECEPTOR SIGNALING IN CLL

Perhaps the most important advancement in CLL biology is the understanding of the role of BCR signaling in the disease. CLL has distinct BCR signaling as compared to normal B cells, which is characterized by low-level IgM expression, variable response to antigen stimulation, and tonic activation of antiapoptotic signaling pathways that promote tumor survival. CLL cells by gene expression profiling share many features with antigen-activated mature B cells, suggesting a role for activation of BCR signaling in the disease pathogenesis. Tissue-based microarrays have revealed upregulation of BCR pathway genes in the lymph nodes and bone marrow compared to the peripheral blood, suggesting a particular importance of this pathway in microenvironmental homing.

Fitting with the role of BCR signaling in CLL, one of the most influential prognostic factors identified in this disease is the mutational status of the immunoglobulin heavy chain variable (IGHV) region. During normal B-cell maturation, the variable regions of the



FIGURE 107-1 Chronic lymphoid leukemia in the peripheral blood. (From M Lichtman et al [eds]: *Williams Hematology*, 7th ed. New York, McGraw-Hill, 2005.)

immunoglobulin heavy chain undergo somatic hypermutation. In CLL, ~60% of patients have IGHV that is ≥2% mutated from germline. This may indicate a more mature, postgerminal center progenitor, and is typically associated with a more indolent disease course. Conversely, ~40% of patients will have IGHV <2% mutated from germline, which is associated with more rapid progression of disease and short survival prior to the era of therapeutics that target BCR. Unfavorable biologic properties including enhanced telomerase activity, overexpression of activation-induced cytidine deaminase, increased nuclear factor- κ B (NF- κ B) activity, high-risk genomic mutations (e.g., *NOTCH1*, *SF3B1*, *TP53*, *ATM*), and clonal evolution are also associated with IGHV unmutated disease.

Because IGHV sequencing was initially cumbersome to perform, a number of surrogate factors have been identified; however, none yet have been shown to be equal or superior to IGHV sequencing. The most prevalent of these surrogate markers are Zap-70 expression, ZAP-70 methylation, and surface CD38 expression. Zap-70 protein is a normal intracellular T-cell signaling protein that is aberrantly expressed in most IGHV unmutated CLL cells. CD38 is a marker that is also more highly expressed on the surface of IGHV unmutated CLL cells. Both of these prognostic factors are widely used but limited in their applicability. Zap-70 protein status is difficult to measure by flow cytometry and has low reproducibility. Measurement of methylation status of the ZAP-70 promoter is much more precise but not widely available. CD38 expression is easier to measure by flow cytometry but not as highly predictive of outcomes and can change during the course of disease.

CYTogenetic Abnormalities

Besides IGHV mutational status, recurrent cytogenetic abnormalities are the most robust prognostic factor clinically available in CLL. These abnormalities are typically identified by fluorescent in situ hybridization (FISH) analysis; however, stimulated metaphase karyotype has a role as well. The most well-characterized abnormalities include del(13)(q14.3), trisomy 12, del(11)(q22.3), and del(17)(p13.1) (Fig. 107-2). The presence of sole del(13)(q14.3) is associated with more indolent disease, prolonged survival, and good response to traditional therapies. Usually, this abnormality is not seen on banded karyotype analysis, and when present on karyotype, it indicates a larger deletion involving the retinoblastoma gene, which negates the favorable prognosis associated with this marker. Trisomy 12 has a more intermediate prognosis. The del(11)(q23.3) results in deletion of the *ATM* gene and is associated with bulky lymphadenopathy and aggressive disease in young patients, with inferior prognosis, and more rapid progression to symptomatic disease. The del(17)(p13.1) results in loss of one allele of the tumor suppressor *TP53* and is associated with the poorest prognosis in CLL with rapid disease progression, poor response to traditional therapies, and shorter survival. Other abnormalities have been shown to be important in smaller studies but are not routinely performed at all centers. Finally, complex karyotype (three or more abnormalities) on stimulated metaphase karyotype analysis has significant adverse impact on time to treatment and overall survival, with

data indicating that increasing complexity is even more deleterious to response and survival.

Clonal evolution, or acquisition of cytogenetic or molecular abnormalities, is common in CLL, especially in patients with IGHV unmutated CLL. Because the tumor cytogenetics can change over time, it is recommended that FISH, with or without cytogenetics, is checked before every line of therapy, mostly to evaluate acquisition of del(17) (p13.1).

Gene Mutations and Mir Alterations

Compared with many other malignancies, the genome in CLL is relatively simple, with an average CLL genome carrying ~20 nonsynonymous alterations and ~5 structural abnormalities. And, unlike many other hematologic malignancies, there is no unifying genetic lesion, and most recurrent genetic driving mutations exist at frequencies of <5%. Whole genome and whole exome sequencing have identified the most common mutations in CLL to be in *SF3B1*, *NOTCH1*, *MYD88*, *ATM*, and *TP53* (Table 107-1). Most of the identified mutations in these genes are common among different malignancies, and with the exception of *MYD88*, they are generally identified with much higher frequency in IGHV unmutated disease.

NOTCH1 mutations are present in ~15% of CLL patients and are commonly associated with trisomy 12. Although multiple different mutations are seen, most are located within the PEST (proline, glutamic acid, serine, and threonine) domain and result in constitutive NOTCH signaling. *NOTCH1* mutations have been associated with lower sensitivity to CD20 antibody therapy and increased risk of transformation to aggressive diffuse large B-cell lymphoma (DLBCL; Richter's transformation), although its relevance in the era of targeted therapies is less clear. *SF3B1* is a component of the RNA spliceosome and is mutated in 10–15% of CLL cases. Mutations appear to be associated with

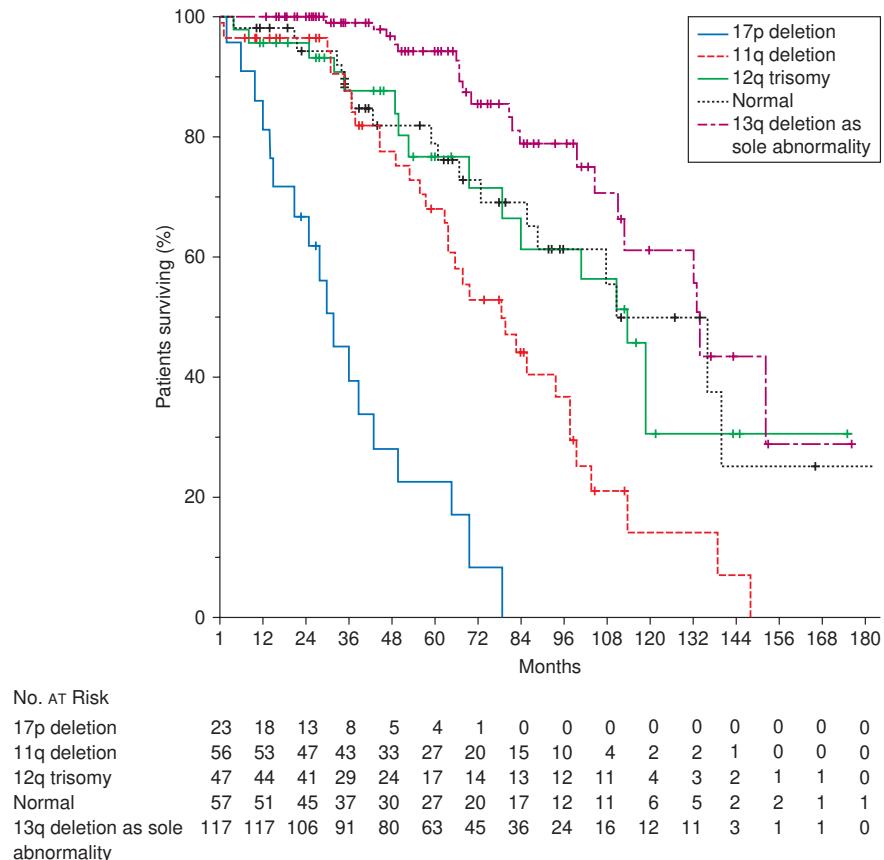


FIGURE 107-2 Outcomes among chronic lymphocytic leukemia patients with various cytogenetic abnormalities. (From H Döhner et al: Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med* 343:1910, 2000. Copyright © (2000) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.)

TABLE 107-1 Recurrent Mutations in CLL

GENE	FREQUENCY OF MUTATIONS (%)
SF3B1	8–14
TP53	5–13
NOTCH1	10–13
MYD88	4–8
ATM	8–11
BIRC3	≤5
XPO1	≤5
FBXW7	≤5
POT1	≤5
BRAF	≤5
EGR2	≤5
IKZF3	≤5

Abbreviation: CLL, chronic lymphocytic leukemia.

intermediate-risk disease, and, functionally, *SF3B1* may be important in the response to DNA damage.

Mutations of the tumor suppressor *TP53* are found in ~5% of CLL patients with previously untreated early-stage disease and up to 40% with later stages. Seventy percent of the time, these mutations coexist with del(17)(p13.1), effectively eliminating *TP53* function. As expected, and consistent with other malignancies, *TP53* mutations are associated with a poor prognosis and expected lack of response to DNA-damaging therapies.

ATM mutations, which are heterogeneous and occur throughout the gene, occur in 10–15% of CLL patients. *ATM* mutations often coexist with del(11)(q22.3), eliminating *ATM* on the alternate allele. Similar to *TP53*, mutations in *ATM* tend to result in impaired response to DNA damage, which can reduce responsiveness to chemotherapy.

In contrast to the aforementioned mutations, those in *MYD88* tend to occur in IGHV mutated CLL and be associated with a more indolent prognosis. This gene is involved in Toll-like receptor signaling, and the most common mutation, L265P, results in constitutive activation and NF-κB activity.

Along with abnormalities in coding genes, it has become apparent that noncoding genes such as microRNAs are recurrently altered in CLL. The most common cytogenetic abnormality, del(13)(q14.3), results in loss of the miR15/16 cluster, which is important in the pathogenesis of CLL. In normal cells, miR15A/miR16A inhibits antiapoptotic gene expression (including *BCL2*, *CCND1*, *CCND3*, and *CDK6*), and this specific deletion allows for overexpression of these genes and thus increased cell survival. Loss of other miR expression such as miR-181a leads to overexpression of antiapoptotic proteins such as MCL-1 and TCL1. Overexpression of miR-155, an onco-miR associated with B cell transformation, has also been documented in the majority of CLL patients.

IMMUNOLOGY

CLL is characterized by dysregulation of the normal immune system in addition to the malignant immune cells. Besides numerical abnormalities due to bone marrow dysfunction, even in the early stages of disease, there are skewed ratios of immune cells and functional abnormalities. Innate immune system defects associated with CLL include

reduced complement proteins and activity, qualitative neutrophil defects, and functional defects of natural killer cells.

More focus has been placed on the impairments in the adaptive immune system in this disease. Within the CD4+ T-cell compartment, a qualitative defect is noted similar to chronic antigen stimulation inducing a phenotype of T-cell exhaustion typical of what is seen in chronic viral infections such as hepatitis. This has been demonstrated to lead to impaired T-cell cytotoxic capacity and reduced proliferative ability. Additionally, there are physical changes in the T-cell cytoskeleton that cause impaired immune synapse formation with antigen presenting cells. In addition to a lack of capacity to respond to pathogens, the T-cell defect in CLL also likely leads to tumor cell tolerance. During the course of the disease, the polarization of the CD4+ T cells shifts from a Th1 (cytotoxic) phenotype to a Th2 phenotype, which leads to expansion of immunosuppressive cytokines such as interleukin 10 (IL-10). Additionally, in the later stage of disease, T regulatory cells are expanded, which contributes to an immunosuppressive phenotype.

Other components of the immune microenvironment are altered as well to form a more supportive environment for the malignant cells. M2 monocytes have been shown to differentiate into a type of tumor-associated macrophage known as a *nurse-like cell* in CLL. These cells promote survival by secreting chemokines and cytokines that increase migration and activation.

The humoral immune system in CLL is also dysregulated, as is expected for a malignancy that results in very few normal B cells. Hypogammaglobulinemia is very common and affects all subclasses of immunoglobulins, occurring in ~85% of patients at some time in their disease course, and is more common as disease progresses. A correlation between low IgG and IgA and infection risk has been established, but isolated IgM reduction does not seem to be associated with excess infection risk. Also, CLL cells can secrete monoclonal IgM or IgG in a small number of cases, and this can correlate with disease progression.

CLINICAL PRESENTATION AND DIAGNOSIS OF CLL

CLINICAL PRESENTATION AND DIAGNOSIS

The presentation of CLL most commonly occurs as an incidental diagnosis made at the time of medical evaluation for another cause. In this regard, CLL is most commonly diagnosed on routine blood work demonstrating an elevated lymphocyte count in asymptomatic individuals, although some patients present with symptoms and require early therapy. When noting either an elevated total white blood cell (WBC) count with lymphocytic predominance or a normal WBC with a differential showing a lymphocytosis, the next step is to perform flow cytometry on the peripheral blood. In CLL, this will reveal the typical immunophenotype that includes the typical B cell markers CD19, CD20, CD22, and CD23; the T-cell marker CD5 (CD5 is also expressed on the B1 subset of B cells that typically has unmutated immunoglobulin and responds to antigens independent of cognate T cell help); and dim surface immunoglobulin of either kappa or lambda type (Table 107-2). Atypical phenotypes can be seen as well and usually can be differentiated on the basis of morphology, cytogenetics, or clinical presentation. In cases in which the clonal B cell count based on flow cytometry is $\geq 5 \times 10^9/L$, no further workup is needed to confirm the diagnosis of CLL.

Some patients will present with a small clonal proliferation of CLL cells in the peripheral blood but will also have lymphadenopathy or

TABLE 107-2 Typical Immunophenotype of CLL Compared with Other B Cell Malignancies

DISEASE	CD5	CD10	CD19	CD20	CD23	CYCLIN D1	SURFACE IG
CLL	+	-	+	+ (dim)	+	-	+ (dim)
Mantle cell lymphoma	+	-	+	+ (mod/bright)	-	+	+ (mod/bright)
Marginal zone lymphoma	-/+	-	+	+ (mod/bright)	-/+	-	+ (mod/bright)
Follicular lymphoma	-	+	+	+	+	-	

Abbreviation: CLL, chronic lymphocytic leukemia.

splenomegaly. In these cases, the likely diagnosis is small lymphocytic lymphoma (SLL), a semantic designation from CLL that denotes a primarily tissue-based disease rather than bone marrow/blood-based disease. The genetic and molecular features of SLL are identical to those of CLL. The retention of the cells in tissues may be related to the expression of a particular adhesion molecule. Thus, SLL patients are managed identically to CLL patients, and often in the later stages of disease, these patients will have blood and bone marrow involvement as well.

MONOCLONAL B CELL LYMPHOCYTOSIS

Patients who do not meet the diagnostic criteria for CLL based on quantification of clonal B cells in the peripheral blood and who do not have associated signs of CLL including lymphadenopathy, organomegaly, or cytopenias have a disorder known as monoclonal B-cell lymphocytosis (MBL), which is now thought to precede every case of CLL. Analogous to monoclonal gammopathy of uncertain significance (MGUS) in myeloma, not all MBL progresses to CLL. MBL is initially characterized by a CLL-like immunophenotype in ~75% of cases but can also be atypical (CD23 negative or bright CD20) or CD5 negative. More relevant for prognosis is characterization by count, with low-count MBL defining those patients with $<0.5 \times 10^9$ clonal B cells/L, and high-count MBL defining those with $>0.5 \times 10^9$ but $<5 \times 10^9$ /L. Patients with low-count MBL have a negligible rate of progression to CLL, whereas those with high count progress to overt CLL at a rate of 1–2% per year, warranting continued monitoring. Population-based studies have estimated the prevalence of MBL to be up to ~12% in the general population, where it is most common in elderly men. It is especially common in first-degree relatives of CLL patients, where the frequency is ~18%.

Although the risk of MBL progression is relatively low, it has become apparent that patients still experience complications that suggest an immune dysfunction in MBL that is similar to that seen with CLL. Rates of serious infections requiring hospitalization appear to be significantly increased in MBL, similar to the rates seen in CLL. In a case-control study, patients with MBL had a 16% chance of hospitalization over a 4-year time period, compared with 18.4% in patients with newly diagnosed CLL. Secondary cancers also appear to be increased in MBL. These data suggest that monitoring for patients with MBL should focus on vaccinations and age-appropriate cancer screening, as the probability of complications appears to be higher than the risk of progression in most of these patients. Follow-up for patients with MBL can occur with the primary care physician as this does not represent a malignancy, whereas CLL is mostly comanaged with both a primary care physician and a hematologist.

COMPLICATIONS OF CLL

A significant amount of morbidity and mortality related to CLL is due to complications of the disease. In general, complications besides disease progression include infections, secondary cancers, autoimmune complications, and transformation to a more aggressive clonally related lymphoma.

INFECTIONS

Infections are a leading cause of both disease-related morbidity and death in patients with CLL, with ~30–50% of deaths in CLL patients attributed to infection. Owing to the immune dysfunction associated with the disease, patients are at risk for both typical and atypical infections. Besides this baseline risk of infections, most CLL therapies can increase infection risk. For many nucleoside analogue-based chemotherapy regimens used in CLL, prophylaxis for *Pneumocystis pneumonia* is indicated for at least 6 months following therapy to allow recovery of functional T cells. Viral prophylaxis is also indicated for many chemotherapy regimens and for patients with a history of varicella-zoster to diminish reactivation and morbidity from this virus.

Because of the abnormalities in cellular and humoral immunity, vaccine responses in CLL are limited in many patients, especially in the later stages of disease. In one study, one dose of 13-valent pneumococcal vaccine produced an adequate immune response in only 58%

of patients compared with 100% in age-matched controls. Despite the known limitations, vaccination against influenza and pneumococcal pneumonia is recommended in CLL. The recombinant zoster vaccine has approximately a 60% response in previously untreated CLL, is safe, and should be considered for this patient group. In contrast, live vaccines should be avoided in the setting of CLL because of the small risk of viral reactivation with an immunocompromised host.

As discussed earlier, hypogammaglobulinemia is common in CLL and can be associated with significant risk for infections, primarily of mucocutaneous etiology such as sinusitis and bronchitis. In addition, women can have frequent urinary tract infections. While administration of prophylactic intravenous immunoglobulin (IVIG) has not been shown to improve survival, it has been shown to reduce the number of minor or moderate bacterial infections and thus is indicated in patients with hypogammaglobulinemia who suffer from recurrent infections or have pulmonary bronchiectasis. We also administer at least one dose of immunoglobulin to CLL patients who develop influenza with coexisting hypogammaglobulinemia to diminish risk of postinfluenza pneumococcal pneumonia. IVIG is also indicated in patients who have been hospitalized for a serious infection and in those whose IgG level is <300 –500 mg/dL.

SECONDARY MALIGNANCIES

Multiple population-based studies have shown that patients with CLL are at an elevated risk to develop other cancers, with a rate up to three times that of the general population, even in the absence of cytotoxic chemotherapy. The most common types of cancers seen in CLL are skin, prostate, and breast cancers, although other cancers are seen as well. Skin cancers are particularly common, with a rate that is 8- to 15-fold higher than in the general population, and may behave more aggressively. All CLL patients should be counseled on the use of sunscreen while outdoors and should undergo preventative skin examinations.

In one single-center study, older age at CLL diagnosis, male sex, high β_2 -microglobulin, high lactate dehydrogenase (LDH), and chronic kidney disease were associated with excess risk of other cancers; other CLL-specific risk factors have not shown association with other cancer risk.

While cancer risk is higher, no specific recommendations for increased cancer screening in CLL patients have been validated. Age- and sex-appropriate screenings should be recommended.

Conflicting data exist regarding the risk of cancers following CLL-specific therapy. Chemoimmunotherapy, in particular alkylator-containing regimens, seems to be associated with an increased risk for secondary cancers. Secondary cancers are also seen in the setting of targeted therapies. Bruton tyrosine kinase (BTK) inhibitors appear to have a secondary cancer risk similar to what is seen in the CLL population in general, but potentially a higher rate of nonmelanoma skin cancers. With short follow-up, the risk of secondary cancers appears to be slightly higher with venetoclax-based regimens than chlorambucil-based chemoimmunotherapy, and further evaluation of this trend is ongoing.

AUTOIMMUNE COMPLICATIONS

Autoimmune complications are frequent in CLL. Most commonly, these include autoimmune cytopenias, but autoimmune complications of other organs including glomerulonephritis, vasculitis, and neuropathies have also been reported. Of the autoimmune cytopenias, the most common is autoimmune hemolytic anemia (AIHA), which is an antibody-mediated destruction of autologous red blood cells (RBCs). Second most common is immune thrombocytopenia (ITP), which shares some features with AIHA and has a similar mechanism targeting platelets. These two syndromes may occur in isolation, occur sequentially in the same patient, or present in combination as Evans' syndrome. Pure red cell aplasia (PRCA) and autoimmune granulocytopenia (AIG) are comparatively rare and can occur alone or in combination with other autoimmune cytopenias. It is difficult to tease out whether autoimmune cytopenias lead to worse prognosis in CLL because of various complicating factors. However, it is clear that these can lead to significant morbidity, both due to the process itself and due to therapies required for management.

AIHA usually presents as an isolated anemia with an elevated reticulocyte count and features of hemolysis including elevated bilirubin and LDH and low haptoglobin. Detection of a warm IgG antibody on the surface of RBCs with a Coombs test can help solidify the diagnosis, although Coombs-negative cases can occur. Immediate therapy is almost always necessary and consists of transfusion and immunosuppression. Glucocorticoids are often used for initial therapy, although in most cases, additional treatment is needed due to either poor response or recurrence with taper of glucocorticoid dosing. Rituximab can be successful, and therapy directed toward the underlying CLL is often effective in more resistant cases. Transfusion of blood in cases of robust AIHA must be initiated with caution as transfusion reactions can be seen due to poorly matched blood, but should be pursued in those with severe, symptomatic anemia. Death from uncontrolled AIHA can occur in the absence of appropriate supportive care ([Chap. 100](#)).

ITP can be more difficult to diagnose as it may be difficult to differentiate from progression of disease due to the lack of laboratory tests that identify platelet destruction from this mechanism. Signs that point toward ITP include isolated thrombocytopenia and rapid decline in platelet levels in the absence of an alternative etiology. A bone marrow biopsy showing normal or increased megakaryocytes can be used to confirm the diagnosis but is often not necessary. In CLL, treatment for ITP is usually instituted when platelet levels drop to 20,000–30,000 or if evidence of bleeding complications or need for invasive procedures develops. Like AIHA, initial therapy consists of glucocorticoids and IVIg, with rituximab also being an effective method to induce long-term remissions. Also, the thrombopoietin receptor agonists romiplostim and eltrombopag are effective in secondary ITP. In many cases, ITP can be successfully treated without treating the underlying CLL. In cases in which anemia or thrombocytopenia appears, it is important to investigate the mechanism because the approach to therapy of autoimmune cytopenias in CLL differs from cytopenias due to marrow replacement ([Chap. 115](#)).

RICHTER'S TRANSFORMATION

One of the most devastating complications of CLL is Richter's transformation, which is transformation of CLL to an aggressive lymphoma, most commonly DLBCL. The World Health Organization also recognizes Hodgkin's lymphoma (HL) as a variant of Richter's transformation; other aggressive lymphomas are rarely identified. Some older series have included prolymphocytic transformation in this category, although this has much less prognostic impact on long-term outcome. The prevalence of Richter's transformation is difficult to estimate based on previous studies, but one prospective observational study estimated a rate of 0.5% per year for DLBCL and 0.05% per year for HL. Risk factors for development include bulky lymphadenopathy, *NOTCH1* mutations, del(17)(p13.1), and a specific stereotyped *IGHV* usage. Lymphomas arising in the setting of CLL can either be clonally related or unrelated to the initial CLL, with prognosis significantly better for clonally unrelated lymphomas. In addition, patients with Hodgkin's transformation have improved outcome, particularly in the absence of prior fludarabine treatment. B-cell prolymphocytic leukemia (PLL) arising from CLL is currently classified as Richter's transformation as well; however, clinical features and therapy are quite different, so these two should be differentiated for therapeutic purposes.

Clinical signs of Richter's transformation include rapid progression in adenopathy, often in a specific area, and constitutional symptoms including fatigue, night sweats, fever, and weight loss. LDH is usually high. In suspected cases, the first step is ¹⁸FDG-PET/CT (fluorodeoxyglucose–positron emission tomography combined with computed tomography) scan to localize an area for biopsy. Standardized uptake values (SUVs) <5 are consistent with CLL and can rule out Richter's transformation in many cases. SUVs >5 are suspicious for Richter's transformation, with SUVs ≥10 being very concerning. Excisional biopsy is the preferred mode of diagnosis, and fine-needle aspiration should be discouraged.

Therapy for DLBCL Richter's transformation usually involves combination chemoimmunotherapy (e.g., R-CHOP [rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone], dose-adjusted

EPOCH-R [etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab]; [Chap. 108](#)). Outcomes may be poor with median survivals of 6–16 months in most series for clonally related Richter's versus ~5 years for clonally unrelated. For fit patients who achieve a response with therapy, stem cell transplantation has the possibility to induce long-term remissions and should be explored. In addition, chimeric antigen receptor T-cell (CAR-T) therapy has shown promising results in small groups of patients and remains an area of active clinical investigation. Patients with Hodgkin's disease can be treated according to the algorithm for this disease, with many individuals being cured.

WORKUP OF CLL AND APPROACH TO THERAPY

WORKUP AND STAGING

Workup of a patient with a new diagnosis of CLL based on typical immunophenotyping includes a detailed history of infectious disease; family history of CLL; and careful physical examination with attention to the lymph nodes, spleen, and liver. In patients desiring to know the expected natural history of their CLL, prognostic testing using FISH and stimulated karyotype and sequencing for *TP53* and *IGHV* mutation status can be performed. Imaging with CT scan is usually not necessary unless there are symptoms and concern for intraabdominal nodes out of proportion to peripheral nodes. Bone marrow biopsy is not undertaken until therapy is initiated except in cases of unexplained cytopenias.

STAGING

There are two widely used staging systems in CLL. The Rai staging system is used more commonly in the United States, whereas the Binet system is more commonly used in Europe. Both characterize CLL on the basis of disease bulk and marrow failure ([Table 107-3](#)). Both rely on physical examination and laboratory studies and do not require imaging or bone marrow analysis. While the initial staging systems could reliably predict survival in CLL with the changes in therapy since the original description of the stages, the impact of initial stage on survival is not as clear. Cytogenetic and genomic testing can help refine outcomes of these staging tests. An international collaboration integrated both clinical and genomic staging to better predict outcome at diagnosis and time of initial treatment, which led to development of the CLL International Prognostic Index ([Table 107-4](#)). This index has been shown to be useful in prediction of both time to first treatment and outcome with chemoimmunotherapy. Validation in the setting of novel targeted therapies has not occurred.

CRITERIA FOR THE INITIATION OF THERAPY

Currently, a watchful waiting strategy is used for most patients with CLL, with therapy reserved for patients with symptomatic disease. This recommendation is based on multiple trials showing no survival advantage with earlier therapy, although this question continues to be a focus of active investigation.

With the exception of patients participating on early intervention studies in CLL, disease-related symptoms that require the initiation of therapy are outlined in [Table 107-5](#). Except for the rare patient who

TABLE 107-3 Staging of CLL

Rai Staging System	
Low risk (stage 0)	Lymphocytosis only
Intermediate risk (stage I/II)	Lymphocytosis with lymphadenopathy, with or without splenomegaly or hepatomegaly
High risk (stage III/IV)	Lymphocytosis with anemia or thrombocytopenia due to bone marrow involvement
Binet Staging System	
A	<3 areas of lymphadenopathy
B	≥3 areas of lymphadenopathy
C	Hemoglobin ≤10 g/dL and/or platelets <100,000/µL

Abbreviation: CLL, chronic lymphocytic leukemia.

TABLE 107-4 CLL International Prognostic Index

Risk Score		
VARIABLE	ADVERSE FACTOR	RISK SCORE
TP53 status	Deleted or mutated	4
IGHV mutational status	Unmutated	2
β_2 -microglobulin concentration	>3.5 mg/L	2
Clinical stage	Rai I–IV or Binet B–C	1
Age	>65 years	1
Implications of Risk Score		
RISK SCORE	RISK CLASSIFICATION	5-YEAR SURVIVAL (TRAINING SET DATA)
0–1	Low	93.2%
2–3	Intermediate	79.3%
4–6	High	63.3%
7–10	Very high	23.3%

Abbreviation: CLL, chronic lymphocytic leukemia.

presents with disease requiring urgent therapy, most times, these symptoms can be monitored over short periods to determine relatedness to CLL and need for therapy.

INITIAL THERAPY FOR CLL

Over the past decade, the initial therapy of CLL has dramatically changed. Whereas chemoimmunotherapy was once standard for all patients, now most patients are treated with oral therapies targeted against BTK or BCL2 with or without a CD20 monoclonal antibody. This continues to be an area of active investigation, with standards of care shifting rapidly. The major classes of these therapies are outlined here.

BTK Inhibitors BTK is an attractive target in CLL because, unlike other kinases in the BCR pathway, BTK does not have natural redundancy and is relatively selective for B cells, so inhibition leads to a predominant B cell-specific phenotype. The first-in-class BTK inhibitor is ibrutinib, which is relatively selective for BTK but also inhibits a number of structurally similar kinases. As initial therapy, ibrutinib was initially compared with chlorambucil (RESONATE 2 study), and there was an 84% lower risk of progression or death with ibrutinib, with 70% of ibrutinib-treated patients alive and progression-free at 5 years. Subsequent studies compared ibrutinib alone or with the anti-CD20 antibody rituximab to standard chemoimmunotherapy with fludarabine plus cyclophosphamide plus rituximab (FCR) in younger patients (<70 years; E1912 study) or bendamustine plus rituximab (BR) in older patients (≥ 65 years; A041202 study). In younger patients, ibrutinib plus rituximab (IR) showed increased progression-free survival (PFS) and overall survival (OS) when compared with FCR, with a 3-year PFS of 89% for IR compared with 71% for FCR. In older patients, ibrutinib alone as well as with rituximab showed superior PFS compared with BR, with 24-month PFS rates of 88% for IR, 87% for ibrutinib alone,

and 74% for BR. IR was not superior to ibrutinib alone, and OS was not different in this trial at 24 months. Side effects distinct to ibrutinib include arthralgias/myalgias, rash, diarrhea, dyspepsia, increased risk of bleeding (particularly when on anticoagulation therapy or with surgery), hypertension, and atrial fibrillation.

The second-generation BTK inhibitor acalabrutinib is more specific for BTK than ibrutinib and consequently shows better tolerability, with less incidence of atrial fibrillation, myalgias/arthritis, and skin and nail changes than reported with ibrutinib. In the frontline setting, acalabrutinib and acalabrutinib plus obinutuzumab were compared with chlorambucil plus obinutuzumab. Both acalabrutinib alone and acalabrutinib with obinutuzumab showed superior 30-month PFS compared with chlorambucil plus obinutuzumab (82%, 90%, and 34%, respectively), with improved PFS for acalabrutinib plus obinutuzumab compared with acalabrutinib alone in an unplanned post hoc analysis.

BCL2 Inhibitor Venetoclax is an orally bioavailable, selective inhibitor of the antiapoptotic protein BCL2, which is upregulated in CLL. Unlike with BTK inhibitors, where many phase 3 studies support benefit over chemoimmunotherapy, only one study has been published with venetoclax. The CLL14 study compared venetoclax plus obinutuzumab (VO) to chlorambucil plus obinutuzumab in previously untreated patients with coexisting medical conditions. Unlike BTK inhibitors, which are administered continuously until disease progression, VO treatment is given for a 1-year fixed duration. At 3 years of follow-up, PFS was 82% in the VO group compared with 50% in the chlorambucil plus obinutuzumab group. No difference has been observed in OS with this follow-up. Side effects associated with venetoclax include tumor lysis syndrome, neutropenia, and nausea.

PI3K Inhibitors Inhibitors of PI3K delta have been studied in CLL due to the specificity of the delta isoform for B lymphocytes. Two agents, idelalisib and duvelisib, are approved for use in relapsed CLL, but trials of idelalisib in frontline CLL demonstrated toxicity that precluded further development in this area. Toxicities seen with idelalisib and duvelisib include pneumonitis, diarrhea/colitis, and transaminitis. More recently, a second-generation PI3K delta inhibitor umbralisib was combined with the anti-CD20 antibody ublituximab in the frontline setting and compared with chlorambucil plus obinutuzumab. Twenty-four-month PFS was 61% for ublituximab plus umbralisib compared with 40% with chlorambucil plus obinutuzumab. PI3K inhibitor-specific toxicities appear to be lower with umbralisib compared with idelalisib and duvelisib, but comparative trials are lacking. As outcome with this combination appears inferior to that with BTK inhibitors or BCL2 inhibitors, it is unlikely this treatment will be used in CLL outside of rare circumstances where other classes of drugs are contraindicated.

Chemoimmunotherapy For the most part, targeted therapy has supplanted chemoimmunotherapy in CLL. However, long-term follow-up of studies of FCR has demonstrated that a subset of patients treated with this regimen can have durable responses over 10 years, with a likely cure of CLL. This group is composed almost exclusively of patients with mutated *IGHV* and favorable cytogenetics. However, despite the efficacy of this regimen, short- and long-term toxicities limit its adaptability to many patients with *IGHV* mutated disease. Short-term toxicities are mostly related to myelosuppression and include neutropenia and infection. Long-term cytopenias are less common, but they do occur. Also, there is about a 3–5% risk of therapy-related myeloid neoplasm with this regimen that is almost always fatal. In the E1912 study of FCR versus IR, at follow-up, there was no difference in PFS or OS between FCR and IR for patients with mutated *IGHV*, suggesting that there may remain a place for this regimen in clinical practice. In addition, current studies are focused on limiting chemotherapy and/or adding novel agents in efforts to achieve cure but limit toxicity.

THERAPY OF RELAPSED CLL

Currently, the mainstays of treatment for relapsed CLL are the same classes as initial therapy. The optimal sequencing of targeted agents in

TABLE 107-5 Criteria for the Initiation of Therapy

Symptoms Indicating Need for Therapy in CLL
Evidence of progressive marrow failure (worsening of anemia or thrombocytopenia not due to autoimmune destruction)
Massive (≥ 6 cm below costal margin), progressive, or symptomatic splenomegaly
Massive (≥ 10 cm), progressive, or symptomatic lymphadenopathy
Progressive lymphocytosis with an increase of $\geq 50\%$ over a 2-month period or lymphocyte doubling time <6 months
Autoimmune anemia or thrombocytopenia not responsive to standard therapy
Symptomatic or functional extranodal involvement
Constitutional symptoms (one or more of the following: unintentional weight loss $\geq 10\%$ over 6 months, significant fatigue, fevers $\geq 100.5^{\circ}\text{F}$ for 2+ weeks without infection, night sweats for >1 month without infection)

Abbreviation: CLL, chronic lymphocytic leukemia.

TABLE 107-6 Response Criteria in CLL

	LYMPHOCYTE COUNT	LYMPH NODES ^a	SPLEEN/LIVER SIZE ^b	BONE MARROW ^c	PERIPHERAL BLOOD COUNTS
CR	<4000/ μ L	None >1.5 cm	Not palpable	Normocellular, <30% lymphocytes, no B lymphoid nodules	<ul style="list-style-type: none"> Platelet count >100,000/μL Hemoglobin >11 g/dL Neutrophils >1500/μL
PR	Decrease \geq 50% from baseline	Decrease \geq 50% from baseline	Decrease \geq 50% from baseline	Infiltrate \leq 50% of baseline	One of the following: <ul style="list-style-type: none"> Platelet count >100,000/μL or \geq50% from baseline Hemoglobin >11 g/dL or \geq50% from baseline Neutrophils >1500/μL or \geq50% from baseline
Stable disease	Not meeting CR/PR/PD criteria	Not meeting CR/PR/PD criteria			
PD	Increase \geq 50%	Increase \geq 50%	Increase \geq 50%		<ul style="list-style-type: none"> Platelet count \leq50% of baseline due to CLL Hemoglobin decrease >2 g/dL due to CLL

^aRefers to sum of the products of multiple lymph nodes evaluated by CT scan. ^bBased on physical examination. ^cBone marrow only required to confirm CR.

Abbreviations: CLL, chronic lymphocytic leukemia; CR, complete response; PD, progressive disease; PR, partial response.

CLL has not been established; however, the available data suggest that the sequence of either BTK inhibitor and then BCL2 inhibitor or the reverse are both acceptable. In a trial of venetoclax for patients who had relapsed after ibrutinib therapy, the overall response rate (ORR) was 65% with a median PFS of ~2 years in a very heavily pretreated patient population. Retrospective data of BTK inhibitor given after venetoclax suggests that this sequence is effective as well, with an ORR of 84% and median PFS of 32 months. PI3K inhibitors also have activity in relapsed CLL; however, activity following both BTK and BCL2 inhibitors is likely minimal. In addition, many new agents are in development in CLL including novel oral targeted therapies, antibodies, and immunobased treatments.

Immune Therapies Immune therapies in CLL are currently focused in the relapsed setting and include allogeneic stem cell transplantation, CAR-T therapy, and oral immunomodulatory agents such as lenalidomide.

Stem cell transplantation is a curative approach to CLL. Because most CLL patients are older and many have significant comorbidities, myeloablative transplantsations incur extensive morbidity and mortality, making them prohibitive in many individuals. Reduced-intensity conditioning (RIC) allogeneic transplantsations have been successfully incorporated into the treatment of patients up to ~75 years in age but still have a \geq 50% frequency of chronic graft-versus-host disease. This is still considered a standard treatment in CLL but has fallen out of favor with the introduction of well-tolerated novel agents, as well as clinical trials of CAR-T cells. CD19 CAR-T cell trials have not been as successful in CLL as they have been in other B cell malignancies due to the immunosuppression associated with the disease. Many current trials are focused on optimizing CD19 CAR-T cells by adding agents such as BTK inhibitors or PI3K inhibitors or modifying the CAR-T structure, and other studies are testing different targets outside of CD19. In addition, recent studies have shown that natural killer (NK) cell CAR cells also can induce clinical response in CLL patients. This area remains a focus of intense investigation in CLL.

ASSESSING RESPONSE TO THERAPY AND MINIMAL RESIDUAL DISEASE IN CLL

Following the completion of therapy or during therapy for indefinite targeted agents, response is initially assessed using physical examination and laboratory studies (Table 107-6). If residual disease is not detected using these methodologies, CT scans are used to assess response. Bone marrow biopsies with flow cytometry are indicated if no disease is detected to confirm complete response.

It has been established in various malignancies that complete tumor eradication is associated with longer survival. In CLL, if no malignant cells can be detected in the bone marrow down to a level of 1 CLL cell

in 10^4 leukocytes (0.01%), the patient is said to be negative for minimal residual disease (MRD). Following combination chemoimmunotherapy, eradication of MRD correlates with long-term survival and potentially cure in a subset of patients receiving FCR chemoimmunotherapy. Undetectable MRD in blood or bone marrow is also associated with improvement in PFS in venetoclax-based regimens. However, eradication of MRD has not been shown to be a meaningful endpoint with BTK or PI3K inhibitors as monotherapy. Higher sensitivity of 1 CLL in 10^6 leukocytes (0.0001%) can be obtained using next-generation sequencing methods such as ClonoSeq. This technique is currently available in clinical practice, although at this point, there are no data confirming that increased sensitivity is clinically meaningful, and studies are underway to support the need for this higher sensitivity with novel combination approaches of BTK/BCL2 inhibitor regimens.

CONCLUSION

CLL is treated only when it becomes symptomatic. At the time of therapy, FCR chemoimmunotherapy in a small subset of young patients with very-good-risk CLL is potentially curative. In the majority of patients with symptomatic CLL, targeted therapy directed at BTK or BCL2 can produce durable remissions and allow patients many years of disease-free survival.

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Non-Hodgkin's lymphomas (NHL) are cancers of mature B, T, and natural killer (NK) cells. They were distinguished from Hodgkin's lymphoma (HL) upon recognition of the Reed-Sternberg (RS) cell and differ from HL with respect to their biologic and clinical characteristics. Whereas 80–85% of patients with HL will be cured of their lymphoma by chemotherapy with or without radiotherapy, the prognosis and natural history of NHL tends to be more variable. NHL can be classified as either a mature B-NHL or a mature T/NK-NHL depending on whether the cancerous lymphocyte is a B, T, or NK cell, respectively. Within each category are lymphomas that grow quickly and behave aggressively, as well as lymphomas that are more indolent, or slow growing, in nature. For a list of the World Health Organization (WHO) classification of lymphoid neoplasms, see Table 108-1.

■ EPIDEMIOLOGY AND ETIOLOGY

In 2020, >77,000 new cases of NHL were diagnosed in the United States, 4% of all new cancers in both males and females, making it the seventh most common cause of cancer-related death in both women and men. The incidence is nearly 10 times the incidence of HL. There is a slight male-to-female predominance and a higher incidence for Caucasians than for African Americans. The incidence rises steadily with age, especially after age 40, but lymphomas are also among the most common malignancies in adolescent and young adult patients. The incidence of NHL has nearly doubled over the past 20–40 years and continues to rise by 1.5–2% each year. Patients with both primary

and secondary immunodeficiency states are predisposed to developing NHL. These include patients with HIV infection, patients who have undergone organ transplantation, and patients with inherited immune deficiencies and autoimmune conditions. The 5-year survival rates for NHL are 72% for Caucasians and 63% for African Americans.

The incidence of NHL and the patterns of expression of the various subtypes differ geographically and across age groups. T-cell lymphomas are more common in Asia than in Western countries, whereas certain subtypes of B-cell lymphomas such as follicular lymphoma (FL) are more common in Western countries. A specific subtype of NHL known as the angiocentric nasal T/NK cell lymphoma has a striking geographic occurrence, being most frequent in southern Asia and parts of Latin America. Another subtype of NHL associated with infection by human T-cell lymphotropic virus (HTLV) 1 is seen particularly in southern Japan and the Caribbean. Likewise, there are differences in the age-dependent incidence of NHL by histologic subtype, with aggressive lymphomas like diffuse large B-cell lymphoma (DLBCL) and Burkitt's lymphoma (BL) being the most common entities in children, and DLBCL and indolent lymphomas including FL being the most common forms in adults. The relative frequencies of the various types of lymphoid malignancies, including HL, plasma cell disorders, and lymphoid leukemias, is shown in Fig. 108-1.

A number of environmental factors have been implicated in the occurrence of NHL, including infectious agents, chemical exposures, and medical treatments. Several studies have demonstrated an association between exposure to agricultural chemicals and an increased incidence of NHL. Patients treated for HL can develop NHL; it is unclear whether this is a consequence of the HL or its treatment, especially radiation.

Several NHLs are associated with infectious agents (Table 108-2). Epstein-Barr virus (EBV) is associated with the development of BL in Central Africa and the occurrence of aggressive NHL in immunosuppressed patients in Western countries. The majority of primary central nervous system (CNS) lymphomas are associated with EBV. EBV infection is strongly associated with the occurrence of extranodal nasal NK/T-cell lymphomas in Asia and South America. HTLV-1 infects T cells and leads directly to the development of adult T-cell lymphoma (ATL) in a small percentage of patients infected as babies through ingestion of breast milk of infected mothers. The median age of patients with ATL is 56 years; thus, HTLV-1 demonstrates a long latency from infection to oncogenesis (Chap. 201). Infection with HIV predisposes to the development of aggressive, B-cell NHL. This may be through overexpression of interleukin 6 by infected macrophages. Infection of the stomach by the bacterium *Helicobacter pylori* induces the development of gastric mucosa-associated lymphoid tissue (MALT) lymphomas. This association is supported by evidence that patients treated with antibiotics to eradicate *H. pylori* have regression of their MALT lymphoma. The bacterium does not transform lymphocytes to produce the lymphoma; instead, a vigorous immune response is made to the bacterium, and the chronic antigenic stimulation leads to the neoplasia. MALT lymphomas of the skin may be related to *Borrelia* sp. infections in Europe, those of the eyes to *Chlamydia psittaci*, and those of the small intestine to *Campylobacter jejuni*. Chronic hepatitis C virus infection has been associated with the development of lymphoplasmacytic lymphoma and splenic marginal zone lymphoma (MZL). Human herpesvirus 8 is associated with primary effusion lymphoma in HIV-infected persons and multicentric Castleman's disease, a diffuse lymphadenopathy associated with systemic symptoms of fever, malaise, and weight loss.

In addition to infectious agents, a number of other diseases or exposures may predispose to developing lymphoma (Table 108-3). Diseases of inherited and acquired immunodeficiency as well as autoimmune diseases are associated with an increased incidence of lymphoma. The association between immunosuppression and induction of NHLs is compelling because if the immunosuppression can be reversed, a percentage of these lymphomas regress spontaneously. The incidence of NHL is nearly a hundredfold increased for patients undergoing organ transplantation necessitating chronic immunosuppression and is greatest in the first year posttransplant. About 30% of these arise as

TABLE 108-1 WHO Classification of Lymphoid Malignancies

B CELL	T CELL
Mature (peripheral) B-cell neoplasms	Mature (peripheral) T-cell neoplasms
Lymphoplasmacytic lymphoma (Waldenström's macroglobulinemia)	T-cell granular lymphocytic leukemia
Hairy cell leukemia	Adult T-cell leukemia/lymphoma (HTLV-1+)
Splenic marginal zone B-cell lymphoma	Extranodal NK/T-cell lymphoma, nasal type
Extranodal marginal zone B-cell lymphoma of MALT type	Enteropathy-associated T-cell lymphoma
Nodal marginal zone B-cell lymphoma	Hepatosplenitic T-cell lymphoma
Follicular lymphoma	Subcutaneous panniculitis-like T-cell lymphoma
Mantle cell lymphoma	Mycosis fungoides
Diffuse large B-cell lymphoma (including subtypes)	Sezary syndrome
High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements	Peripheral T-cell lymphoma NOS
High-grade B-cell lymphoma NOS	Angioimmunoblastic T-cell lymphoma
Burkitt's lymphoma/Burkitt's cell leukemia	Anaplastic large-cell lymphoma, ALK+
Primary mediastinal large B-cell lymphoma	Anaplastic large-cell lymphoma, ALK-
Plasmablastic lymphoma	
Primary effusion lymphoma	
HHV8+ DLBCL NOS	
Intravascular large B-cell lymphoma	
ALK+ large B-cell lymphoma	

Abbreviations: DLBCL, diffuse large B-cell lymphoma; HHV, human herpesvirus; HTLV, human T-cell lymphotropic virus; MALT, mucosa-associated lymphoid tissue; NK, natural killer; NOS, not otherwise specified; WHO, World Health Organization.

Source: Adapted from SH Swerdlow et al: *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 5th ed. IARC, 2016.

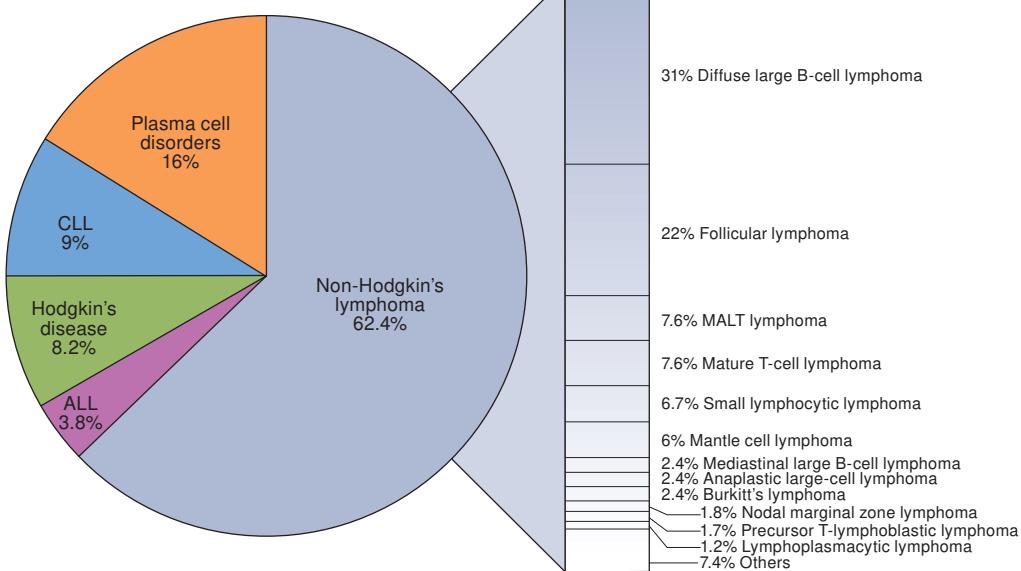


FIGURE 108-1 Relative frequency of lymphoid malignancies. ALL, acute lymphoid leukemia; CLL, chronic lymphocytic leukemia; MALT, mucosa-associated lymphoid tissue.

a polyclonal B-cell proliferation that evolves into a clonal B cell malignancy. The NHLs that occur in the context of immunosuppression or immunodeficiency, including HIV infection, are frequently associated with EBV. Histologically, DLBCLs are most frequently associated with immunosuppression and autoimmune diseases, although almost all histologies can be seen, especially MALT lymphomas in the context of autoimmune diseases such as Sjögren's syndrome and Hashimoto's thyroiditis. The rare inherited immunodeficiency diseases X-linked lymphoproliferative syndrome, Wiskott-Aldrich syndrome, Chédiak-Higashi syndrome, ataxia-telangiectasia, and common variable immunodeficiency syndrome are complicated by highly aggressive lymphomas. The elevated incidence of lymphoma in iatrogenic immunosuppression, AIDS, and autoimmune disease argues strongly for immune dysregulation contributing in the pathogenesis of some lymphomas. An increased risk of NHL has been observed in first-degree relatives with NHL, HL, or chronic lymphocytic leukemia (CLL). In large database studies, 9% of patients with lymphoma or CLL have a first-degree relative with a lymphoproliferative disorder.

TABLE 108-2 Infectious Agents Associated with the Development of Lymphoid Malignancies

INFECTIOUS AGENT	LYMPHOID MALIGNANCY
Epstein-Barr virus	Burkitt's lymphoma
	Post-organ transplant lymphoma
	Primary CNS diffuse large B-cell lymphoma
	Hodgkin's lymphoma
	Extranodal NK/T-cell lymphoma, nasal type
HTLV-1	Adult T-cell leukemia/lymphoma
HIV	Diffuse large B-cell lymphoma
	Burkitt's lymphoma
Hepatitis C virus	Lymphoplasmacytic lymphoma
<i>Helicobacter pylori</i>	Gastric MALT lymphoma
Human herpesvirus 8	Primary effusion lymphoma
	Multicentric Castleman's disease

Abbreviations: CNS, central nervous system; HIV, human immunodeficiency virus; HTLV, human T-cell lymphotropic virus; MALT, mucosa-associated lymphoid tissue; NK, natural killer.

IMMUNOLOGY

All lymphoid cells are derived from a common hematopoietic progenitor that gives rise to lymphoid, myeloid, erythroid, monocyte, and megakaryocyte lineages. Through the ordered and sequential activation of a series of transcription factors, the cell first becomes committed to the lymphoid lineage and then gives rise to B and T cells.

About 90% of all lymphomas are of B cell origin. A cell becomes committed to B cell development when it expresses the master B lineage transcription factor PAX5, which ultimately results in a transcriptional program that leads to the rearrangement of its immunoglobulin genes, which involves chromosomal recombination as well as somatic hypermutation to create an immunoglobulin gene that is unique to that B cell. The sequence of cellular changes, including changes in cell-surface phenotype that characterizes normal B cell development, is shown in Fig. 108-2. Most B-cell lymphomas arise following the process of immunoglobulin gene recombination and somatic hypermutation, which leads to class switching and affinity maturation of the mature immunoglobulin, respectively, suggesting that it is the error-prone nature of these genetic events that contributes to oncogenesis. Certainly the frequency of chromosomal translocations that result in the activation of an oncogene or the inactivation of a tumor-suppressor gene in B-cell NHL may be the result of these normal cellular processes gone awry (see below). In addition, the key roles of the transcription factors MYC and BCL6 and the antiapoptotic protein BCL2 in the

TABLE 108-3 Diseases or Exposures Associated with Increased Risk of Development of Malignant Lymphoma

Inherited immunodeficiency disease	Autoimmune disease
Klinefelter's syndrome	Sjögren's syndrome
Chédiak-Higashi syndrome	Celiac sprue
Ataxia-telangiectasia syndrome	Rheumatoid arthritis and systemic lupus erythematosus
Wiskott-Aldrich syndrome	Chemical or drug exposures
Common variable immunodeficiency disease	Phenytoin
Acquired immunodeficiency diseases	Dioxin, phenoxy herbicides
Iatrogenic immunosuppression	Radiation
HIV-1 infection	Prior chemotherapy and radiation therapy
Acquired hypogammaglobulinemia	

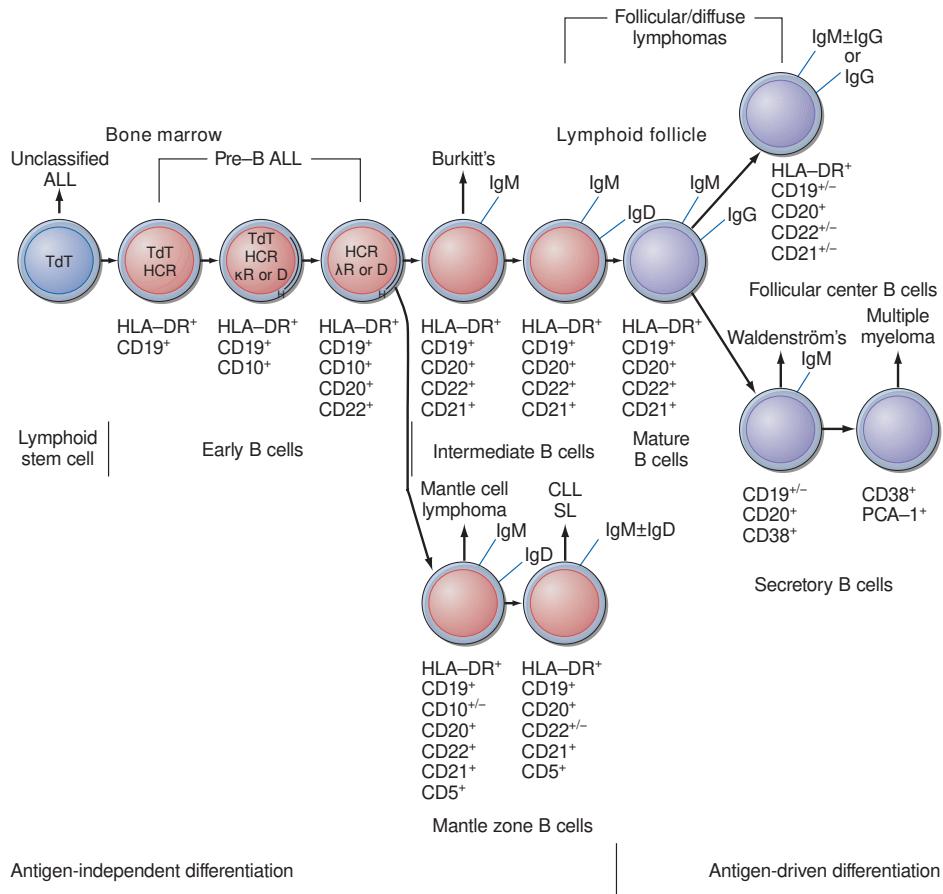


FIGURE 108-2 Pathway of normal B-cell differentiation and relationship to B-cell lymphomas. HLA-DR, CD10, CD19, CD20, CD21, CD22, CD5, and CD38 are cell markers used to distinguish stages of development. Terminal transferase (TdT) is a cellular enzyme. Immunoglobulin heavy chain gene rearrangement (HCR) and light chain gene rearrangement or deletion (κR or D, λR or D) occur early in B-cell development. The approximate normal stage of differentiation associated with particular lymphomas is shown. ALL, acute lymphoid leukemia; CLL, chronic lymphocytic leukemia; SL, small lymphocytic lymphoma.

process of B cell development explain why the genes encoding these proteins are commonly mutated in B-cell lymphomas.

A cell becomes committed to T-cell differentiation upon migration to the thymus and rearrangement of T-cell receptor (TCR) genes. This requires the expression of the T-cell master regulatory transcription factor, NOTCH-1. As in B cells, the development of the mature TCR involves the rearrangement and recombination of the TCR loci, which is error-prone and potentially oncogenic. The sequence of the events that characterize T-cell development is depicted in Fig. 108-3.

Although lymphoid malignancies often retain the cell-surface phenotype of lymphoid cells at particular stages of differentiation, this information is of little clinical or prognostic consequence. The so-called stage of differentiation of a malignant lymphoma does not predict its natural history. The antigen footprint, or immunophenotype, of the cell, however, is valuable diagnostically as it allows for the distinguishing of specific NHL subtypes. It can be detected by flow cytometry of single-cell suspension from blood, bone marrow, body fluid, or disaggregated tissue using fluorescently labeled antibodies against these antigens or by immunohistochemical staining of paraffin-embedded tissue sections with enzyme-linked antibodies against these antigens followed by a colorimetric reaction.

As already mentioned, malignancies of lymphoid cells are associated with recurring genetic abnormalities including chromosomal translocations and genetic mutations that may in part be the result of aberrant immunoglobulin or TCR development. While specific genetic abnormalities have not been identified for all subtypes of lymphoid malignancies, it is presumed that they exist. As previously discussed, B cells are even more susceptible to acquiring mutations during their

maturity in germinal centers; the generation of antibody of higher affinity requires the introduction of mutations into the variable region genes in the germinal centers. Given this, other nonimmunoglobulin genes, e.g., *bcl-6*, may acquire mutations as well. Likewise, many lymphomas contain balanced chromosomal translocations involving the antigen receptor genes; immunoglobulin genes on chromosomes 2, 14, and 22 in B cells; and T-cell antigen receptor genes on chromosomes 7 and 14 in T cells. The rearrangement of chromosome segments to generate mature antigen receptors must create a site of vulnerability to aberrant recombination. Examples of this type of event include the (8;14)(q24;q32) translocation in BL, involving the *MYC* proto-oncogene and the IgH gene; the (14;18)(q32;q32) translocation in FL, involving the *BCL2* proto-oncogene and the IgH gene; and the (11;14)(q13;q32) translocation in mantle cell lymphoma (MCL), involving the gene encoding cyclin D1 (*CCND1*) and the IgH gene. Less commonly, chromosomal translocations produce fusion genes that encode chimeric oncogenic proteins. Examples of this include the (2;5)(p23;q35) translocation involving the *ALK* and *NPM1* genes in anaplastic large-cell lymphoma (ALCL) and the t(11;18)(q21;q21) translocation involving the *API2* and *MLT* genes in MALT lymphoma. Table 108-4 presents the most common translocations and associated oncogenes for various subtypes of lymphoid malignancies.

Gene profiling using array technology allows the simultaneous assessment of the expression of thousands of genes. This technology provides the possibility to identify new genes with pathologic importance in lymphomas, the identification of patterns of gene expression with diagnostic and/or prognostic significance, and the identification of new therapeutic targets. Recognition of patterns of gene expression

T-CELL DIFFERENTIATION

Stage I
Prothymocyte

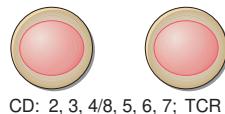
THYMUS



CD: 2, 7, 38, 71

Stage II
Thymocyte

CD: 1, 2, 4, 7, 8, 38

Stage III
Thymocyte

CD: 2, 3, 4/8, 5, 6, 7; TCR

T-CELL MALIGNANCIES

Majority of
T-cell ALLMinority of T-ALL
Majority of T-LLMinority of T-LL
Rare T-ALL

PERIPHERAL BLOOD AND NODES

Mature T Helper
Cell

CD: 2, 3, 4, 5, 6, 7; TCR

Majority of
T-CLL, CTCL,
Sezary Cell, NHLMature T Cytotoxic/
Suppressor Cell

CD: 2, 3, 4, 5, 6, 7; TCR

Minority of
T-CLL, NHL

FIGURE 108-3 Pathway of normal T-cell differentiation and relationship to T-cell lymphomas. CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD38, and CD71 are cell markers used to distinguish stages of development. T-cell antigen receptors (TCR) rearrange in the thymus, and mature T cells emigrate to nodes and peripheral blood. ALL, acute lymphoid leukemia; T-ALL, T-cell ALL; T-LL, T-cell lymphoblastic lymphoma; T-CLL, T-cell chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; NHL, non-Hodgkin's lymphoma.

TABLE 108-4 Genetic Features of B- and T-Cell Lymphomas

GENETIC FEATURE	GENES	LYMPHOMA
t(8;14)	<i>MYC/IgH</i>	Burkitt's lymphoma
t(2;8)	<i>MYC/IgK</i>	
t(8;22)	<i>MYC/Igλ</i>	
t(11;14)	<i>BCL1 (COND1)/IgH</i>	Mantle cell lymphoma; multiple myeloma
t(14;18)	<i>BCL2/IgH</i>	Follicular lymphoma, diffuse large B-cell lymphoma (DLBCL)
t(3;14)	<i>BCL6/IgH</i>	
t(11;18)	<i>API2/MALT1</i>	MALT lymphoma
t(1;14)	<i>BCL10/IgH</i>	
t(14;18)	<i>MALT1/IgH</i>	
t(3;14)	<i>FOXP1/IgH</i>	
Trisomy 3 7q21 deletion	Unknown <i>CDK6</i>	Splenic marginal zone lymphoma
t(9;14) 6q21 deletion	<i>PAX5/IgH</i> Unknown	Lymphoplasmacytic lymphoma
inv(14) t(14;14)	<i>TCRγ/TCL1</i>	Peripheral T-cell lymphoma, NOS; T-PLL
t(2;5)	<i>NPM1/ALK</i>	Anaplastic large-cell lymphoma (ALCL)
t(1;2)	<i>TPM3/ALK</i>	
t(2;3)	<i>TFG/ALK</i>	
t(2;17)	<i>CTLC/ALK</i>	
inv(2)	<i>ATIC/ALK</i>	
Trisomy 3	Unknown	Angioimmunoblastic T-cell lymphoma
Trisomy 5	Unknown	
Isochromosome 7q	Unknown	Hepatosplenial T-cell lymphoma

Abbreviations: MALT, mucosa-associated lymphoid tissue; NOS, not otherwise specified; T-PLL, T-cell prolymphocytic leukemia.

is complicated and requires sophisticated mathematical techniques. Early successes using this technology in lymphoma include the identification of previously unrecognized subtypes of DLBCL whose gene expression patterns resemble either those of follicular or germinal center B (GCB) cells or activated peripheral blood B cells (ABC). Patients whose lymphomas have a GCB-like pattern of gene expression have a considerably better prognosis than those whose lymphomas have a pattern resembling ABCs. This improved prognosis is independent of other known prognostic factors. These subcategories have been more specifically refined into five subcategories, using more advanced genetic sequencing techniques, that differ with respect to biology and driver genes, as well as prognosis, and may have important treatment implications in the future. Similar information is being generated in FL and MCL. The challenge remains to provide information from such techniques in a clinically useful time frame.

APPROACH TO THE PATIENT

Regardless of the type of lymphoid malignancy, the initial evaluation of the patient should include performance of a careful history and physical examination. These will help confirm the diagnosis, identify those manifestations of the disease that might require prompt attention, and aid in the selection of further studies to optimally characterize the patient's status to allow the best choice of therapy. It is difficult to overemphasize the importance of a carefully done history and physical examination. They might provide observations that lead to reconsidering the diagnosis, provide hints at etiology, clarify the stage, and allow the physician to establish rapport with the patient that will make it possible to develop and carry out a therapeutic plan.

The duration of symptoms and pace of symptomatic progression are important in distinguishing aggressive from more indolent lymphomas, as are the presence or absence of "B" symptoms, such as fevers, night sweats, or unexplained weight loss. Patients should be asked about localizing symptoms that may point toward lymphomatous involvement of specific sites, such as the chest, abdomen, or CNS. Comorbid diagnoses that may impact therapy or monitoring on therapy should be reviewed and acknowledged, including a history of diabetes or congestive heart failure. A physical examination should pay close attention to all the peripherally accessible sites of lymph nodes; the liver and spleen size; Waldeyer's ring; whether there is a pleural or pericardial effusion or abdominal ascites; whether there is an abdominal, testicular, or breast mass; and whether there is cutaneous involvement because all of these findings may influence further evaluation and disease management.

Laboratory studies should include a complete blood count, routine chemistries, liver function tests, and serum protein electrophoresis to document the presence of circulating monoclonal paraproteins. The serum β_2 -microglobulin level and serum lactate dehydrogenase (LDH) are important independent prognostic factors in NHL. Staging of certain diseases may involve a bone marrow biopsy; results of other laboratory and staging studies may also warrant a marrow evaluation. A lumbar puncture for evaluation of lymphomatous involvement may be indicated in the setting of concerning neurologic signs or symptoms or diseases that are high risk for CNS involvement. The latter may include disease involving the paranasal sinuses, testes, breast, kidneys, adrenal glands, and epidural space, as well as highly aggressive histologies like BL. Since HIV and hepatitis B and C infection can be risk factors for developing NHL, and since treatment for some NHLs can result in the potentially life-threatening reactivation of hepatitis B, patients with a new diagnosis of NHL should be screened for these viruses as well.

Lymphoma histology and clinical presentation dictate which imaging studies should be ordered. Chest, abdominal, and pelvic computed tomography (CT) scans are essential for accurate staging to assess lymphadenopathy for indolent lymphomas, whereas positron emission tomography (PET) using ^{18}F -fluorodeoxyglucose

TABLE 108-5 Staging Evaluation for Non-Hodgkin's Lymphoma

Physical examination
Documentation of B symptoms
Laboratory evaluation
Complete blood counts
Liver function tests
Uric acid
Calcium
Serum protein electrophoresis
Serum β_2 -microglobulin
Chest radiograph
CT scan of abdomen, pelvis, and usually chest
Bone marrow biopsy
Lumbar puncture in lymphoblastic, Burkitt's, and diffuse large B cell lymphoma with positive marrow biopsy
Gallium scan (SPECT) or PET scan in large-cell lymphoma

Abbreviations: CT, computed tomography; PET, positron emission tomography; SPECT, single-photon emission computed tomography.

(FDG-PET) is useful for aggressive lymphomas, including BL, DLBCL, plasmablastic lymphoma, and the aggressive T-cell NHLs. FDG-PET is highly sensitive for detecting both nodal and extranodal sites involved by NHL. The intensity of FDG avidity, or standardized uptake value (SUV), correlates with histologic aggressiveness, and may be useful in cases when disease transformation of an indolent lymphoma to a diffuse aggressive lymphoma is suspected. PET scanning can also differentiate between treated disease and active disease at the end of therapy in patients with residual masses on CT scans. Consensus recommendations regarding PET scanning were published as a result of an International Harmonization Project and state that PET should only be used for DLBCL and HL, that scanning during therapy should only be done as part of clinical trials, and that the end-of-treatment scan should not be done before 3 weeks but preferably 6–8 weeks after chemotherapy and 8–12 weeks after radiation or chemoradiotherapy. There is no evidence that long-term follow-up should include PET scanning. More recently, though, PET scan results at the end of therapy for FL have been associated with prognosis, with patients with residual PET-avid disease at the end of treatment having a poorer prognosis than those who are PET negative, and so it may be used for this prognostic purpose. Finally, magnetic resonance imaging (MRI) is useful in detecting bone, bone marrow, and CNS disease in the brain and spinal cord. The staging evaluation is outlined in **Table 108-5**.

The Ann Arbor staging system developed in 1971 for HL was adapted for staging NHLs (**Table 108-6**). This staging system focuses on the number of tumor sites (nodal and extranodal),

TABLE 108-6 Ann Arbor Staging for Lymphoma^a

STAGE	DESCRIPTION
I	Involvement of a single lymph node region (I) or single extranodal site (IE)
II	Involvement of two or more lymph node regions or lymphatic structures on the same side of the diaphragm alone (II) or with involvement of limited, contiguous, extralymphatic organ or tissue (IIE)
III	Involvement of lymph node regions on both sides of the diaphragm (III), which may include the spleen (IIIS), or limited, contiguous, extralymphatic organ or tissue (IIIE), or both (IIIES)
IV	Diffuse or disseminated foci of involvement of one or more extralymphatic organs or tissues, with or without associated lymphatic involvement

^aAll stages are further subdivided according to the absence (A) or presence (B) of systemic B symptoms including fevers, night sweats, and/or weight loss (>10% of body weight over 6 months prior to diagnosis).

TABLE 108-7 International Prognostic Index for NHL

Five Clinical Risk Factors

- Age ≥60 years
- Serum lactate dehydrogenase levels elevated
- Performance status ≥2 (ECOG) or ≤70 (Karnofsky)
- Ann Arbor stage III or IV
- >1 site of extranodal involvement

For Diffuse Large B Cell Lymphoma

0, 1 factor = low risk	35% of cases; 5-year survival, 73%
2 factors = low-intermediate risk	27% of cases; 5-year survival, 51%
3 factors = high-intermediate risk	22% of cases; 5-year survival, 43%
4, 5 factors = high risk	16% of cases; 5-year survival, 26%

For Diffuse Large B Cell Lymphoma Treated With R-CHOP

0 factor = good	10% of cases; 4-year survival, 94%
1, 2 factors = intermediate	45% of cases; 4-year survival, 80%
3, 4, 5 factors = poor	45% of cases; 4-year survival, 53%

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NHL, non-Hodgkin's lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.

location, and the presence or absence of systemic, or B, symptoms. Table 108-6 summarizes the essential features of the Ann Arbor system.

This anatomic based system is less useful in NHL, which disseminates widely, not in an ordered stepwise fashion. A majority of patients with NHL have advanced-stage disease at diagnosis. Apart from early-stage disease limited to a radiation field where local therapy with radiation is an option, all other disease is treated the same regardless of stage. Histology and clinical parameters at presentation are more important than stage with respect to prognosis. The International Prognostic Index (IPI) is perhaps the best predictor of outcome (**Table 108-7**). The IPI was developed based on the analysis of >2000 patients with aggressive NHLs treated with an anthracycline-containing regimen. Age (≤60 vs >60), serum LDH (≤normal vs > normal), performance status (0 or 1 vs 2–4), stage (I or II vs III or IV), and extranodal involvement (<1 site vs >1 site) were identified as independently prognostic for overall survival (OS). A point is awarded for each risk factor and then summed, defining four risk groups: low (0 or 1); low-intermediate (2); high-intermediate (3); and high (4–5). The 5-year OS rates for patients with scores of 0 to 1, 2, 3, and 4–5 were 73, 51, 43, and 26%, respectively. The age-adjusted IPI separates patients ≤60 from patients >60. For the age-adjusted IPI, only stage, LDH, and performance status were important. Younger patients with 0, 1, 2, or 3 risk factors had 5-year survival rates of 83, 69, 46, and 32%, compared to 56, 44, 37, and 21% for older patients. When factoring in the introduction and clinical benefit of rituximab, the 4-year progression-free survival rates are 94, 80, and 53% for 0 and 1, 2, or 3 or more risk factors, respectively.

The Follicular Lymphoma International Prognostic Index (FLIPI) is a similar predictive model for FL, derived from the analysis of >4000 patients. Age >60, stage III/IV disease, the presence of >4 nodal sites, an elevated serum LDH concentration, and a hemoglobin <12 were identified as independent prognostic variables, and summation of each variable identified three risk groups. The median 10-year survival rates for patients with zero to one (low risk), two (intermediate risk), or three or more (high risk) of these adverse factors were 71, 51, and 36%, respectively. Similar disease-specific IPIs have been developed for MCL and peripheral T-cell lymphoma (PTCL) as well. These prognostic indices take into account the proliferative index and cell-surface markers, respectively.

Finally, as mentioned previously, gene expression profiling has identified DLBCLs with differential prognoses: GCB and ABC, where GCB-like DLBCL is associated with a significantly better

OS. A more readily accessible immunohistochemical algorithm has been developed, based on the presence or absence of CD10, BCL6, and MUM1 that correlates closely with gene expression profiles and can differentiate the majority of GCB from non-GCB-like DLBCL. These profiles have prognostic importance but, to date, do not alter treatment recommendations for the primary treatment of DLBCL. Current clinical trials do stratify by DLBCL subtype, and it appears that agents like the Bruton tyrosine kinase (BTK) inhibitor ibrutinib and lenalidomide are most active in non-GCB DLBCL in the relapsed setting. Treatment may then be differentiated by these subtypes in the future.

CLINICAL FEATURES, TREATMENT, AND PROGNOSIS OF SPECIFIC NHL

MATURE B CELL NEOPLASMS

B-cell NHLs can be characterized into two broad groups—those that behave aggressively, require immediate or urgent treatment with combination chemotherapy regimens, and are potentially curable; and those that are more indolent in nature, can be observed and treated only when they cause symptoms or signs of organ function impairment, are very responsive to therapy, but are not ultimately curable in the vast majority of cases. Among the aggressive diseases, the most common are NHL and DLBCL, and the most rapidly proliferative are NHL and BL. FL is the second most common NHL and the most common indolent NHL. Other indolent NHLs include MZL, lymphoplasmacytic lymphoma (LPL), and hairy cell leukemia (HCL). MCL is an intermediate-grade lymphoma that shares some characteristics with the aggressive lymphomas (fairly urgent need for treatment and aggressive upfront combination chemotherapy regimens), but like the indolent lymphomas, it is not readily curable with conventional-dose therapies.

Burkitt's Lymphoma Burkitt's lymphoma/leukemia (BL) is a rare disease in adults in the United States, making up <1% of NHL, but it makes up 30% of childhood NHL. It is one of the fastest growing neoplasms, with a doubling time of <24 h. In general, it is a pediatric tumor that has three major clinical presentations. The endemic (African) form presents as a jaw or facial bone tumor that spreads to extranodal sites including ovary, testis, kidney, breast, and especially the bone marrow and meninges. The nonendemic form has an abdominal presentation with massive disease, ascites, and renal, testis, and/or ovarian involvement and, like the endemic form, also spreads to the bone marrow and CNS. Immunodeficiency-related cases more often involve lymph nodes and may present as acute leukemia. BL has a male predominance and is typically seen in patients <35 years of age.

On biopsy, there is a monotonous infiltration of medium-sized cells with round nuclei, multiple nucleoli, and basophilic cytoplasm with vacuoles. The proliferation rate is 100%, and tingible body macrophages give rise to the classic “starry sky” appearance of this tumor (Fig. 108-4). Tumor cells are positive for B-cell antigens CD19 and CD20 and surface immunoglobulin. They are also uniformly positive for CD10 and BCL6 but negative for BCL2. Endemic BLs are EBV positive, whereas the majority of nonendemic BLs are EBV negative. BL is associated with a translocation involving MYC on chromosome 8q24 in >95% of the cases. The most common partners are chromosomes 14, 2, or 22, rearrangements that produce fusions of MYC with either the IgH (80%), kappa (15%), or lambda (5%) light chain genes, respectively.

While exquisitely chemosensitive, it is imperative that treatment for BL be initiated quickly given the rapid doubling time and high morbidity of this disease. There are several effective intensive combination chemotherapy regimens, all of which incorporate high doses of cyclophosphamide. Prophylactic therapy to the CNS is mandatory. Cure can be expected in 80–90% of patients when treated promptly and correctly. Dose-adjusted EPOCH-R (rituximab, infusional etoposide/vincristine/doxorubicin, cyclophosphamide, prednisone) is highly effective. Salvage therapy has been generally ineffective in patients whose disease progresses after upfront therapy, emphasizing the

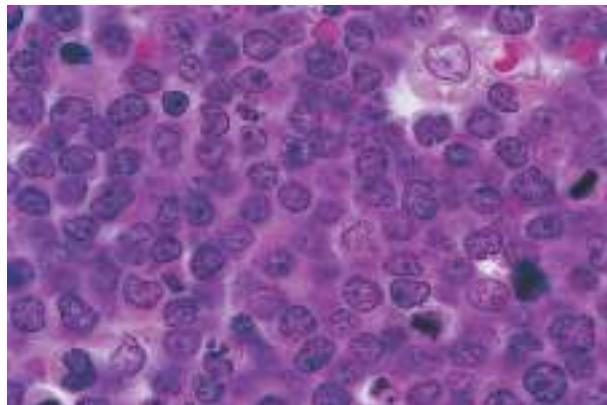


FIGURE 108-4 Burkitt's lymphoma. The neoplastic cells are homogeneous, medium-sized B cells with frequent mitotic figures, a morphologic correlate of high growth fraction. Reactive macrophages are scattered through the tumor, and their pale cytoplasm in a background of blue-staining tumor cells gives the tumor a so-called starry sky appearance.

importance of the initial treatment approach and referral to a tertiary cancer center with experience treating this disease.

Diffuse Large B-Cell Lymphoma DLBCL is the most common histologic subtype of NHL diagnosed, representing about one-third of all cases. Previously felt to be “one disease,” it is now recognized as a heterogeneous collection of multiple entities. It is slightly more common in Caucasians and men, and the median age at diagnosis is 64. The relative risk (RR) of DLBCL is higher among people with affected first-degree relatives (RR 3.5-fold), and patients with congenital or acquired immunodeficiency, patients on immunosuppression, and patients with autoimmune disorders also have a higher risk of developing DLBCL, often EBV-related. The majority of patients present with advanced-stage disease, with only 30–40% of patients having stage I or II disease; 40% of patients will have “B” symptoms, and 50% of patients will have an elevated LDH. Up to 40% of patients will have involvement of non-lymph node sites including bone marrow, CNS, gastrointestinal tract, thyroid, liver, and skin. Patients with extensive bone marrow involvement or involvement of the testes, breast, kidney, adrenal gland, paranasal sinus, or epidural space are at increased risk of CNS dissemination.

The tumor consists of a diffuse proliferation of large, atypical lymphocytes with a high proliferative index (Fig. 108-5). These cells typically express the B-cell antigens CD19, CD20, and CD79a. Expression of CD10 and BCL6 is consistent with the tumor cell being of germinal center origin (GCB), while the expression of MUM1 corresponds with

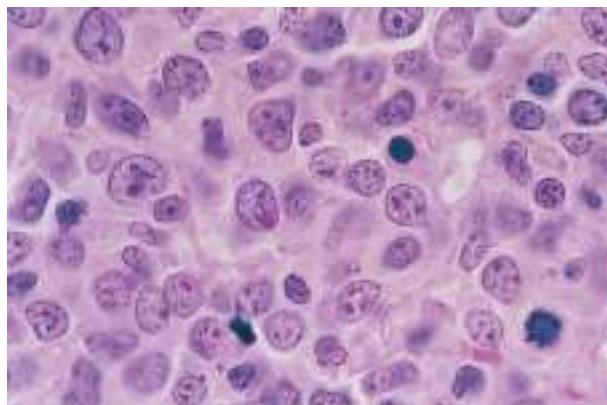


FIGURE 108-5 Diffuse large B-cell lymphoma. The neoplastic cells are heterogeneous but predominantly large cells with vesicular chromatin and prominent nucleoli.

the non–germinal center or activated B cell (ABC) subtype. BCL2 is overexpressed in anywhere from 25 to 80% of DLBCLs, whereas BCL6 is positive in more than two-thirds of cases, either as the result of translocations, gain of copy number, or promoter mutations. MYC is rearranged in 10% of DLBCLs, and 20% of MYC-rearranged cases have concurrent BCL2 or BCL6 rearrangements, a combination referred to as “double-hit lymphoma.” These double-hit lymphomas are associated with an extremely poor prognosis with a median OS of only 12–18 months. Amplification and/or overexpression of MYC independent of rearrangements or amplification have also been described and are also associated with a poor, albeit better, prognosis.

Combination chemotherapy offers potentially curative therapy for DLBCL, regardless of the stage. The addition of the anti-CD20 antibody rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) improved survival beyond CHOP alone and is the standard first-line chemotherapy for this disease. For patients with early-stage disease localized to a radiation field, treatment options include full-course chemotherapy with R-CHOP every 3 weeks for six cycles or abbreviated chemotherapy for three to four cycles followed by involved field radiotherapy. For advanced-stage DLBCL, therapy is with a full course of chemotherapy. On average, 60–65% of patients with DLBCL can be expected to be cured with this approach, and the likelihood of cure is predicted by the IPI, gene expression profile cell of origin, and/or MYC cytogenetics and expression. Several studies have investigated alternative anthracycline-containing chemotherapy regimens and/or consolidation autologous stem cell transplantation in first remission for higher-risk disease without improvement over R-CHOP alone. Dose-adjusted R-EPOCH is one such regimen. Although this regimen did not appear to be better than R-CHOP for DLBCL in one multicenter clinical trial, it is often used to treat primary mediastinal large B-cell lymphoma and double-hit DLBCL based on results from phase 2 and retrospective studies, respectively. CNS prophylaxis with either intrathecal chemotherapy or high-dose systemic methotrexate and leucovorin rescue should be considered for patients with high risk of CNS dissemination. This includes patients with primary testicular involvement and breast involvement, as well as patients with several IPI risk factors and diffuse bone marrow involvement, renal involvement, or adrenal involvement. The use of CNS prophylaxis for disease involving the paranasal sinuses or the epidural space is less clear but may be considered.

Over one-third of patients will either have primary refractory disease or disease that relapses after first-line chemotherapy. These patients may still be cured with salvage chemotherapy regimens followed by autologous stem cell transplantation. However, patients with a poor performance status or advanced age who are not candidates for such an approach are often managed with palliative intentions. Radiation to symptomatic areas of disease can be transiently helpful. Less intensive chemotherapy with drugs like gemcitabine, cytarabine, or bendamustine can help control disease and symptoms for a limited period of time. These patients should be referred for clinical trials when applicable. For patients in whom more aggressive therapy is an option, treatment is with combination chemotherapy using various combinations of drugs primarily in order to identify patients with chemosensitive disease. Patients with chemosensitive disease have the greatest likelihood of benefiting from high-dose chemotherapy and autologous stem cell transplant, which improves response duration and survival over salvage chemotherapy alone and leads to long-term disease-free survival in 40–50% of patients. For patients with chemorefractory disease, chimeric antigen receptor T cells (CAR-T cells) offer a potentially curative option. For this therapy, T cells are collected from a patient and are then genetically modified to express a receptor that will bind to a surface antigen expressed on the patient’s own tumor cells. In the case of B cell malignancies, CD19 has been targeted most commonly. After infusion, autologous CAR-T cells home to sites of disease and also persist over time. The CARs consist of an extracellular antigen recognition domain (typically a single chain Fv variable fragment from a monoclonal antibody) linked via a transmembrane domain to an intracellular signaling domain (usually the CD3 ζ endo-domain), resulting in the redirection of T cell specificity toward target

antigen-positive cells, and one or more costimulatory domains including CD28, 4-1BB, or OX40 to enhance cytokine secretion and effector cell expansion and prevent activation-induced apoptosis and immune suppression by tumor-related metabolites. Anti-CD19 CAR-T cells have been approved for the treatment of relapsed/refractory DLBCL following two prior systemic therapies. This would include patients with chemotherapy-insensitive disease following second-line salvage chemotherapy for whom autologous stem cell transplant is not an option or patients who relapse after autologous stem cell transplant. In this setting, the response rate of CAR-T cells is >80%, with >50% of patients achieving a complete response. These responses appear to be durable, with 40% of patients in remission at long-term follow-up.

Targeting CD19 with the monoclonal antibody tafasitamab in combination with lenalidomide also yielded high response rates and prolonged response durability, leading to approval of this regimen in relapsed disease. Reports of ongoing studies exploring bispecific antibodies that target CD20 on malignant B cells while also binding CD3 on T cells, thereby activating T cells to attack the malignant B cell, have been very promising in both aggressive and indolent B-cell NHL. The antibody-drug conjugate polatuzumab vedotin, which combines an anti-CD79b antibody with the microtubule toxin monomethyl auristatin E (MMAE), was approved for the treatment of relapsed/refractory DLBCL in combination with bendamustine and rituximab based on the results of a randomized clinical trial against bendamustine and rituximab alone. The oral drug selinexor, a selective inhibitor of nuclear export, has modest activity in relapsed DLBCL as a single agent and is approved for this indication. These drugs, along with drugs such as lenalidomide alone or ibrutinib, should be viewed as a bridge to allogeneic stem cell transplant for eligible patients in whom curative therapy is the goal because they are unlikely to lead to durable or permanent remissions.

Other large B-cell lymphomas include intravascular large B-cell lymphoma, T-cell/histiocyte-rich large B-cell lymphoma, EBV-positive DLBCL of the elderly, and ALK-positive large B-cell lymphoma. Patients with the latter two diseases tend to have a poor prognosis, whereas the addition of rituximab to CHOP chemotherapy has dramatically improved outcomes with intravascular large B-cell lymphoma, and the outcomes in T-cell/histiocyte-rich large B-cell lymphoma are similar to DLBCL. R-CHOP remains the treatment of choice for each of these lymphomas.

Follicular Lymphoma FLs are the second leading NHL diagnosis in the United States and Europe and make up 22% of NHLs worldwide and at least 30% of NHLs diagnosed in the United States. This type of lymphoma can be diagnosed accurately on morphologic findings alone and has been the diagnosis in the majority of patients in therapeutic trials for “low-grade” lymphoma in the past.

Evaluation of an adequate biopsy by an expert hematopathologist is sufficient to make a diagnosis of FL. The tumor is composed of small cleaved and large cells in varying proportions organized in a follicular pattern of growth (Fig. 108-6). Confirmation of B-cell immunophenotype (monoclonal immunoglobulin light chain, CD19, CD20, CD10, and BCL6 positive, and CD5 and CD23 negative) and the existence of the t(14;18) and abnormal expression of BCL2 protein are confirmatory. While >85% of FLs will harbor a t(14;18) and overexpress the antiapoptotic protein BCL2, this genetic event is necessary but not sufficient for malignant transformation of the B lymphocytes, and multiple genetic events are required for the development of FL. Studies have identified the most common recurrent genetic events in FL, and they included mutations in several epigenetic modifying genes, including *MLL2*, *EZH2*, *CREBBP*, and *EP300*. The major differential diagnosis is between lymphoma and reactive follicular hyperplasia. The coexistence of DLBCL must be considered. Patients with FL are often subclassified, or graded, into those with predominantly small cells, those with a mixture of small and large cells, and those with predominantly large cells. The WHO classification adopted grading from I to III based on the number of centroblasts, or large cells, counted per high-power field (hpf): grade I, from 0 to 5 centroblasts/hpf; grade II, from 6 to 15 centroblasts/hpf; and grade III, >15 centroblasts/hpf. Grade III has

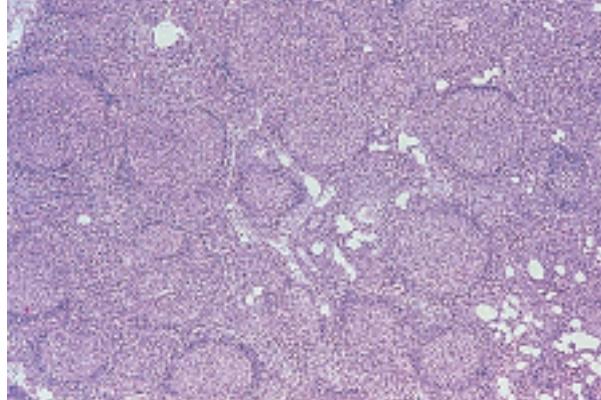


FIGURE 108-6 Follicular lymphoma. The normal nodal architecture is effaced by nodular expansions of tumor cells. Nodules vary in size and contain predominantly small lymphocytes with cleaved nuclei along with variable numbers of larger cells with vesicular chromatin and prominent nucleoli.

been subdivided into grade IIIa, in which centrocytes predominate, and grade IIIb, in which there are sheets of centroblasts. While this distinction cannot be made simply or very reproducibly, these subdivisions do have prognostic significance. Patients with FL with predominantly large cells have a higher proliferative fraction, progress more rapidly, and have a shorter OS with simple chemotherapy regimens. Grade IIIb FL is an aggressive disease and considered most similar to DLBCL and treated as such with curative intent.

The most common presentation for FL is with new, painless lymphadenopathy. Multiple sites of lymphoid involvement are typical, and unusual sites such as epitrochlear nodes are sometimes seen. However, essentially any organ can be involved, and extranodal presentations do occur. Most patients do not have an elevated LDH or fevers, night sweats, or weight loss, although histologic transformation to DLBCL does occur at a rate of 3% per year and can be associated with these signs or symptoms. As discussed previously, prognosis is best predicted by the FLIPI. Staging is typically done with CT scans of the chest, abdomen, and pelvis, as well as the neck if neck disease is suspected, although PET/CT scans can be helpful in cases where disease transformation is suspected, as transformed disease will be more FDG avid than indolent disease, or for confirmation of early-stage disease, where definitive local therapy with radiation may be considered.

Although FL is highly sensitive to chemotherapy and radiotherapy, these therapies are usually not ultimately curative, except in the setting of early-stage disease. If the disease can be encompassed in a radiation field, involved field radiotherapy at a dose of 24–30 Gy may be curative, with 5-, 10-, and 15-year freedom from treatment failure rates of 72, 46, and 39%, and overall 5-, 10-, and 15-year survival rates of 93, 75, and 62%, respectively. If radiation therapy would not be tolerated or if a patient prefers not to receive radiation, observation is a reasonable alternative with a median time to treatment not reached at 7 years of follow-up in one study. Many of these patients are diagnosed incidentally or at a time when their lymphoma is not causing symptoms or signs of organ function impairment. Numerous studies have shown that treating patients with asymptomatic disease does not improve survival compared with a program of close observation, with treatment reserved for symptomatic disease progression or organ dysfunction. Thus, asymptomatic patients should be observed.

When treatment is indicated, there are a variety of treatment options, including the use of the monoclonal antibody against CD20, rituximab, alone or in combination with chemotherapy. Treatment decisions are often determined by the indication for treatment and/or by the volume of disease being treated. For patients requiring therapy for inflammatory or autoimmune phenomenon thought to be driven by FL, or for patients with low-volume disease, single-agent rituximab is associated with a response rate of 70% and a median response

duration of >2 years. This response duration is improved with the addition of maintenance rituximab following a favorable response to rituximab induction therapy. For patients with a larger volume of disease at the time of treatment initiation, the addition of rituximab to chemotherapy regimens such as CHOP or cyclophosphamide, vincristine, and prednisone (CVP) has improved survival in this disease. The combination of bendamustine and rituximab (BR) has been compared to R-CHOP and results in longer response duration and less toxicity. Thus, BR has become the standard of care for the first-line therapy of medium- to high-volume FL. Similarly, the addition of maintenance rituximab following a good response to R-CHOP or R-CVP improves response duration when used in newly treated FL patients. A newer anti-CD20 antibody, obinutuzumab, has been tested in combination with chemotherapy in a randomized trial against rituximab plus chemotherapy in previously untreated FL. The obinutuzumab combinations resulted in improvements in minimal residual disease (MRD) negativity as well as progression-free survival at the expense of more infection and infusion reactions. Based on these results, both rituximab plus chemotherapy and obinutuzumab plus chemotherapy are options for untreated FL in need of treatment. The superiority of one over the other has not been established.

In patients with FL, the disease nearly always recurs following therapy, after which retreatment is again reserved for symptomatic disease or disease interfering with organ function. Single-agent rituximab or alternative chemotherapy regimens, with both rituximab and obinutuzumab, can again be employed. Both autologous and allogeneic hematopoietic stem cell transplantsations yield high complete response rates in patients with relapsed FL, and long-term remissions can occur in 40 and 60% of patients, respectively. The latter is associated with considerable treatment-related morbidity and mortality and so is usually reserved for patients with multiply relapsed FL that is no longer responsive to chemotherapy. More targeted oral therapies like lenalidomide and the PI3 kinase inhibitors idelalisib, duvelisib, and copanlisib are active in both untreated and relapsed FL. Inhibitors of one of the most commonly mutated genes in FL, *EZH2*, have activity in both *EZH2* mutated as well as unmutated lymphomas, and one, tazemetostat, is approved for this indication. Anti-CD19-directed CAR-T cell therapies are also being tested in FL, with complete responses seen in >80% of patients with multiply relapsed disease, and with many of those responses proving durable, albeit with limited follow-up. Longer follow-up is needed to determine if this may be a definitive treatment strategy for a subset of relapsed FL patients. On average, most patients will live with FL for 15–20 years, a number that is increasing given our improved understanding of the genetics and microenvironment of FL and the increasing number of drugs and therapies being tested in this disease. However, in addition to a high-risk FLIPI, patients who do not have a complete metabolic response by PET/CT scanning to their primary therapy and patients who relapse within 2 years of the completion of their primary chemotherapy tend to do poorly with chemotherapy.

Patients with FL have a high rate of histologic transformation to DLBCL (3% per year). This is recognized 40% of the time during the course of the illness by repeat biopsy and is present in almost all patients at autopsy. This transformation is usually heralded by rapid growth of lymph nodes—often localized—and the development of systemic symptoms such as fevers, sweats, and weight loss. When this happens in patients who have had previously untreated FL, treatment with R-CHOP chemotherapy, as for DLBCL, can be curative for the aggressive component while the FL may eventually recur. In patients with previously treated FL that transforms to DLBCL, prognosis is poor, and successful therapy with an aggressive combination chemotherapy regimen should be consolidated with an autologous stem cell transplant. Finally, as discussed previously, grade IIIb FL is more similar to DLBCL than it is to FL and should be treated as such.

Marginal Zone Lymphoma The second most common indolent B-cell NHL is MZL. There are three main types: splenic MZL, extranodal MZL of MALT, and nodal MZL.

Nodal MZL most closely resembles FL clinically, and much of the way we manage and treat it is based on studies done in FL. Tumor biopsies in this disease show parafollicular and perivascular infiltration by monocyteoid-appearance atypical lymphocytes with folded nuclear contours that are positive for CD19, CD20, and CD79a but negative for CD10 and largely negative for CD5. Some cases can have plasmacytoid differentiation and can be associated with a monoclonal expression of kappa or lambda light chains and with small monoclonal immunoglobulin spikes. Treatment is often similar to that of FL, with the exception that the BTK inhibitor ibrutinib is highly active in this disease, while largely disappointing in FL, and is a good treatment option for relapsed nodal MZL as well as other MZL subtypes.

Splenic MZL is largely a disease of older Caucasian patients; infection with hepatitis C is a risk factor for this disease, and treatment of hepatitis C can result in regression of the lymphoma. Patients present with a lymphocytosis with or without cytopenias and splenomegaly. Bone marrow involvement is common. Diagnosis can be made by flow cytometry of the peripheral blood; malignant lymphocytes will be positive for surface immunoglobulin, CD19, and CD20 and will generally lack CD5 and CD10. On peripheral smear, they have small nuclei and abundant cytoplasm with "shaggy" or villous projections. It can be differentiated from HCL by the absence of CD25, CD103, and annexin A1. Recurrent cytogenetic abnormalities include trisomy 3 and abnormalities of chromosome 7q. Therapy is indicated for symptomatic disease or significant cytopenias. Splenectomy is reasonable for selected patients with excellent relief of symptoms and cytopenias. Splenectomy is associated with an overall response rate of 85% and estimated progression-free survival and OS rates at 5 years of 58 and 77%, respectively. Single-agent rituximab can improve splenomegaly and cytopenias in >90% of patients. In a study of induction with weekly rituximab followed by maintenance, the response rate was 95%, with overall and progression-free survival rates at 5 years of 92 and 73%, respectively. Other options for therapy at relapse are similar to those used for FL and include retreatment with rituximab, alkylating agents, and purine analogues in combination with rituximab. The survival rate of patients is in excess of 70% at 10 years.

MALT lymphoma is an MZL lymphoma of extranodal tissue, most commonly the stomach, but other common sites include the skin, salivary glands, lung, small bowel, ocular adnexa, breasts, bladder, thyroid, dura, and synovium. It is associated with states of chronic inflammation due to either autoimmune diseases like Sjögren's syndrome or Hashimoto's thyroiditis or chronic infections with organisms like *H. pylori* (gastric), *Borrelia burgdorferi* (skin), *C. psittaci* (conjunctiva), *C. jejuni* (intestines), and hepatitis C virus. The essential pathologic feature of MALT lymphoma is the presence of lymphoepithelial lesions, which result from invasion of mucosal glands and crypts by the neoplastic lymphocytes. These cells are positive for CD19, CD20, and CD79a and negative for CD5 and CD10. Recurrent cytogenetic abnormalities include t(11;18), t(14;18), t(1;14), t(3;14), and trisomy 8. The t(11;18) is most common, occurring in up to 50% of MALT lymphomas. It results in the fusion of the apoptosis inhibitor 2 (*API2*) gene and the *MALT1* gene, resulting in activation of nuclear factor- κ B (NF- κ B). Unlike other indolent B-cell lymphomas, MALT lymphomas present most commonly with stage I or II disease. In these cases, radiation therapy may be curative. Alternatively, patients may respond to antibiotics for the associated underlying infection. Treatment of symptomatic or organ-impairing relapsed, refractory, or advanced-stage disease is similar to approaches used in FL with chemotherapy, immunotherapy, or chemoimmunotherapy.

Lymphoplasmacytic Lymphoma About 1% of all NHLs will be LPLs, which are indolent B-cell NHLs with lymphoplasmacytic differentiation, most commonly associated with a monoclonal IgM paraprotein. Nearly all patients will have stage IV disease at diagnosis with bone marrow involvement. Patients with high levels of circulating IgM paraproteins constitute a specific entity known as Waldenström's macroglobulinemia and can have symptoms due to hyperviscosity as a result of the circulating IgM. Activating mutations in MYD88, an

adaptor protein that is involved in signaling downstream of the Ig receptor leading to NF- κ B activation, are present in >90% of cases. Tumor biopsies are notable for proliferation of small lymphocytes, lymphoplasmacytic cells, and plasma cells, and malignant lymphocytes are positive for CD19, CD20, and surface IgM but generally negative for CD5 and CD10. Like the other indolent NHLs, treatment is indicated for disease that causes symptoms or interferes with organ function; hyperviscosity related to elevated serum IgM and paraneoplastic neuropathy are additional indications for therapy. Single-agent rituximab may be useful for low-volume disease but can be associated with a transient rise in serum IgM concentrations that can cause or exacerbate hyperviscosity. Chemoimmunotherapy with regimens such as BR and rituximab, cyclophosphamide, and dexamethasone is active, as are myeloma therapies such as bortezomib. Ibrutinib in combination with rituximab is highly active in this disease and is an option for both previously untreated and relapsed disease. Given that 85% of IgM remains intravascular, acute relief of hyperviscosity symptoms can be obtained by plasmapheresis. For recurrent disease, one can often use agents that were previously used. For patients with more refractory LPL, the mammalian target of rapamycin (mTOR) inhibitor everolimus and the oral BTK ibrutinib are active. Selected patients with relapsed disease are considered for high-dose therapy with autologous or allogeneic stem cell transplantation. The results seen are similar to those of other indolent lymphomas.

Mantle Cell Lymphoma MCL composes 6% of NHLs. It is an intermediate-grade lymphoma that, like the indolent B-cell NHLs, is not curable with conventional therapies but, like the aggressive lymphomas, often requires more aggressive chemoimmunotherapy regimens with or without an autologous stem cell transplant to achieve a reasonable response duration. This therapy is not curative, however, and median survival with this disease is on the order of 5–10 years. An exception to this is a more indolent SOX11 variant that often presents with circulating disease with splenomegaly but without significant lymphadenopathy and with a low Ki67 (<10%). This subset behaves more like the indolent B-cell NHLs and can be observed until treatment is indicated by symptoms or organ function impairment. Similarly, there is a blastic variant with a high Ki67 index that is associated with a poor prognosis and a median OS of only 18 months. For other patients, prognosis is best predicted by the biologic MCL International Prognostic Index (MIPI), which factors in age, performance status, LDH, white blood cell count, and Ki67 expression to determine a risk group. This disease is more common in men, and the average age of diagnosis is 63. MCLs with a mutation in *TP53* or a complex karyotype are particularly high risk as well. Over two-thirds of patients will have stage IV disease, mostly with bone marrow and peripheral blood involvement, at the time of diagnosis. Another common extranodal site of involvement is the gastrointestinal tract, where diffuse lymphomatous polyposis may be seen.

The pathognomonic cytogenetic finding in MCL is t(11;14), which brings the gene for the cell cycle control protein cyclin D1 under the control of the immunoglobulin heavy chain gene promoter on chromosome 14. This translocation is present in >90% of cases. The remaining cases usually overexpress cyclin D2, cyclin D3, or cyclin E. Tumor cells also are positive for B cell markers CD19 and CD20, as well as CD5. They usually lack CD10 and CD23.

Therapies for MCL are evolving. Patients with localized disease might be treated with combination chemotherapy followed by radiotherapy; however, these patients are exceedingly rare. Similarly, patients with the indolent variant can be observed until disease progresses to cause symptoms or signs of organ function impairment. For the usual presentation with disseminated disease, standard lymphoma treatments like R-CHOP have been unsatisfactory, with the minority of patients achieving complete remission. The addition of high-dose cytarabine to an R-CHOP-like backbone with or without consolidation autologous stem cell transplantation in first remission has improved progression-free survival, but it has not elicited cures in this disease. These include the Nordic regimens and R-HyperCVAD (rituximab,

cyclophosphamide, vincristine, doxorubicin, dexamethasone, cytarabine, and methotrexate). BR has activity in this disease and is more effective and better tolerated than R-CHOP. Newer studies with short follow-up suggest that strategies that combine BR with cytarabine with or without autologous stem cell transplant may be effective and well tolerated. Maintenance rituximab, following a good response to induction chemotherapy or after autologous stem cell transplant, also improves outcomes over observation alone. For relapsed disease, the BTK inhibitors ibrutinib and acalabrutinib have single-agent activity with a response rate of almost 70% but a response duration of only 18 months. These drugs are being explored in combination with chemotherapy as well as with the BCL2 antagonist venetoclax. Anti-CD19-directed CAR-T cell therapies are approved for the treatment of relapsed/refractory MCL; two-thirds of patients who had progressed after chemoimmunotherapy (with or without an autologous stem cell transplant) and BTK inhibition have achieved complete responses, many of which are durable through limited follow-up. As in FL, longer follow-up is needed to determine if some of these patients may be cured, which would make this the only curative therapy for this disease outside of an allogeneic stem cell transplantation. Drugs such as lenalidomide, venetoclax, bortezomib, and temsirolimus can similarly induce transient partial responses. Appropriate patients who respond to salvage therapy, with the exception of CAR-T cell therapy, should be considered for allogeneic stem cell transplant, which can lead to long-term disease-free survival in 30–50% of patients.

MATURE PERIPHERAL T CELL DISORDERS

Mature T cell disorders include cutaneous lymphomas, such as mycosis fungoïdes, and the PTCLs, some of which are distinguished based on specific clinical presentations or contexts or by molecular or biologic features, but many of which fall into the category of PTCL not otherwise specified (NOS). T-cell NHLs are significantly rarer than B-cell NHLs, and as such, our understanding of their biology is less advanced and our therapies are less well developed. While some T-cell lymphomas, like mycosis fungoïdes, can behave indolently and some, like ALK-positive ALCL, can be cured with chemotherapy, the majority are associated with a poor prognosis. The advent of genomic technologies is enhancing our ability to understand the genetic and biologic basis of these neoplasms.

Mycosis Fungoïdes Mycosis fungoïdes is also known as cutaneous T-cell lymphoma. This lymphoma is more often seen by dermatologists than internists. The median age of onset is in the mid-fifties, and the disease is more common in males and in blacks.

Mycosis fungoïdes is an indolent lymphoma, with patients often having several years of eczematous or dermatitic skin lesions before the diagnosis is finally established. The skin lesions progress from patch stage to plaque stage to cutaneous tumors. Early in the disease, biopsies are often difficult to interpret, and the diagnosis may only become apparent by observing the patient over time. Adenopathy may reflect involvement with mycosis fungoïdes or be read as dermatopathic change. In advanced stages, the lymphoma can spread to lymph nodes and visceral organs. Patients with this lymphoma may develop generalized erythroderma and circulating tumor cells, called *Sézary's syndrome*.

Rare patients with localized early-stage mycosis fungoïdes can be cured with radiotherapy, often total-skin electron beam irradiation. More advanced disease has been treated with topical glucocorticoids, topical nitrogen mustard, phototherapy, psoralen with ultraviolet A (PUVA), extracorporeal photopheresis, retinoids (bexarotene), electron beam radiation, interferon, antibodies, fusion toxins, histone deacetylase inhibitors, brentuximab (for CD30+ disease), and systemic cytotoxic therapy. Mogamulizumab, an anti-CCR4 antibody, has activity in this disease and has been approved by the U.S. Food and Drug Administration for this indication. Unfortunately, these treatments are palliative.

Peripheral T-Cell Lymphoma, Not Otherwise Specified PTCLs include a number of entities, which constitute 15% of all NHLs in adults. PTCL NOS, which composes 6% of all NHLs, is the

term used for cases that are not other entities defined in the WHO classification. Named varieties include ALCL, angioimmunoblastic T-cell lymphoma (AITL), hepatosplenic T-cell lymphoma, enteropathy-associated T-cell lymphoma, and subcutaneous panniculitis T-cell lymphoma. PTCL NOS is a disease of older individuals, with a median age at presentation of 65, and the majority of patients will have advanced-stage disease at diagnosis, with involvement of the bone marrow, liver, spleen, and skin being common. Associated "B" symptoms and pruritis are also common. These lymphomas can be associated with a reactive eosinophilia as well as hemophagocytic syndrome. The IPI has been applied to PTCL NOS and provides some assessment of outcomes, but even the low-risk group has a median OS of just >2 years.

This diagnostic category is a collection of heterogeneous lymphomas that vary widely and lack typical findings of other specific PTCL subgroups. Because of this heterogeneity, histology, immunophenotype, and genetics are variable. Often lymph nodes are effaced by atypical lymphoid cells of various sizes, sometimes associated with vascular proliferation or an infiltrate of eosinophils and/or macrophages. As most of these lymphomas behave aggressively, note is often made of mitotic and apoptotic figures as well as geographic necrosis. The cells often are positive for CD3, and the majority of PTCL NOS is positive for CD4 rather than CD8, but some are negative for both markers. There can be loss of more mature T-cell markers like CD5 and CD7, and this is associated with a more aggressive course. There are some recurrent translocations, including t(7;14), t(11;14), inv(14), and t(14;14), all of which involve the TCR genes.

The most common primary therapy for PTCL NOS involves a CHOP-like chemotherapy backbone—either CHOP alone or CHOP in combination with etoposide (CHOEP). The latter may provide the most benefit to younger patients and patients with more favorable disease risk factors. Brentuximab in combination with cyclophosphamide, doxorubicin, and prednisone (CHP) has been tested in a randomized clinical trial against CHOP in CD30+ T-cell lymphomas; progression-free survival was improved with the brentuximab-containing arm, and this was most pronounced for patients with ALCL (see below). Autologous stem cell transplant has been investigated for patients in their first remission and does seem to improve progression-free survival in certain contexts. Drugs such as gemcitabine, bendamustine, and pralatrexate have activity in relapsed disease, as do the histone deacetylase inhibitors romidepsin and belinostat. The PI3 kinase inhibitor duvelisib is being investigated in these diseases with early signals of activity. All of these agents are associated with transient responses in a minority of patients. Patients should be considered for clinical trials. For patients who do achieve remission, reduced-intensity allogeneic stem cell transplantation can yield long-term nonrelapse survival rates of 40–50%.

Angioimmunoblastic T-Cell Lymphoma AITL constitutes 20% of T-cell NHLs and 4% of all NHLs diagnosed. Patients present with a variety of signs and symptoms, most often including lymphadenopathy, hepatosplenomegaly, "B" symptoms, rash, polyarthritis, and hemolytic anemia. Over 80% of patients have advanced-stage disease at diagnosis, and bone marrow involvement is common. Polyclonal hypergammaglobulinemia is common, as are elevated LDH, eosinophilia, a positive Coombs test, and opportunistic infections.

On biopsy, lymph nodes are effaced by a polymorphous infiltrate of lymphocytes, ranging in size and shape, and of immunoblasts. The neoplastic lymphocytes are positive for CD3 as well as CXCL13, PD-1, CD10, and BCL6, most closely resembling CD4-positive follicular helper T cells. There is an expanded follicular dendritic cell network surrounding tumor cells. Scattered immunoblasts are often EBV positive and may give rise to secondary EBV-positive B-cell lymphomas at a later time. Genetic analysis of this disease has revealed recurrent mutations in *TET2* (76%), *DNMT3* (33%), and *IDH2* (20%).

There is a subset of AITL that can remit with immunosuppression with agents like glucocorticoids or methotrexate. Most patients, however, will need combination chemotherapy with regimens like those used in PTCL NOS. Median response duration is short, and median OS

is only 15–36 months. Treatment of relapsed disease is similar to that of relapsed PTCL NOS.

Anaplastic Large-Cell Lymphoma ALCL is the next most common T-cell lymphoma after AITL but is more common in children, accounting for up to 10% of pediatric lymphomas. Approximately 40–60% of cases harbor t(2;5), which fuses a portion of the nucleolar protein nucleophosmin-1 (*NPM1*) gene to a part of the anaplastic lymphoma kinase (*ALK*) gene, the product of which has constitutive tyrosine kinase activity. These patients have a much more favorable prognosis compared to ALK-negative ALCL, akin to that of DLBCL. There is an additional, more indolent and favorable subtype that occurs in the breast tissue of patients with breast implants, and there is a cutaneous variant. In general, this is a disease that is more common in men. ALK-positive disease is a disease of younger patients, with a median age at diagnosis of 34 years, whereas the median age at diagnosis of ALK-negative patients is 58. With the exception of the cutaneous variant and the variant associated with breast implants, most patients present with rapidly growing lymphadenopathy with or without extranodal involvement; “B” symptoms are common.

Most cases of ALCL involve large atypical lymphocytes with horsehoe-shaped nuclei with prominent nucleoli (“hallmark” cells). Tumor cells tend to be localized within the lymph node sinuses, and almost all are positive for CD30 but negative for CD15. A majority will also express CD3, CD25, CD43, and CD4. ALK-rearranged ALCL can be diagnosed by fluorescence in situ hybridization (FISH) cytogenetics for t(2;5) or by immunohistochemical staining for ALK.

ALCL is generally treated with CHOP, although like PTCL NOS, CHOEP may benefit younger patients, particularly with ALK-positive disease. Overall, ALCL has a better prognosis than PTCL, and this is particularly true for ALK-positive disease, which has an 8-year OS rate of 82%, versus 49% for ALK-negative disease. Relapsed ALK-positive ALCL is treated similarly to relapsed DLBCL, with salvage combination chemotherapy to identify chemotherapy sensitivity followed by autologous stem cell transplant. For patients with chemotherapy-insensitive disease or for ALK-negative disease, the conjugated anti-CD30 antibody to MMAE brentuximab is highly active, with a response rate of 86% and a complete response rate of 57%. As mentioned earlier, brentuximab in combination with CHP chemotherapy is an approved frontline regimen for the treatment of CD30+ T-cell lymphomas, including ALCL. The ALK inhibitors, including crizotinib, are active in refractory ALK-positive ALCL with excellent outcomes.

Other PTCL Subtypes Enteropathy-associated T-cell lymphoma, hepatosplenic T-cell lymphoma, and subcutaneous panniculitis-like T-cell lymphoma are other less common PTCL subtypes. *Enteropathy-type intestinal T-cell lymphoma* is a rare disorder. Type I occurs in patients with a history of gluten-sensitive enteropathy and is associated with HLADQA1 0501, DQB1 0201; a gluten-free diet can prevent the development of this lymphoma. Type II is not associated with celiac disease and may be a separate disease entity. Patients are frequently cachectic and sometimes present with intestinal perforation. The prognosis is poor, with a median survival of 10 months. Therapy is often with combination chemotherapy, including high-dose methotrexate, and autologous stem cell transplant in first remission.

Hepatosplenic γδ T-cell lymphoma is a systemic illness that presents with sinusoidal infiltration of the liver, spleen, and bone marrow by malignant T cells. Tumor masses generally do not occur. The disease is associated with systemic symptoms and is often difficult to diagnose. Recurrent genetic events include isochromosome 7q and trisomy 8. Treatment outcome is poor, but regimens that include ifosfamide, such as ifosfamide, carboplatin, and etoposide (ICE) or ifosfamide, etoposide, and cytarabine (IVAC), are associated with better outcomes in small series of patients. Responding patients should be considered for allogeneic stem cell transplantation.

Subcutaneous panniculitis-like T-cell lymphoma is a rare disorder that is often confused with panniculitis. Patients present with multiple subcutaneous nodules, which progress and can ulcerate.

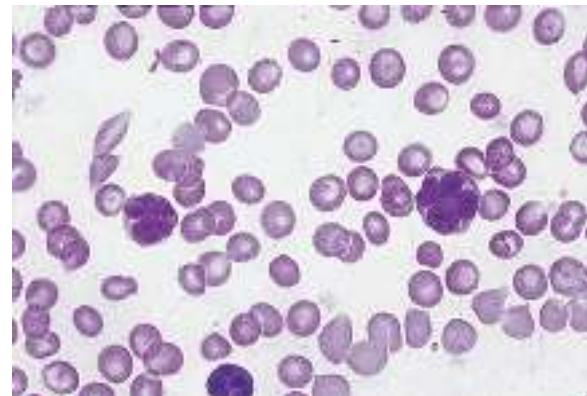


FIGURE 108-7 Adult T-cell leukemia/lymphoma. Peripheral blood smear showing leukemia cells with typical “flower-shaped” nucleus.

There is a more indolent form that tends to express α/β TCRs and can be managed with immune suppression, whereas lymphomas that express γ/δ TCRs are more aggressive and are associated with a worse prognosis and coincident hemophagocytic syndrome. This is a disease of young men in their fifth and sixth decades of life. Patients with aggressive disease are managed with multiagent chemotherapy, and responding patients should be considered for allogeneic stem cell transplantation.

Adult T-Cell Leukemia/Lymphoma Adult T-cell leukemia/lymphoma (ATLL) is a disease that is most prevalent in Japan and the Caribbean basin. It is a neoplasm that is driven by HTLV-1, often contracted through the breast milk of infected mothers. The average age at diagnosis is 60, so there is a long latency between viral infection and viral transformation, and only 4% of infected patients will develop the disease. This suggests that HTLV-1 may not be sufficient to cause the malignant phenotype. There are four disease variants: acute (60% of patients), lymphomatous (20% of patients), chronic (15% of patients), and smoldering (5% of patients); prognosis varies across these groups, with median survival times of 6, 10, and 24 months, and not yet reached, respectively. Presentation depends on the subtype, but most commonly, patients present with circulating disease and bone marrow involvement, hypercalcemia, lytic bone lesions, lymphadenopathy, hepatosplenomegaly, skin lesions, and opportunistic infections.

The pathognomonic finding is the malignant “flower cell” that is positive for CD4 and CD25, as well as CD2, CD3, and CD5 but lacking CD7 (Fig. 108-7). Combination chemotherapy is generally used, but for patients fortunate enough to respond, response durations are very short. Other active agents in this disease include the antiretroviral agent zidovudine, interferon α, and arsenic. In any patients who do respond to therapy, allogeneic stem cell transplant should be considered.

Extranodal NK/T-Cell Lymphoma, Nasal Type Extranodal NK/T-cell lymphoma, nasal type, is a lymphoma that is associated with EBV infection in nearly all cases and more common in Asia and native populations in Peru. It usually presents with a mass and obstructive symptoms in the upper aerodigestive tract with occasional extranodal sites, but over two-thirds of patients will have localized disease. It is more common in men, and the median age at diagnosis is 60. This disease has its own prognostic score, which takes into account the presence or absence of “B” symptoms, disease stage, whether LDH is elevated, and whether there is lymph node involvement. EBV viral load at diagnosis and at the end of therapy is also predictive.

Treatment for early-stage disease is usually with combined-modality therapy of chemotherapy (commonly using etoposide, ifosfamide, cisplatin, and dexamethasone) and intensity-modulated radiation therapy (50–55 Gy), and patients with localized disease involving the nasal passages do quite well, with 3-year OS of 85%. Patients with

more advanced-stage disease do poorly, with disseminated extranodal relapse occurring frequently, and the median OS is only 4.3 months. The most commonly used treatment regimen is the SMILE regimen (dexamethasone, methotrexate, ifosfamide, -asparaginase, and etoposide).

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109 Hodgkin's Lymphoma

Caron A. Jacobson, Dan L. Longo



Hodgkin's lymphoma (HL) is a malignancy of mature B lymphocytes. It represents ~10% of all lymphomas diagnosed each year. The majority of HL diagnoses are classical HL (cHL), but there is a second subtype of HL, nodular lymphocyte-predominant HL (NLPHL). While this diagnosis does resemble cHL morphologically in certain respects, there is some evidence that it is more related to the indolent B-cell non-Hodgkin's lymphomas (NHLs) biologically than it is to cHL. The majority of this chapter will be specific to cHL, with a discussion of NLPHL at the end.

cHL is one of the success stories of modern oncology. Until the advent of extended-field radiotherapy in the mid-twentieth century, it was a highly fatal disease of young people. Radiation therapy cured some patients with early-stage disease, and the introduction of multiagent chemotherapy in the 1970s resulted in further improved cure rates, both for patients with early- and advanced-stage disease. Cure rates now are >85%. The new challenge in the treatment of HL is late therapy-related toxicity, including a high rate of secondary malignancies and cardiovascular disease. Current clinical trials are aimed at minimizing this risk while preserving efficacy.

EPIDEMIOLOGY AND ETIOLOGY

HL is of B-cell origin. The incidence of HL appears fairly stable, with 8480 new cases diagnosed in 2020 in the United States. HL is more common in whites than in blacks and more common in males than in females. A bimodal distribution of age at diagnosis has been observed, with one peak incidence occurring in patients in their twenties and the other in those in their eighties. Some of the late age peak may be attributed to confusion among entities with similar appearance such as anaplastic large-cell lymphoma and T-cell/histiocyte-rich B-cell lymphoma. There are four distinct subtypes of cHL that are differentiated based on their histopathologic features (Table 109-1): nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted. Patients in the younger age groups diagnosed in the United States largely have the nodular sclerosing subtype of HL. Elderly patients, patients infected with HIV, and patients in developing countries more commonly have mixed-cellularity HL or lymphocyte-depleted HL. Together, nodular sclerosis and mixed-cellularity types account for nearly 95% of cases. Infection by HIV is a risk factor for developing

TABLE 109-1 World Health Organization Classification of Hodgkin's Lymphoma

Nodular lymphocyte-predominant Hodgkin's lymphoma
Classical Hodgkin's lymphoma
Nodular sclerosis
Lymphocyte-rich
Mixed cellularity
Lymphocyte-depleted

HL. In addition, an association between infection by Epstein-Barr virus (EBV) and HL has been suggested. A monoclonal or oligoclonal proliferation of EBV-infected cells in 20–40% of the patients with HL has led to proposals for this virus having an etiologic role in HL. However, the matter is not settled definitively. Viral oncogenesis appears to play a greater role in HIV-related cHL: EBV can be detected in nearly all cases of HIV-associated cHL, compared to only one-third of cases of non-HIV-associated cHL. Reed-Sternberg (HRS) cells are the malignant cells in HL. HRS cells in HIV-associated cHL express the EBV-transforming protein latent membrane protein 1 (LMP-1), and the EBV genomes from multiple disease sites in the same HIV-associated cHL patient are episomal and clonal, suggesting that EBV is directly involved in early lymphomagenesis.

Histologically, the HRS cell is diagnostic of cHL (Fig. 109-1). These cells are large cells with abundant cytoplasm with bilobed and/or multiple nuclei. By immunohistochemistry, they are often PAX-5 positive but have low to no expression of other B-cell antigens like CD19 and CD20. They express CD15 and CD30 in 85 and 100% of cases, respectively. These cells, though, comprise <1% of the tumor cellularity, with the majority of the tumor made up of a surrounding inflammatory infiltrate of polyclonal lymphocytes, eosinophils, neutrophils, macrophages, plasma cells, fibroblasts, and collagen. The HRS cell interacts with its microenvironment via cell-cell contact and elaboration of growth factors and cytokines, which results in a surrounding cellular milieu that protects it from host immune attack. The surrounding environmental cells likewise support the HRS cells via cell-cell signaling and cytokine production, which provides signals that promote proliferation and survival of the HRS cell itself. Interestingly, 97% of HRS cells in cHL harbor genetic aberrations in the PD-L1 locus on chromosome 9p24.1, resulting in overexpression of PD-L1, the ligand for the inhibitory PD-1 receptor on immune cells. This is one mechanism whereby the HRS cell may be able to avoid immune destruction in its inflammatory microenvironment and may contribute to the generalized immune suppression in HL patients.

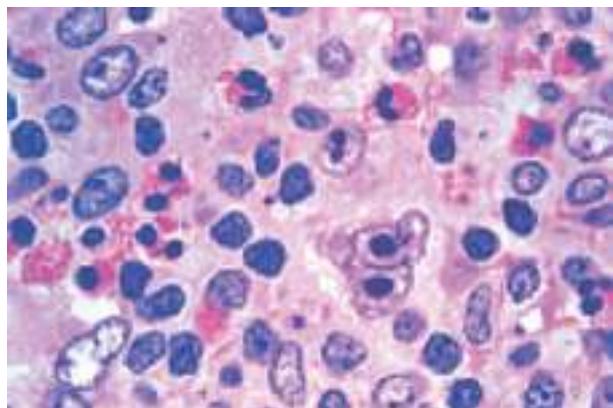


FIGURE 109-1 Hodgkin's disease: A classic Reed-Sternberg (RS) cell is present near the center of the field. RS cells are large cells with a bilobed nucleus and prominent nucleoli surrounded by a pleiomorphic cellular infiltrate. (From DL Kasper: *Harrison's Principles of Internal Medicine*, 16th ed. New York, NY: McGraw-Hill, 2005, Fig. 97-11, p. 654.)

APPROACH TO THE PATIENT

Classical Hodgkin's Lymphoma

Most patients with cHL present with palpable lymphadenopathy that is nontender; in most patients, these lymph nodes are in the neck, supraclavicular area, and axilla. More than half of the patients will have mediastinal adenopathy at diagnosis, and this is sometimes the initial manifestation. Subdiaphragmatic presentation of cHL is unusual and more common in older males. One-third of patients present with fevers, night sweats, and/or weight loss, or “B” symptoms. Occasionally, HL can present as a fever of unknown origin. This is more common in older patients who are found to have mixed-cellularity HL in an abdominal site. Rarely, the fevers persist for days to weeks, followed by afebrile intervals and then recurrence of the fever. This pattern is known as *Pel-Ebstein* fever. HL can occasionally present with unusual manifestations. These include severe and unexplained itching, cutaneous disorders such as erythema nodosum and ichthyosiform atrophy, paraneoplastic cerebellar degeneration and other distant effects on the CNS, nephrotic syndrome, immune hemolytic anemia and thrombocytopenia, hypercalcemia, and pain in lymph nodes on alcohol ingestion.

Evaluation of patients with HL will typically begin with a careful history and physical examination. Patients should be asked about the presence or absence of “B” symptoms. Comorbid diagnoses that may impact therapy should be reviewed, including a history of pulmonary disease and congestive heart failure given the use of chemotherapy drugs that can cause both lung and heart toxicity. A physical examination should pay attention to the peripherally accessible sites of lymph nodes and to the liver and spleen size. Laboratory evaluation should include a complete blood count with differential; erythrocyte sedimentation rate (ESR); chemistry studies reflecting major organ function including serum albumin; and HIV and hepatitis virus testing. A positron emission tomography (PET)/computed tomography (CT) scan is used for staging and is more accurate than a bone marrow biopsy for evaluation of bone marrow involvement as the bone marrow involvement in cHL tends to be patchy and therefore potentially missed on a unilateral bone marrow biopsy. The initial evaluation of a patient with HL or NHL is similar. In both situations, the determination of an accurate anatomic stage is an important part of the evaluation. Staging is done using the Ann Arbor staging system (Table 109-2).

TABLE 109-2 The Ann Arbor Staging System for Hodgkin's Lymphoma

STAGE	DEFINITION
I	Involvement of a single lymph node region or lymphoid structure (e.g., spleen, thymus, Waldeyer's ring)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site; hilar lymph nodes should be considered “lateralized” and, when involved on both sides, constitute stage II disease)
III	Involvement of lymph node regions or lymphoid structures on both sides of the diaphragm
III ₁	Subdiaphragmatic involvement limited to spleen, splenic hilar nodes, celiac nodes, or portal nodes
III ₂	Subdiaphragmatic involvement includes paraaortic, iliac, or mesenteric nodes plus structures in III ₁
IV	Involvement of extranodal site(s) beyond that designated as “E”
	More than one extranodal deposit at any location
	Any involvement of liver or bone marrow
A	No symptoms
B	Unexplained weight loss of >10% of the body weight during the 6 months before staging investigation
	Unexplained, persistent, or recurrent fever with temperatures >38°C during the previous month
	Recurrent drenching night sweats during the previous month
E	Localized, solitary involvement of extralymphatic tissue, excluding liver and bone marrow

The diagnosis of HL is established by review of an adequate biopsy specimen by an expert hematopathologist. HL is a tumor characterized by rare neoplastic cells of B-cell origin (immunoglobulin genes are rearranged but not expressed) in a tumor mass that is largely polyclonal inflammatory infiltrate, probably a reaction to cytokines produced by the tumor cells. The differential diagnosis of a lymph node biopsy suspicious for HL includes inflammatory processes, mononucleosis, NHL, phenytoin-induced adenopathy, and nonlymphomatous malignancies.

Staging for cHL is anatomically based given the propensity of the disease to march from one lymph node group to the next group, often contiguous to the first. Staging is important for selecting therapy of appropriate intensity, but the outcome of optimal therapy for all the stages is excellent. Patients are stratified based on whether they have early-stage disease (stage I or II) or advanced-stage disease (stage III or IV). Patients with early-stage disease have a better prognosis overall but are further classified as favorable or unfavorable based on a variety of factors. These factors vary from study to study but include bulky disease, number of lymph node areas involved, an elevated ESR (>30 if “B” symptoms are present; >50 if “B” symptoms are absent), and age. Prognosis in advanced-stage disease is best predicted by the International Prognostic Score (IPS), which ascribes 1 point for male sex, older age (>45 years), stage IV disease, serum albumin <4 g/dL, hemoglobin <10.5 g/dL, white blood cell count ≥15,000/µL, and a lymphocyte count <600/µL and/or <8% of white blood cell count. Five-year progression-free survival ranges from 88% for patients with no risk factors to 62% for patients with four or more factors, but very few patients have multiple risk factors.

TREATMENT

Classical Hodgkin's Lymphoma

The overwhelming majority of patients with HL will be cured with either chemotherapy alone or a combination of chemotherapy and radiation therapy. It has long been appreciated that patients with advanced-stage disease do not benefit from the addition of radiation therapy to chemotherapy and are thus treated with chemotherapy alone. For early-stage disease, however, treatment with combined-modality therapy has been associated with a small decrease in risk of relapse but with an increased risk of late toxicity including secondary malignancies, thyroid disease, and premature cardiovascular disease and stroke resulting in minimal or no improvement in long-term survival. Much of this risk can be attributed to radiation therapy. Thus, investigation into the treatment of early-stage HL at present is aimed at trying to maximize treatment outcome without using radiotherapy. This is an area of controversy in the treatment of HL.

EARLY-STAGE DISEASE

The most common chemotherapy regimen used to treat HL in the United States is ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine). This regimen is given every other week, with each cycle including two treatments. In patients with low-risk, or favorable, disease, the use of four to six cycles of ABVD alone, without radiation therapy, results in progression-free and overall survival rates of 88–92% and 97–100%, respectively, at 5–7 years. This may be associated with a slightly increased risk of relapse when compared with abbreviated chemotherapy (ABVD for four cycles) followed by involved field radiation therapy (30 Gy), but with no difference in overall survival owing to the excellent salvage strategies used for relapsed HL and to the late toxicities seen following radiation therapy to the chest. German studies have examined a very abbreviated chemotherapy regimen (ABVD for two cycles) and low-dose radiation (20 Gy) for particularly good-risk disease with two or fewer lymph node areas involved and found that this was equally effective to standard combined-modality therapy of ABVD for four cycles and 30 Gy of radiation. However, long-term follow-up is not yet available to assess the impact of the lower

radiotherapy dose on late toxicities. Finally, the use of an early interim PET/CT scan can aid decisions regarding the duration and extent of therapy. In one study, a negative PET/CT scan after three cycles of ABVD predicted for excellent outcomes with no additional therapy; in another, a negative PET/CT scan after two cycles of ABVD predicted for good outcomes with two additional cycles of ABVD alone, without radiation therapy.

For unfavorable-risk disease, the omission of radiation therapy following chemotherapy is associated with a more significant increased risk of relapse compared to favorable-risk disease, but again with no change in overall survival. For these patients, treatment options would include ABVD for four cycles followed by involved field radiation therapy or ABVD alone for six cycles. Treatment decisions are often based on the extent of the radiation field and the unfavorable risk factor, with patients with nonbulky disease being candidates for chemotherapy alone if radiation would be contraindicated for another reason. Combined modality therapy has typically been used for patients with bulky disease, although patients with bulky disease who have a negative PET/CT scan after chemotherapy may not benefit from additional radiation therapy.

Alternative chemotherapy regimens to ABVD have been developed and include the Stanford V regimen and escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone). Neither of these regimens has resulted in improved outcomes in patients with early-stage disease.

ADVANCED-STAGE DISEASE

Patients with advanced-stage disease do not benefit from the addition of radiation therapy after a complete response to chemotherapy alone and should be treated with chemotherapy alone. The most common regimen used in the United States is ABVD for six cycles. Again, Stanford V and escalated BEACOPP have been evaluated in advanced-stage disease and are not associated with an improvement in overall survival but are associated with increased toxicity. The small fraction of patients who do not achieve complete remission with chemotherapy alone (partial responders with persistent PET scan positivity account for <10% of patients) may benefit from the addition of involved field radiotherapy.

Newer drugs have been developed for the treatment of relapsed HL (see "Relapsed Disease," below). These include the antibody-drug conjugate brentuximab vedotin, which is an antibody against CD30 conjugated to the microtubule inhibitor monomethyl auristatin E (MMAE). This drug has been combined with doxorubicin, bleomycin, and dacarbazine in early-phase studies for advanced-stage HL with favorable efficacy compared to historical controls. Eschelon-1, a randomized trial of doxorubicin, vinblastine, and dacarbazine (AVD) plus brentuximab compared to ABVD, was a positive study in that it demonstrated an improvement in progression-free survival for AVD plus brentuximab, especially among younger patients, patients from North America, and patients with higher risk disease. Drugs that target the PD-1/PD-L1 axis have been developed in an attempt to boost the host immune recognition of tumors. This was particularly attractive in HL given the overexpression of PD-L1 on the HRS cell surface. In the setting of relapsed disease, these drugs, which include pembrolizumab and nivolumab, have very high response rates and are associated with durable responses. These are now being tested in conjunction with chemotherapy both as salvage therapy for relapsed disease and in previously untreated patients, including in a multicenter randomized trial against AVD plus brentuximab as initial therapy for advanced-stage disease.

RELAPSED DISEASE

Patients who relapse after primary therapy of HL can frequently still be cured. Patients who relapse after an effective chemotherapy regimen are usually not curable with subsequent chemotherapy administered at standard doses. Alternative salvage chemotherapy administered at standard doses, then, is given in order to document sensitivity to chemotherapy and to achieve maximum reduction of tumor mass. For patients who respond completely or nearly so, autologous stem cell transplantation can cure over

half of patients. Standard salvage chemotherapy regimens include ICE (ifosfamide, carboplatin, and etoposide) and GND (gemcitabine, vinorelbine, and doxorubicin). Newer combinations, including brentuximab with either chemotherapy or immune checkpoint inhibitors such as nivolumab, have also been tested with promising early results. For patients with early-stage disease who do not respond sufficiently to salvage chemotherapy, radiation therapy can be very effective to achieve a remission; whether to consolidate such a remission with an autologous stem cell transplant is debated. For patients with advanced-stage disease in whom salvage chemotherapy fails, the antibody-drug conjugate brentuximab vedotin, a CD30-directed antibody linked to the microtubule toxin MMAE, is active and can be tried as a bridge to allogeneic transplant. It is also used as a maintenance therapy following successful autologous stem cell transplantation based on results of a randomized trial versus observation. The anti-PD-1 immune checkpoint inhibitors, nivolumab and pembrolizumab, have efficacy in relapsed HL, and many responses are durable. Increasingly, there is an appreciation that use of checkpoint inhibitors restores the HRS cell's sensitivity to chemotherapy by unknown mechanisms; autologous stem cell transplantation may be a potentially curative option for patients who had previously been felt to have chemotherapy-resistant disease. Finally, anti-CD30 chimeric antigen receptor (CAR) T-cell therapy has been tested in multiply relapsed cHL with promising early results; these products are now being tested in multicenter phase 2 clinical trials.

Two other options may be useful in the setting of disease relapse after ABVD chemotherapy. Alkylating agent-based combinations such as ChIVPP (chlorambucil, vincristine, prednisone, and procarbazine) may be active in patients with disease resistant to ABVD. In addition, relapse following bone marrow transplant can be responsive to weekly low-dose single-agent vinblastine.

SURVIVORSHIP

Because of the very high cure rate in patients with HL, long-term complications have become a major focus for clinical research. In fact, in some series of patients with early-stage disease, more patients died from late complications of therapy than from HL itself. This is particularly true in patients with localized disease. The most serious late side effects include second malignancies and cardiac injury. Patients are at risk for the development of acute leukemia in the first 10 years after treatment with combination chemotherapy regimens that contain alkylating agents plus radiation therapy. The risk for development of acute leukemia is greater after MOPP-like (mechlorethamine, vincristine, procarbazine, and prednisone) and BEACOPP-like regimens than with ABVD. The risk of development of acute leukemia after treatment for HL is also related to the number of exposures to potentially leukemogenic agents (i.e., multiple treatments after relapse) and the age of the patient being treated, with those aged >60 years at particularly high risk. The development of carcinomas as a complication of treatment for HL is a major problem. These tumors usually occur ≥10 years after treatment and are associated with use of radiotherapy. For this reason, young women treated with thoracic radiotherapy for HL should institute screening mammograms 5–10 years after treatment, and all patients who receive thoracic radiotherapy for HL should be discouraged from smoking. Mediastinal radiation also accelerates coronary artery disease, and patients should be encouraged to minimize risk factors for coronary artery disease such as smoking and elevated cholesterol levels. Cervical radiation therapy increases the risk of carotid atherosclerosis and stroke and thyroid disease, including cancer.

A number of other late side effects from the treatment of HL are well known. Patients who receive thoracic radiotherapy are at very high risk for the eventual development of hypothyroidism and should be observed for this complication; intermittent measurement of thyrotropin should be made to identify the condition before it becomes symptomatic. Lhermitte's syndrome occurs in 15% of patients who receive thoracic radiotherapy. This syndrome is manifested by an "electric shock" sensation into the lower extremities on flexion of the neck. Because of the young age at which HL is often diagnosed, infertility is a concern for patients undergoing treatment for HL. Chemotherapy

regimens containing alkylating agents induce permanent infertility in nearly all men. The risk of permanent infertility in women treated with alkylating agent-containing chemotherapy is age-related, with younger women more likely to recover fertility. Infertility is very rare after treatment with ABVD.

NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN'S LYMPHOMA

NLPHL is now recognized as an entity distinct from cHL. Previous classification systems recognized that biopsies from a small subset of patients diagnosed as having HL contained a predominance of small lymphocytes and rare Reed-Sternberg-like cells; tumors had a nodular growth pattern and a clinical course that varied from that of patients with cHL. This is an unusual clinical entity and represents <5% of cases of HL and defines NLPHL.

NLPHL has a number of characteristics that suggest its relationship to NHL, rather than cHL, however. The HRS-like cell, or L&H (lymphocyte and histiocyte) or "popcorn" cell, is a clonal proliferation of B-cells that are positive for B-cell markers CD45, CD79a, CD20, CD19, and BCL2. They do not express two markers normally found on HRS cells, CD30 and CD15. This lymphoma tends to have a chronic, relapsing course and sometimes transforms to diffuse large B-cell lymphoma, including a specific subtype of diffuse large B-cell lymphoma known as T-cell/histiocyte-rich B-cell lymphoma, which shares an immunophenotype with the L&H cell. This natural history most closely resembles that of the indolent B-cell NHLs outlined in Chaps. 108 and 110.

Patients with NLPHL are more commonly male (75%). Like cHL, the age distribution of patients with this disease has two peaks, but unlike cHL, these peaks include children and adults ages 30–40 years, respectively. The majority of patients diagnosed have stage I or II disease (75%), with a minority having advanced-stage disease at diagnosis. "B" symptoms are uncommon.

Patients with early-stage disease at diagnosis should be treated with definitive radiotherapy. This is associated with a 15-year nonrelapse survival rate of 82%. The treatment of patients with advanced-stage NLPHL is controversial. Some clinicians favor no treatment of asymptomatic disease and merely close follow-up, akin to the indolent B-cell NHLs. For patients who need therapy due to symptoms or signs of organ function impairment, both cHL regimens and B-cell NHL regimens have been used, including ABVD and R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). A single-institution experience with R-CHOP resulted in a 100% response rate in a small group of patients without a single relapse with 42 months of follow-up. Although this is short follow-up for an indolent disease, some believe R-CHOP may be curative in this disease and advocate treating patients with advanced-stage disease at diagnosis, regardless of symptoms or organ function.

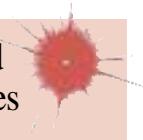
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110

Less Common Lymphoid and Myeloid Malignancies

Ayalew Tefferi, Dan L. Longo



The most common lymphoid malignancies are discussed in Chaps. 106, 107, 108, 109, and 111, myeloid leukemias in Chaps. 104 and 105, myelodysplastic syndromes (MDS) in Chap. 102, and myeloproliferative syndromes in Chap. 103. This chapter will focus on the more unusual forms of hematologic malignancy. The diseases discussed here are listed in Table 110-1. Each of these entities accounts for <1% of hematologic neoplasms.

RARE LYMPHOID MALIGNANCIES

All the lymphoid tumors discussed here are mature B-cell or T-cell natural killer (NK) cell neoplasms.

MATURE B CELL NEOPLASMS

B-Cell Prolymphocytic Leukemia (B-PLL) This is a malignancy of medium-sized (about twice the size of a normal small

TABLE 110-1 Unusual Lymphoid and Myeloid Malignancies

Lymphoid

Mature B-cell neoplasms
B-cell prolymphocytic leukemia
Splenenic marginal zone lymphoma
Hairy cell leukemia
Nodal marginal zone B-cell lymphoma
Mediastinal large B-cell lymphoma
Intravascular large B-cell lymphoma
Primary effusion lymphoma
Lymphomatoid granulomatosis
Mature T-cell and natural killer (NK) cell neoplasms
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Aggressive NK cell leukemia
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-type T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Blastic NK cell lymphoma
Primary cutaneous CD30+ T-cell lymphoma
Angioimmunoblastic T-cell lymphoma

Myeloid

Chronic neutrophilic leukemia
Chronic eosinophilic leukemia/hypereosinophilic syndrome

Histiocytic and Dendritic Cell Neoplasms

Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma

Mast Cells

Mastocytosis
Cutaneous mastocytosis
Systemic mastocytosis
Mast cell sarcoma
Extracutaneous mastocytoma

lymphocyte), round lymphocytes with a prominent nucleolus and light blue cytoplasm on Wright's stain. It predominantly affects the blood, bone marrow (BM), and spleen and usually does not cause adenopathy. The median age of affected patients is 70 years, and men are more often affected than women (male-to-female ratio is 1.6). This entity is distinct from chronic lymphoid leukemia (CLL) and does not develop as a consequence of that disease.

Clinical presentation is generally from symptoms of splenomegaly or incidental detection of an elevated white blood cell (WBC) count. The clinical course can be rapid. The cells express surface IgM (with or without IgD) and typical B-cell markers (CD19, CD20, CD22). CD23 is absent, and about one-third of cases express CD5. The CD5 expression along with the presence of the t(11;14) translocation in 20% of cases leads to confusion in distinguishing B-PLL from the leukemic form of mantle cell lymphoma. No reliable criteria for the distinction have emerged, and gene expression studies suggest a close relationship between mantle cell lymphoma and B-PLL and significant differences with CLL. About half of patients have mutation or loss of p53, and deletions have been noted in 11q23 and 13q14. Nucleoside analogues like fludarabine and cladribine and combination chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone [CHOP]) have produced responses. CHOP plus rituximab may be more effective than CHOP alone, but the disease is sufficiently rare that large series have not been reported. Splenectomy can produce palliation of symptoms but appears to have little or no impact on the course of the disease. BM transplantation may be curative. Imatinib may also have activity.

Splenic Marginal Zone Lymphoma (SMZL) This tumor of mainly small lymphocytes originates in the marginal zone of the spleen white pulp, grows to efface the germinal centers and mantle, and invades the red pulp. Splenic hilar nodes, BM, and peripheral blood (PB) may be involved. The circulating tumor cells have short surface villi and are called villous lymphocytes. Table 110-2 shows differences in tumor cells of a number of neoplasms of small lymphocytes that aid in the differential diagnosis. SMZL cells express surface immunoglobulin and CD20 but are negative for CD5, CD10, CD43, and CD103. Lack of CD5 distinguishes SMZL from CLL, and lack of CD103 separates SMZL from hairy cell leukemia.

The median age of patients with SMZL is mid-fifties, and men and women are equally represented. Patients present with incidental or symptomatic splenomegaly or incidental detection of lymphocytosis in the PB with villous lymphocytes. Autoimmune anemia or thrombocytopenia may be present. The immunoglobulin produced by these cells contains somatic mutations that reflect transit through a germinal center, and ongoing mutations suggest that the mutation machinery has remained active. About 40% of patients have either deletions or translocations involving 7q21, the site of the *FLNC* gene (filamin C γ , involved in cross-linking actin filaments in the cytoplasm). *NOTCH2* mutations are seen in 25% of patients. Chromosome 8p deletions may

TABLE 110-2 Immunophenotype of Tumors of Small Lymphocytes

	CD5	CD20	CD43	CD10	CD103	sIG	CYCLIN D1
Follicular lymphoma	neg	pos	pos	pos	neg	pos	neg
Chronic lymphoid leukemia	pos	pos	pos	neg	neg	pos	neg
B-cell prolymphocytic leukemia	pos	pos	pos	neg	neg	pos	pos
Mantle cell lymphoma	pos	pos	pos	neg	neg	pos	pos
Splenic marginal zone lymphoma	neg	pos	neg	neg	neg	pos	neg
Hairy cell leukemia	neg	pos	?	neg	pos	pos	neg

Abbreviations: neg, negative; pos, positive.

TABLE 110-3 Differential Diagnosis of "Dry Tap" Inability to Aspirate Bone Marrow

Dry taps occur in about 4% of attempts and are associated with:	
Metastatic carcinoma infiltration	17%
Chronic myeloid leukemia	15%
Myelofibrosis	14%
Hairy cell leukemia	10%
Acute leukemia	10%
Lymphomas, Hodgkin's disease	9%
Normal marrow	Rare

also be noted. The genetic lesions typically found in extranodal marginal zone lymphomas (e.g., trisomy 3 and t[11;18]) are uncommon in SMZL.

The clinical course of disease is generally indolent with median survivals exceeding 10 years. Patients with elevated lactate dehydrogenase (LDH) levels, anemia, and hypoalbuminemia generally have a poorer prognosis. Long remissions can be seen after splenectomy. Rituximab, ibrutinib, and PI3 kinase inhibitors are also active. A small fraction of patients undergo histologic progression to diffuse large B-cell lymphoma with a concomitant change to a more aggressive natural history. Experience with combination chemotherapy in SMZL is limited.

Hairy Cell Leukemia Hairy cell leukemia is a tumor of small lymphocytes with oval nuclei, abundant cytoplasm, and distinctive membrane projections (hairy cells). Patients have splenomegaly and diffuse BM involvement. While some circulating cells are noted, the clinical picture is dominated by symptoms from the enlarged spleen and pancytopenia. The mechanism of the pancytopenia is not completely clear and may be mediated by both inhibitory cytokines and marrow replacement. The marrow has an increased level of reticulin fibers; indeed, hairy cell leukemia is a common cause of inability to aspirate BM or so-called "dry tap" (Table 110-3). Monocytopenia is profound and may explain a predisposition to atypical mycobacterial infection that is observed clinically. The tumor cells have strong expression of CD22, CD25, and CD103; soluble CD25 level in serum is an excellent tumor marker for disease activity. The cells also express tartrate-resistant acid phosphatase. The immunoglobulin genes are rearranged and mutated, indicating the influence of a germinal center. No specific cytogenetic abnormality has been found, but most cases contain the activating *BRAF* mutation V600E.

The median age of affected patients is mid-fifties, and the male-to-female ratio is 5:1. Treatment options are numerous. Splenectomy is often associated with prolonged remission. Nucleosides including cladribine and deoxycyformycin are highly active but are also associated with further immunosuppression and can increase the risk of certain opportunistic infections. However, after brief courses of these agents, patients usually obtain very durable remissions during which immune function spontaneously recovers. Interferon α is also an effective therapy but is not as effective as nucleosides. Chemotherapy-refractory patients have responded to vemurafenib, a BRAF inhibitor. Vemurafenib does not appear to be curative, but responses can be maintained with chronic treatment. More durable remissions occur when rituximab is added to vemurafenib.

Nodal Marginal Zone B Cell Lymphoma This rare node-based disease bears an uncertain relationship with extranodal marginal zone lymphomas, which are often mucosa-associated and are called mucosa-associated lymphoid tissue (MALT) lymphomas, and SMZLs. Patients may have localized or generalized adenopathy. The neoplastic cell is a marginal zone B cell with monocyteoid features and has been called monocyteoid B cell lymphoma in the past. Up to one-third of the patients may have extranodal involvement, and involvement of the lymph nodes can be secondary to the spread of a mucosal primary lesion. In authentic nodal primaries, the cytogenetic abnormalities associated with MALT lymphomas (trisomy 3 and t[11;18]) are very rare. The clinical course is indolent. Patients often respond

to combination chemotherapy, although remissions have not been durable. Few patients have received CHOP plus rituximab, which is likely to be an effective approach to management.

Mediastinal (Thymic) Large B-Cell Lymphoma This entity was originally considered a subset of diffuse large B-cell lymphoma; however, additional study has identified it as a distinct entity with its own characteristic clinical, genetic, and immunophenotypic features. This is a disease that can be bulky in size but usually remains confined to the mediastinum. It can be locally aggressive, including progressing to produce a superior vena cava obstruction syndrome or pericardial effusion. About one-third of patients develop pleural effusions, and in 5–10% of cases, disease can disseminate widely to kidney, adrenal, liver, skin, and even brain. The disease affects women more often than men (male-to-female ratio is 1:2–3), and the median age is 35–40 years.

The tumor is composed of sheets of large cells with abundant cytoplasm accompanied by variable, but often abundant, fibrosis. It is distinguished from nodular sclerosing Hodgkin's disease by the paucity of normal lymphoid cells and the absence of lacunar variants of Reed-Sternberg cells. However, more than one-third of the genes that are expressed to a greater extent in primary mediastinal large B-cell lymphoma than in usual diffuse large B-cell lymphoma are also overexpressed in Hodgkin's disease, suggesting a possible pathogenetic relationship between the two entities that affect the same anatomic site. Tumor cells may overexpress *MAL*. The genome of tumor cells is characterized by frequent chromosomal gains and losses. The tumor cells in mediastinal large B-cell lymphoma express CD20, but surface immunoglobulin and human leukocyte antigen (HLA) class I and class II molecules may be absent or incompletely expressed. Expression of lower levels of class II HLA identifies a subset with poorer prognosis. The cells are CD5 and CD10 negative but may show light staining with anti-CD30. The cells are CD45 positive, unlike cells of classical Hodgkin's disease.

Methotrexate, leucovorin, doxorubicin, cyclophosphamide, vin-cristine, prednisone, and bleomycin (MACOP-B) and rituximab plus CHOP are effective treatments, achieving 5-year survival of 75–87%. Dose-adjusted therapy with prednisone, etoposide, vincristine, cyclophosphamide, and doxorubicin (EPOCH) plus rituximab has produced 5-year survival of 97%. A role for mediastinal radiation therapy has not been definitively demonstrated, but it is frequently used, especially in patients whose mediastinal area remains positron emission tomography–avid after 4–6 cycles of chemotherapy.

Intravascular Large B-Cell Lymphoma This is an extremely rare form of diffuse large B-cell lymphoma characterized by the presence of lymphoma in the lumen of small vessels, particularly capillaries. It is also known as malignant angiogenesis or angiotropic large-cell lymphoma. It is sufficiently rare that no consistent picture has emerged to define a clinical syndrome or its epidemiologic and genetic features. It is thought to remain inside vessels because of a defect in adhesion molecules and homing mechanisms, an idea supported by scant data suggesting absence of expression of β -1 integrin and ICAM-1. Patients commonly present with symptoms of small-vessel occlusion, skin lesions, or neurologic symptoms. The tumor cell clusters can promote thrombus formation. A subset of patients have tumors with *MYD88* or *CD79B* mutations. In general, the clinical course is aggressive and the disease is poorly responsive to therapy. Often a diagnosis is not made until very late in the course of the disease or at autopsy.

Primary Effusion Lymphoma This entity is another variant of diffuse large B-cell lymphoma that presents with pleural effusions, usually without apparent tumor mass lesions. It is most common in the setting of immune deficiency disease, especially AIDS, and is caused by human herpes virus 8 (HHV-8)/Kaposi's sarcoma herpes virus (KSHV). It is also known as *body cavity-based lymphoma*. Some patients have been previously diagnosed with Kaposi's sarcoma. It can also occur in the absence of immunodeficiency in elderly men of Mediterranean heritage, similar to Kaposi's sarcoma but even less common.

The malignant effusions contain cells positive for HHV-8/KSHV, and many are also co-infected with Epstein-Barr virus. The cells

are large with large nuclei and prominent nucleoli that can be confused with Reed-Sternberg cells. The cells express CD20 and CD79a (immunoglobulin-signaling molecule), although they often do not express immunoglobulin. Some cases aberrantly express T-cell markers such as CD3 or rearranged T-cell receptor genes. No characteristic genetic lesions have been reported, but gains in chromosome 12 and X material have been seen, similar to other HIV-associated lymphomas. The clinical course is generally characterized by rapid progression and death within 6 months. CHOP plus lenalidomide or bortezomib may produce responses. Highly active antiretroviral therapy for HIV should be maintained during treatment.

Lymphomatoid Granulomatosis This is an angiocentric, angiolytic lymphoproliferative disease comprised by neoplastic Epstein-Barr virus-infected monoclonal B cells accompanied and outnumbered by a polyclonal reactive T-cell infiltrate. The disease is graded based on histologic features such as cell number and atypia in the B cells. It is most often confused with extranodal NK/T-cell lymphoma, nasal type, which can also be angiolytic and is Epstein-Barr virus-related. The disease usually presents in adults (males > females) as a pulmonary infiltrate. Involvement is often entirely extranodal and can include kidney (32%), liver (29%), skin (25%), and brain (25%). The disease often but not always occurs in the setting of immune deficiency.

The disease can be remitting and relapsing in nature or can be rapidly progressive. The course is usually predicted by the histologic grade. The disease is highly responsive to combination chemotherapy and is curable in most cases. Some investigators have claimed that low-grade disease (grade I and II) can be treated with interferon α .

■ MATURE T CELL AND NK CELL NEOPLASMS

T-Cell Prolymphocytic Leukemia This is an aggressive leukemia of medium-sized prolymphocytes involving the blood, marrow, nodes, liver, spleen, and skin. It accounts for 1–2% of all small lymphocytic leukemias. Most patients present with elevated WBC count (often >100,000/ μ L), hepatosplenomegaly, and adenopathy. Skin involvement occurs in 20%. The diagnosis is made from PB smear, which shows cells about 25% larger than those in small lymphocytes, with cytoplasmic blebs and nuclei that may be indented. The cells express T-cell markers like CD2, CD3, and CD7; two-thirds of patients have cells that are CD4+ and CD8-, and 25% have cells that are CD4+ and CD8+. T-cell receptor β chains are clonally rearranged. In 80% of patients, inversion of chromosome 14 occurs between q11 and q32. Ten percent have t(14;14) translocations that bring the T-cell receptor alpha/beta gene locus into juxtaposition with oncogenes *TCL1* and *TCL1b* at 14q32.1. Chromosome 8 abnormalities are also common. Deletions in the *ATM* gene are also noted. Activating *JAK3* mutations have also been reported.

The course of the disease is generally rapid, with median survival of about 12 months. Responses have been seen with the anti-CD52 antibody alemtuzumab, nucleoside analogues, and CHOP chemotherapy. Histone deacetylase inhibitors like vorinostat and romidepsin may also have activity. Small numbers of patients with T-cell prolymphocytic leukemia have also been treated with high-dose therapy, and allogeneic BM transplantation after remission has been achieved with alemtuzumab or conventional-dose therapy.

T-Cell Large Granular Lymphocytic Leukemia T-cell large granular lymphocytic (LGL) leukemia is characterized by increases in the number of LGLs in the PB (2000–20,000/ μ L) often accompanied by severe neutropenia, with or without concomitant anemia. Patients may have splenomegaly and frequently have evidence of systemic autoimmune disease, including rheumatoid arthritis, hypergamma-globulinemia, autoantibodies, and circulating immune complexes. BM involvement is mainly interstitial in pattern, with <50% lymphocytes on differential count. Usually the cells express CD3, T-cell receptors, and CD8; NK-like variants may be CD3-. The leukemic cells often express Fas and Fas ligand.

The course of the disease is generally indolent and dominated by the neutropenia. Paradoxically, immunosuppressive therapy with cyclosporine, methotrexate, or cyclophosphamide plus glucocorticoids can produce an increase in granulocyte counts. Nucleosides have been used anecdotally. Occasionally the disease can accelerate to a more aggressive clinical course.

Aggressive NK Cell Leukemia NK neoplasms are very rare, and they may follow a range of clinical courses from very indolent to highly aggressive. They are more common in Asians than whites, and the cells frequently harbor a clonal Epstein-Barr virus episome. The PB white count is usually not greatly elevated, but abnormal large lymphoid cells with granular cytoplasm are noted. The aggressive form is characterized by symptoms of fever and laboratory abnormalities of pancytopenia. Hepatosplenomegaly is common; node involvement is less common. Patients may have hemophagocytosis, coagulopathy, or multiorgan failure. Serum levels of Fas ligand are elevated.

The cells express CD2 and CD56 and do not have rearranged T-cell receptor genes. Deletions involving chromosome 6 are common. The disease can be rapidly progressive. Some forms of NK neoplasms are more indolent. They tend to be discovered incidentally with LGL lymphocytosis and do not manifest the fever and hepatosplenomegaly characteristic of the aggressive leukemia. The cells are also CD2 and CD56 positive, but they do not contain clonal forms of Epstein-Barr virus and are not accompanied by pancytopenia or autoimmune disease.

Extranodal NK/T-Cell Lymphoma, Nasal Type Like lymphomatoid granulomatosis, extranodal NK/T-cell lymphoma tends to be an angiocentric and angiolytic lesion, but the malignant cells are not B cells. In most cases, they are CD56+ Epstein-Barr virus-infected cells; occasionally, they are CD56+ Epstein-Barr virus-infected cytotoxic T cells. They are most commonly found in the nasal cavity. Historically, this illness was called lethal midline granuloma, polymorphic reticulosis, and angiocentric immunoproliferative lesion. This form of lymphoma is prevalent in Asia, Mexico, and Central and South America; it affects males more commonly than females. When it spreads beyond the nasal cavity, it may affect soft tissue, the gastrointestinal tract, or the testis. In some cases, hemophagocytic syndrome (HPS) may influence the clinical picture. Patients may have B symptoms. Many of the systemic manifestations of disease are related to the production of cytokines by the tumor cells and the cells responding to their signals. Deletions and inversions of chromosome 6 are common.

Many patients with extranodal NK/T-cell lymphoma, nasal type, have excellent antitumor responses with combination chemotherapy regimens, particularly those with localized disease. Radiation therapy is often used after completion of chemotherapy. Four risk factors have been defined, including B symptoms, advanced stage, elevated LDH, and regional lymph node involvement. Patient survival is linked to the number of risk factors: 5-year survival is 81% for zero risk factors, 64% for one risk factor, 32% for two risk factors, and 7% for three or four risk factors. Combination regimens without anthracyclines have been touted as superior to CHOP, but data are sparse. High-dose therapy with stem cell transplantation has been used, but its role is unclear.

Enteropathy-Type T-Cell Lymphoma Enteropathy-type T-cell lymphoma is a rare complication of longstanding celiac disease. It most commonly occurs in the jejunum or the ileum. In adults, the lymphoma may be diagnosed at the same time as celiac disease, but the suspicion is that the celiac disease was a longstanding precursor to the development of lymphoma. The tumor usually presents as multiple ulcerating mucosal masses, but may also produce a dominant exophytic mass or multiple ulcerations. The tumor expresses CD3 and CD7 nearly always and may or may not express CD8. The normal-appearing lymphocytes in the adjacent mucosa often have a similar phenotype to the tumor. Most patients have the HLA genotype associated with celiac disease, HLA DQA1 0501 or DQB1 0201.

The prognosis of this form of lymphoma is typically poor (median survival is 7 months), but some patients have a good response to CHOP chemotherapy. Patients who respond can develop bowel perforation

from responding tumor. If the tumor responds to treatment, recurrence may develop elsewhere in the celiac disease-affected small bowel.

Hepatosplenic T-Cell Lymphoma Hepatosplenic T-cell lymphoma is a malignancy derived from T cells expressing the gamma/delta T-cell antigen receptor that affects mainly the liver and fills the sinusoids with medium-size lymphoid cells. When the spleen is involved, dominantly the red pulp is infiltrated. It is a disease of young people, especially young people with an underlying immunodeficiency or with an autoimmune disease that demands immunosuppressive therapy. The use of thiopurine and infliximab is particularly common in the history of patients with this disease. The cells are CD3+ and usually CD4- and CD8-. The cells may contain isochromosome 7q, often together with trisomy 8. The lymphoma has an aggressive natural history. Combination chemotherapy may induce remissions, but most patients relapse. Median survival is about 2 years. The tumor does not appear to respond to reversal of immunosuppressive therapy.

Subcutaneous Panniculitis-Like T-Cell Lymphoma Subcutaneous panniculitis-like T-cell lymphoma involves multiple subcutaneous collections of neoplastic T cells that are usually cytotoxic cells in phenotype (i.e., contain perforin and granzyme B and express CD3 and CD8). The rearranged T-cell receptor is usually alpha/beta-derived, but occasionally, the gamma/delta receptors are involved, particularly in the setting of immunosuppression. The cells are negative for Epstein-Barr virus. Patients may have an HPS in addition to the skin infiltration; fever and hepatosplenomegaly may also be present. Nodes are generally not involved. Patients frequently respond to combination chemotherapy, including CHOP. When the disease is progressive, the HPS can be a component of a fulminant downhill course. Effective therapy can reverse the HPS.

Blastic NK Cell Lymphoma The neoplastic cells express NK cell markers, especially CD56, and are CD3 negative. They are large blastic-appearing cells and may produce a leukemia picture, but the dominant site of involvement is the skin. Morphologically, the cells are similar to the neoplastic cells in acute lymphoid and myeloid leukemia. No characteristic chromosomal abnormalities have been described. The clinical course is rapid, and the disease is largely unresponsive to typical lymphoma treatments.

Primary Cutaneous CD30+ T-Cell Lymphoma This tumor involves the skin and is composed of cells that appear similar to the cells of anaplastic T-cell lymphoma. Among cutaneous T-cell tumors, ~25% are CD30+ anaplastic lymphomas. If dissemination to lymph nodes occurs, it is difficult to distinguish between the cutaneous and systemic forms of the disease. The tumor cells are often CD4+, and the cells contain granules that are positive for granzyme B and perforin in 70% of cases. The typical t(2;5) of anaplastic T-cell lymphoma is absent; indeed, its presence should prompt a closer look for systemic involvement and a switch to a diagnosis of anaplastic T-cell lymphoma. This form of lymphoma has sporadically been noted as a rare complication of silicone or saline breast implants. The natural history of breast implant-associated lymphoma is generally indolent. Cutaneous CD30+ T-cell lymphoma often responds to therapy. The anti-CD30 immunotoxin conjugate brentuximab vedotin is active. Radiation therapy can be effective, and surgery can also produce long-term disease control. Five-year survival exceeds 90%.

Angioimmunoblastic T-Cell Lymphoma Angioimmunoblastic T-cell lymphoma is a systemic disease that accounts for ~15% of all T-cell lymphomas. Patients frequently have fever, advanced stage, diffuse adenopathy, hepatosplenomegaly, skin rash, polyclonal hypergammaglobulinemia, and a wide range of autoantibodies including cold agglutinins, rheumatoid factor, and circulating immune complexes. Patients may have edema, arthritis, pleural effusions, and ascites. The nodes contain a polymorphous infiltrate of neoplastic T cells and nonneoplastic inflammatory cells together with proliferation of high endothelial venules and follicular dendritic cells (FDCs). The most common chromosomal abnormalities are trisomy 3, trisomy 5, and an extra X chromosome. Aggressive combination chemotherapy can

induce regressions. The underlying immune defects make conventional lymphoma treatments more likely to produce infectious complications.

RARE MYELOID MALIGNANCIES

The World Health Organization (WHO) system uses PB counts and smear analysis, BM morphology, and cytogenetic and molecular genetic tests in order to classify myeloid malignancies into several major categories (Table 110-4). Among them, acute myeloid leukemia (AML) is discussed in Chap. 104, myelodysplastic syndromes (MDS) in Chap. 102, chronic myeloid leukemia (CML) in Chap. 105, and *JAK2* mutation-enriched myeloproliferative neoplasms (MPNs) in Chap. 103. In this chapter, we focus on the rest (listed in Table 110-4) including chronic neutrophilic leukemia (CNL); atypical CML, *BCR-ABL1* negative (aCML); chronic myelomonocytic leukemia (CMML); juvenile myelomonocytic leukemia (JMML); chronic eosinophilic leukemia, not otherwise specified (CEL-NOS); mastocytosis; MPN, unclassifiable (MPN-U); MDS/MPN, unclassifiable (MDS/MPN-U); MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T); and myeloid/lymphoid neoplasms with eosinophilia and rearrangements of *PDGFRA*, *PDGFRB*, or *FGFR1* or with *PCM1-JAK2*. This chapter also includes histiocytic and dendritic cell neoplasms, transient myeloproliferative disorders, and a broader discussion on primary eosinophilic disorders including hypereosinophilic syndrome (HES).

■ CHRONIC NEUTROPHILIC LEUKEMIA

CNL is a clonal proliferation of mature neutrophils with few or no circulating immature granulocytes. In 2013, CNL was described to be associated with activating mutations of the gene (*CSF3R*) encoding

TABLE 110-4 World Health Organization Classification of Myeloid Malignancies

1. Acute myeloid leukemia (AML) and related precursor neoplasms
2. Myeloproliferative neoplasms (MPN)
 - 2.1. Chronic myeloid leukemia (CML), *BCR-ABL1* positive
 - 2.2. *JAK2* mutation-enriched MPN
 - 2.2.1. Polycythemia vera
 - 2.2.2. Primary myelofibrosis
 - 2.2.3. Essential thrombocythemia
 - 2.3. Chronic neutrophilic leukemia (CNL)
 - 2.4. Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS)
 - 2.5. Myeloproliferative neoplasm, unclassifiable (MPN-U)
3. Myelodysplastic syndromes (MDS)
 - 3.1. MDS with single lineage dysplasia
 - 3.2. MDS with ring sideroblasts (MDS-RS)
 - 3.3. MDS with multilineage dysplasia
 - 3.4. MDS with excess blasts
 - 3.5. MDS with isolated del(5q)
 - 3.6. MDS, unclassifiable (MDS-U)
 - 3.7. *Provisional entity: Refractory cytopenia of childhood*
4. MDS/MPN overlap
 - 4.1. Chronic myelomonocytic leukemia (CMML)
 - 4.2. Atypical chronic myeloid leukemia (aCML), *BCR-ABL1* negative
 - 4.3. Juvenile myelomonocytic leukemia (JMML)
 - 4.4. MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
 - 4.5. MDS/MPN, unclassifiable (MDS/MPN-U)
5. Mastocytosis
6. Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1* or with *PCM1-JAK2*
 - 6.1. Myeloid/lymphoid neoplasms with *PDGFRA* rearrangement
 - 6.2. Myeloid/lymphoid neoplasms with *PDGFRB* rearrangement
 - 6.3. Myeloid/lymphoid neoplasms with *FGFR1* rearrangement
 - 6.4. *Provisional entity: Myeloid/lymphoid neoplasms with PCM1-JAK2 translocation*
7. Myeloid neoplasms with germline predisposition

for the receptor for granulocyte colony-stimulating factor (G-CSF), also known as colony-stimulating factor 3 (CSF3). Patients with CNL might be asymptomatic at presentation but also display constitutional symptoms, splenomegaly, anemia, and thrombocytopenia. Median survival is approximately 2 years and causes of death include leukemic transformation, progressive disease associated with severe cytopenias and marked treatment-refractory leukocytosis. The true incidence of CNL is not known due to diagnostic uncertainty with >200 currently reported cases. Median age at diagnosis is approximately 67 years with a slight male preponderance in gender distribution.

Pathogenesis CSF3 is the main growth factor for granulocyte proliferation and differentiation. Accordingly, recombinant CSF3 is used for the treatment of severe neutropenia, including severe congenital neutropenia (SCN). Some patients with SCN acquire *CSF3R* mutations, and the frequency of such mutations is significantly higher (~80%) in patients who experience leukemic transformation. SCN-associated *CSF3R* mutations occur in the region of the gene coding for the cytoplasmic domain of CSF3R and result in truncation of the C-terminal-negative regulatory domain. In 2013, Maxson et al described a different class of *CSF3R* mutations in ~90% of patients with CNL; these were mostly membrane proximal, the most frequent being a C-to-T substitution at nucleotide 1853 (T618I). In a subsequent confirmatory study, *CSF3R* mutations were found to be specific to WHO-defined CNL. About 40% of the T618I-mutated cases also harbored *SETBP1* mutations. *CSF3R* T618I has been shown to induce lethal myeloproliferative disorder in a mouse model and in vitro sensitivity to JAK inhibition.

Diagnosis Diagnosis of CNL requires exclusion of the more common causes of neutrophilia including infections and inflammatory processes. In addition, one should be mindful of the association between some forms of metastatic cancer or plasma cell neoplasms with secondary neutrophilia. Neoplastic neutrophilia also occurs in other *BCR-ABL1*-negative myeloid malignancies including aCML and CMML. Accordingly, the WHO diagnostic criteria for CNL are designed to exclude the possibilities of both secondary/reactive neutrophilia and leukocytosis associated with myeloid malignancies other than CNL (Table 110-5): leukocytosis ($\geq 25 \times 10^9/L$), $\geq 80\%$ segmented/band neutrophils, $<10\%$ immature myeloid cells, $<1\%$ circulating blasts, and absence of dysgranulopoiesis or monocytosis (monocyte count $<1 \times 10^9/L$). BM in CNL is hypercellular and displays increased number and percentage of neutrophils with a very high myeloid-to-erythroid ratio and minimal left shift, myeloid dysplasia, or reticulin fibrosis.

The recent discovery of *CSF3R* mutations (see above) and their almost invariable association with WHO-defined CNL has allowed its incorporation in the WHO diagnostic criteria (Table 110-5). In practical terms, the presence of a membrane proximal *CSF3R* mutation in a patient with predominantly neutrophilic granulocytosis should be sufficient for the diagnosis of CNL, regardless of the degree of leukocytosis. Unfortunately, several exclusionary criteria still need to be met for diagnosing CNL in the absence of *CSF3R* mutations (Table 110-5).

Treatment Current treatment in CNL is largely palliative and suboptimal in its efficacy. Several drugs alone or in combination have been tried, and none have shown remarkable efficacy. As such, allogeneic hematopoietic stem cell transplant (AHSCT) is reasonable to consider in the presence of symptomatic disease, especially in younger patients. Otherwise, cytoreductive therapy with hydroxyurea is probably as good as anything, and a more intensive combination chemotherapy may not have additional value. However, response to hydroxyurea therapy is often transient, and some have successfully used interferon α as an alternative drug. Response to treatment with ruxolitinib (a JAK1 and JAK2 inhibitor) has been reported in several case reports, but as is the case with hydroxyurea treatment, the response was often incomplete and temporary. In a recently reported phase 2 study of ruxolitinib in 44 patients with CNL or aCML, 21 patients had CNL (76% harbored *CSF3R* mutations), of whom only 4 (20%) experienced complete or partial response according to conventional response criteria.

TABLE 110-5 2016 World Health Organization (WHO) Diagnostic Criteria for Chronic Neutrophilic Leukemia (CNL), Atypical Chronic Myeloid Leukemia, *BCR-ABL1*-Negative (aCML), and Chronic Myelomonocytic Leukemia (CMML)

VARIABLES	CNL	aCML	CMML
PB leukocyte count	$\geq 25 \times 10^9/L$	Granulocytosis	
PB segmented neutrophils/bands	$\geq 80\%$		
PB immature granulocytes ^a	<10%	$\geq 10\%$	
PB blast count	<1%	<20%	<20%
PB monocyte count	$<1 \times 10^9/L$	No or minimal monocytosis	$\geq 1 \times 10^9/L$ Persistent and lasting for at least 3 months
Dysgranulopoiesis	No	Yes	
PB basophil percentage		<2%	
PB monocyte percentage		<10%	$\geq 10\%$
BM	Hypercellular \uparrow Neutrophils, number and % <5% blasts Normal neutrophilic maturation	Hypercellular \uparrow Granulocyte proliferation Granulocytic dysplasia \pm erythroid/megakaryocyte Dysplasia <20% blasts	Dysplasia in ≥ 1 myeloid lineages or Clonal cytogenetic/molecular abnormality <20% blasts or promonocytes
<i>BCR-ABL1</i>	No	No	No
<i>PDGFRA</i> , <i>PDGFRB</i> , <i>FGFR1</i> , or <i>PCM1-JAK2</i> rearrangement	No	No	No
<i>CSF3RT618I</i> or other activating <i>CSF3R</i> mutation or persistent neutrophilia, splenomegaly, and no identifiable cause of reactive neutrophilia	Yes		
PB and BM blasts/promonocytes		<20%	<20%
Evidence for other MPN: CML, PV, ET, or PMF	No	No	No
Evidence for reactive leukocytosis ^b or monocytosis	No		No

^aImmature granulocytes include myeloblasts, promyelocytes, myelocytes, and metamyelocytes. ^bCauses of reactive neutrophilia include plasma cell neoplasms, solid tumor, infections, and inflammatory processes.

Abbreviations: BM, bone marrow; CML, chronic myeloid leukemia; ET, essential thrombocythemia; MPN, myeloproliferative neoplasms; PB, peripheral blood; PMF, primary myelofibrosis; PV, polycythemia vera.

■ ATYPICAL CHRONIC MYELOID LEUKEMIA

“Atypical chronic myeloid leukemia, *BCR-ABL1* negative (aCML)” is formally classified under the MDS/MPN category of myeloid malignancies and is characterized by left-shifted granulocytosis and dysgranulopoiesis. The differential diagnosis of aCML includes CML, which is distinguished by the presence of *BCR-ABL1*; CNL, which is distinguished by the absence of dysgranulopoiesis and presence of *CSF3R* mutations; and CMML, which is distinguished by the presence of monocytosis (absolute monocyte count $\geq 1 \times 10^9/L$). The WHO diagnostic criteria for aCML are listed in Table 110-5 and include granulocytosis; dysgranulopoiesis; $\geq 10\%$ immature granulocytes; <20% PB or BM myeloblasts; <10% PB monocytes; <2% basophils; absence of otherwise specific mutations such as *BCR-ABL1*, *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2*; and not meeting WHO criteria for CML, primary myelofibrosis (PMF), polycythemia vera (PV), or essential thrombocytopenia (ET). The BM in aCML is hypercellular with granulocyte proliferation and dysplasia with or without erythroid or megakaryocytic dysplasia.

The molecular pathogenesis of aCML is incompletely understood; about a fourth of patients express *SETBP1* mutations, which are, however, also found in several other myeloid malignancies, including CNL and CMML. *SETBP1* mutations in aCML are prognostically detrimental and mostly located between codons 858 and 871; similar mutations are seen with Schinzel-Giedion syndrome (a congenital disease with severe developmental delay and various physical stigmata including midface retraction, large forehead, and macroglossia). More recently, a somatic missense mutation in ethanolamine kinase 1 (*ETNK1* N244S) was described in 9% of patients with aCML but was also seen in 14% of patients with CMML, 6% of patients with mastocytosis (especially in association with eosinophilia), and rarely in other MPNs.

In a series of 55 patients with WHO-defined aCML, median age at diagnosis was 62 years with female preponderance (57%); splenomegaly was reported in 54% of the patients, red cell transfusion requirement in 65%, abnormal karyotype in 20% (20q- and trisomy 8 being the most frequent), and leukemic transformation in 40%. Median survival was 25 months. Outcome was worse in patients with marked leukocytosis, transfusion requirement, and increased immature cells in the PB. In a more recent Mayo Clinic study of 25 molecularly annotated and strictly WHO-defined aCML patients, median age was 70 years and 84% were male. Cytogenetic abnormalities were seen in 36% and gene mutations in 100%. Mutational frequencies were as follows: *ASXL1* 28%, *TET2* 16%, *NRAS* 16%, *SETBP1* 12%, *RUNX1* 12%, *ETNK1* 8%, and *PTPN11* 4%. Median survival was 10.8 months, and at last follow-up (median 11 months), 17 deaths (68%) and 2 leukemic transformations (8%) were documented. In multivariable analysis, advanced age, low hemoglobin, and *TET2* mutations were shown to carry independent prognostic significance; other mutations, including *ASXL1* and *SETBP1*, lacked prognostic significance. Conventional chemotherapy is largely ineffective in the treatment of aCML. Similarly, treatment response to the JAK1/2 inhibitor ruxolitinib has not been impressive. However, a favorable experience with autologous stem cell transplantation (ASCT) was reported in nine patients; after a median follow-up of 55 months, the majority of the patients remained in complete remission.

■ CHRONIC MYELOMONOCYTIC LEUKEMIA

CMML is classified under the WHO category of MDS/MPN and is defined by an absolute monocyte count (AMC) of $\geq 1 \times 10^9/L$ in the PB and accounting for $\geq 10\%$ of the leukocyte count. Median age at diagnosis ranges from 65 to 75 years, and there is a 2:1 male predominance.

Clinical presentation is variable and depends on whether the disease presents with MDS-like or MPN-like phenotype; the former is associated with cytopenias and the latter with splenomegaly and features of myeloproliferation such as fatigue, night sweats, weight loss, and cachexia. About 20% of patients with CMML experience serositis involving the joints (arthritis), pericardium (pericarditis and pericardial effusion), pleura (pleural effusion), or peritoneum (ascites).

Pathogenesis Almost all patients with CMML harbor somatic mutations involving epigenetic regulator genes (e.g., *ASXL1*, *TET2*), spliceosome pathway genes (e.g., *SRSF2*), DNA damage response genes (e.g., *TP53*), and tyrosine kinases/transcription factors (e.g., *KRAS*, *NRAS*, *CBL*, and *RUNX1*). However, none of these mutations are specific to CMML, and their precise pathogenetic contribution is unclear. Clonal cytogenetic abnormalities are seen in about a third of patients with CMML and include trisomy 8 and abnormalities of chromosome 7. More recent studies have demonstrated the presence of BM dendritic cell aggregates suggesting systemic immune dysregulation and distinct phenotypic features of monocytes in CMML.

Diagnosis Reactive monocytosis is uncommon but has been reported in association with certain infections and inflammatory conditions. Clonal (i.e., neoplastic) monocytosis defines CMML but is also seen with JMML and AML with monocytic differentiation. The WHO diagnostic criteria for CMML are listed in Table 110-5 and include persistent PB monocyte count of $\geq 1 \times 10^9/L$ with monocyte percentage of $\geq 10\%$; absence of *BCR-ABL1*, *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2* rearrangements; not meeting WHO criteria for CML, PV, ET, or PMF; $<20\%$ blasts and promonocytes in the PB and BM; and dysplasia involving one or more myeloid lineages or, in the absence of dysplasia, presence of an acquired clonal cytogenetic or molecular genetic abnormality or nonreactive monocytosis lasting for at least 3 months.

The BM in CMML is hypercellular with granulocytic and monocytic proliferation. Dysplasia is often present and may involve one, two, or all myeloid lineages. On immunophenotyping, the abnormal cells often express myelomonocytic antigens such as CD13 and CD33, with variable expression of CD14, CD68, CD64, and CD163. Monocytic-derived cells are almost always positive for the cytochemical non-specific esterases (e.g., butyrate esterase), while normal granulocytic precursors are positive for lysozyme and chloroacetate esterase. In CMML, it is common to have a hybrid cytochemical staining pattern with cells expressing both chloroacetate and butyrate esterases simultaneously (dual esterase staining). Monocytosis can be associated with reactive as well as other myeloid neoplasms. Based on flow cytometric expression of CD14/CD16, monocytes can be classified into classical MO1 (CD14+/CD16-), intermediate MO2 (CD14+/CD16+), and nonclassical MO3 (CD14-/CD16+) fractions, with MO1 constituting the major monocyte population (85%) in healthy conditions. Recent studies have suggested characteristic increase in classical monocytes in CMML patients, distinguishing them from other causes of reactive and clonal monocytosis.

Prognosis A recent meta-analysis showed median survival of 1.5 years in CMML. Numerous prognostic systems have attempted to better define and stratify the natural history of CMML. One of these, the Mayo prognostic model, assigns one point each to the following four independent prognostic variables: $AMC > 10 \times 10^9/L$, presence of circulating immature cells, hemoglobin $< 10 \text{ g/dL}$, and platelet count $< 100,000/\text{mL}$. This model stratified patients into three risk groups: low (0 points), intermediate (1 point), and high (≥ 2 points), translating to median survival of 32, 18, and 10 months, respectively. Another prognostic model referred to as the CMML-specific prognostic scoring system (CPSS) identified four variables as being prognostic for both overall survival and leukemia-free survival: French-American-British (FAB) and WHO CMML subtypes, red blood cell transfusion dependency, and the Spanish cytogenetic risk stratification system. A French study incorporated *ASXL1* mutational status in 312 CMML patients; in a multivariable model, independent predictors of poor survival were WBC $> 15 \times 10^9/L$ (3 points), *ASXL1* mutations (2 points), age > 65 years (2 points), platelet count $< 100,000/\text{mL}$ (2 points), and

hemoglobin $< 10 \text{ g/dL}$ in females and $< 11 \text{ g/dL}$ in males (2 points). This model stratified patients into three groups: low (0–4 points), intermediate (5–7 points), and high risk (8–12 points), with median survivals of not reached and 38.5 and 14.4 months, respectively. More recent studies have highlighted the adverse prognostic effect of *ASXL1* and *DNMT3A* mutations in CMML. To further clarify the prognostic relevance of *ASXL1* mutations, an international collaborative cohort of 466 CMML patients was analyzed. In multivariable analysis, *ASXL1* mutations, $AMC > 10 \times 10^9/L$, hemoglobin $< 10 \text{ g/dL}$, platelets $< 100 \times 10^9/L$, and circulating immature myeloid cells were independently predictive of shortened overall survival. More recently, the aforementioned CPSS model was updated to include molecular abnormalities including *ASXL1*, *RUNX1*, *NRAS*, and *SETBP1* mutations (CPSS-Mol). In a report of 171 patients with blast phase CMML (median age 71 years), treatment included best supportive care in 25%, hypomethylating agent therapy in 10%, AML-like induction chemotherapy in 38%, AML-like induction chemotherapy followed by AHSCT in 15%, upfront AHSCT in 2%, and clinical trials in 11%. After a median follow-up of 4.4 months, 141 deaths (82%) were recorded. Median overall survival was 6 months with 1-, 3-, and 5-year survival rates of 25%, 9%, and 6%, respectively.

Treatment Current treatment in CMML consists of hydroxyurea and supportive care, including red cell transfusions and use of erythropoiesis-stimulating agents (ESAs). The value of hydroxyurea was reinforced by a randomized trial against oral etoposide. No other single or combination chemotherapy has been shown to be superior to hydroxyurea. AHSCT is a viable treatment option for transplant-eligible patients with poor prognostic features. Given the MDS/MPN overlap phenotype and the presence of MDS-like genetic/methylation abnormalities in CMML, hypomethylating agents such as 5-azacitidine and decitabine have been used with limited efficacy; in a recent study using decitabine in CMML, overall response rate was 48% with 17% complete remissions and median survival of 17 months. The experience with 5-azacytidine was somewhat similar. In a recent Mayo Clinic report, among 406 consecutive CMML patients (age ≤ 75 years at diagnosis) seen between January 1990 and December 2018, 70 (17%) underwent AHSCT (median age 58 years) including 46 (66%) in chronic phase and 24 (34%) in blast phase. At a median follow-up of 70 months, there were 22 deaths (31%) in the chronic phase transplant group, 11 (24%) from disease relapse and 9 (20%) from nonrelapse mortality. Posttransplant median survival was 67 months in the chronic phase and 16 months in the blast phase ($p < .01$) transplant groups; 5-year survival rates were 51% and 19%, respectively.

JUVENILE MYELOMONOCYTIC LEUKEMIA

JMML is primarily a disease of early childhood and is included, along with CMML, in the MDS/MPN WHO category. Both CMML and JMML feature leukocytosis, monocytosis, and hepatosplenomegaly. Additional characteristic features in JMML include thrombocytopenia and elevated fetal hemoglobin. Myeloid progenitors in JMML display granulocyte-macrophage colony-stimulating factor (GM-CSF) hypersensitivity that has been attributed to dysregulated RAS/MAPK signaling. The latter is believed to result from mutually exclusive mutations involving *RAS*, *PTPN11*, and *NFI*. A third of patients with JMML that is not associated with Noonan syndrome carry *PTPN11* mutations, whereas the incidence of *NFI* in patients without neurofibromatosis type 1 (NF1) and *RAS* mutations is ~15% each. In general, ~85% of JMML cases have one of the classical RAS pathway mutations (*PTPN11*, *NRAS*, *KRAS*, *NFI*, or *CBL*); in addition, a myriad of other mutations, such as *ASXL1*, *RUNX1*, *SETBP1*, *JAK3*, and *CUX1*, have recently been reported. Drug therapy is relatively ineffective in JMML, and the treatment of choice is AHSCT, which results in a 5-year survival of approximately 50%.

The 2016 revised WHO diagnostic criteria for JMML require the presence of PB monocyte count $\geq 1 \times 10^9/L$, $< 20\%$ blasts in blood or BM, splenomegaly, and absence of *BCR-ABL1*. Diagnosis also requires the presence of one of the following: somatic mutation of *PTPN11*,

KRAS, or *NRAS*; clinical diagnosis of NF1 or *NF1* mutation; germline mutation of *CBL*; and loss of heterozygosity. Diagnosis of JMML can still be considered without the aforementioned genetic features in the presence of monosomy 7 or any other cytogenetic abnormality or in the presence of two of the following: increased hemoglobin F, presence of myeloid or erythroid precursors in the PB, GM-CSF hypersensitivity in colony assay, and hyperphosphorylation of STAT5.

MDS/MPN, UNCLASSIFIABLE MDS/MPN U

The WHO classifies patients with morphologic and laboratory features that resemble both MDS and MPN as “MDS/MPN overlap.” This category includes CMML, aCML, and JMML, which have been discussed above. In addition, MDS/MPN includes a fourth category referred to as MDS/MPN, unclassifiable (MDS/MPN-U). Diagnosis of MDS/MPN-U requires the presence of both MDS and MPN features that are not adequate to classify patients as CMML, aCML, or JMML. MDS/MPN also includes the provisional category of refractory anemia with ring sideroblasts and thrombocytosis (RARS-T); the 2016 revision of the WHO classification document has changed the term *RARS-T* to *MDS/MPN-RS-T*.

In a representative study of 85 patients with MDS/MPN-U, median age was 70 years and 72% were male. Splenomegaly at presentation was present in 33%, thrombocytosis in 13%, leukocytosis in 18%, *JAK2* mutations in 30%, and abnormal karyotype in 51%; the most frequent cytogenetic abnormality was trisomy 8. Median survival was 12.4 months and favorably affected by thrombocytosis. Treatment with hypomethylating agents, immunomodulators, or AHSCT did not appear to favorably affect survival.

MDS/MPN WITH RING SIDEROBLASTS AND THROMBOCYTOSIS MDS/MPN RS T

MDS/MPN-RS-T is classified in the MDS/MPN category because it shares dysplastic features with MDS-RS and myeloproliferative features with ET. The 2016 revised WHO diagnostic criteria for MDS/MPN-RS-T includes anemia associated with erythroid lineage dysplasia, presence of $\geq 5\%$ ring sideroblasts, blast count of $< 5\%$ in BM and $< 1\%$ in the PB, platelet count of $\geq 450 \times 10^9/L$, and absence of *BCR-ABL1*, *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2* mutations or t(3;3) (q21;q26), inv(3)(q21q26), or del(5q). These new diagnostic criteria also require the absence of history of MPN, MDS, or other type of MDS/MPN and also either the presence of *SF3B1* mutation or absence of exposure to cytotoxic or other treatment that could be blamed for the morphologic abnormalities.

In a recent study, 111 patients with MDS/MPN-RS-T were compared with 33 patients with RARS. The frequency of *SF3B1* mutations in MDS/MPN-RS-T-T (87%) was similar to that in MDS-RS (85%). *JAK2* V617F mutation was detected in 49% of MDS/MPN-RS-T patients (including 48% of those mutated for *SF3B1*) but none of those with MDS-RS. In MDS/MPN-RS-T, *SF3B1* mutations were more frequent in females (95%) than in males (77%), and mean ring sideroblast counts were higher in *SF3B1*-mutated patients. Median overall survival was 6.9 years in *SF3B1* mutated cases versus 3.3 years in unmutated cases. Six-year survival was 67% in *JAK2* mutated cases versus 32% in unmutated cases. Multivariable analysis identified younger age and *JAK2* and *SF3B1* mutations as favorable factors. Predictors of poor survival in MDS/MPN-RS-T include anemia, abnormal karyotype, and presence of *ASXL1* or *SETBP1* mutations. Interestingly, the presence of *SF3B1* mutations in MDS/MPN-RS-T was recently shown to be associated with increased risk of thrombosis. Several case reports have suggested that treatment with lenalidomide might induce red cell transfusion independency and complete remissions in MDS/MPN-RS-T. Most recently, luspatercept, a recombinant fusion protein that binds transforming growth factor β superfamily ligands to reduce SMAD signaling, has also been shown to benefit some patients with MDS-RS-T; in a recently published phase 3 trial involving 229 patients with transfusion-dependent very-low- to intermediate-risk MDS-RS-T, transfusion independence for ≥ 8 weeks was achieved in 38% of the patients receiving luspatercept versus 13% of patients in the placebo group ($p < .01$).

MYELOPROLIFERATIVE NEOPLASM, UNCLASSIFIABLE MPN U

The category of MPN-U includes MPN-like neoplasms that cannot be clearly classified as one of the other seven subcategories of MPN (Table 110-4). Examples include patients presenting with unusual thrombosis or unexplained organomegaly with normal blood counts but found to carry MPN-characteristic mutations such as *JAK2* and *CALR* or display BM morphology that is consistent with MPN. It is possible that some cases of MPN-U represent earlier disease stages in PV or ET, which however fail to meet the threshold hemoglobin levels or platelet counts that are required per WHO diagnostic criteria. Specific treatment interventions might not be necessary in asymptomatic patients with MPN-U, whereas patients with arterial thrombotic complications might require cytoreductive and aspirin therapy and those with venous thrombosis might require systemic anticoagulation.

MYELOID NEOPLASMS WITH GERMLINE PREDISPOSITION

The 2016 WHO revision on the classification of myeloid neoplasms added a section referred to as “myeloid neoplasms with germline predisposition” and that includes cases of AML, MDS, and MDS/MPN that arise in the setting of a germline predisposition mutation, such as *CEBPA*, *DDX41*, *RUNX1*, *ANKRD26*, *ETV6*, or *GATA2*. This particular category of diseases also includes myeloid neoplasms that arise in the background of BM failure syndromes, Down syndrome, Noonan syndrome, neurofibromatosis, and telomeropathies.

TRANSIENT MYELOPROLIFERATIVE DISORDER TMD

TMD, also referred to as transient abnormal myelopoiesis (TAM), constitutes an often but not always transient phenomenon of abnormal megakaryoblast proliferation, which occurs in $\sim 10\%$ of infants with Down syndrome. TMD is usually recognized at birth and either undergoes spontaneous regression (75% of cases) or progresses to acute megakaryoblastic leukemia (AMKL) (25% of cases). Almost all patients with TMD and TMD-derived AMKL display somatic *GATA1* mutations. TMD-associated *GATA1* mutations constitute exon 2 insertions, deletions, or missense mutations, affecting the N-terminal transactivation domain of GATA-1, and result in loss of full-length (50-kD) GATA-1 and its replacement with a shorter isoform (40-kD) that retains friend of GATA-1 (FOG-1) binding. In contrast, inherited forms of exon 2 *GATA1* mutations produce a phenotype with anemia, whereas exon 4 mutations that affect the N-terminal, FOG-1-interactive domain produce familial dyserythropoietic anemia with thrombocytopenia or X-linked macrothrombocytopenia.

PRIMARY EOSINOPHILIA

Eosinophilia refers to a PB absolute eosinophil count (AEC) that is above the upper normal limit of the reference range. The term *hyper-eosinophilia* is used when the AEC is $> 1500 \times 10^9/L$. Eosinophilia is operationally classified into secondary (nonneoplastic proliferation of eosinophils) and primary (proliferation of eosinophils that is either neoplastic or otherwise unexplained). Secondary eosinophilia is by far the most frequent cause of eosinophilia and is often associated with infections, especially those related to tissue-invasive helminths, allergic/vasculitic diseases, drugs, and metastatic cancer. Primary eosinophilia is the focus of this chapter and is considered when a cause for secondary eosinophilia is not readily apparent.

Primary eosinophilia is classified as clonal or idiopathic. Diagnosis of clonal eosinophilia requires morphologic, cytogenetic, or molecular evidence of a myeloid neoplasm. Idiopathic eosinophilia is considered when both secondary and clonal eosinophilias have been ruled out as a possibility. HES is a subcategory of idiopathic eosinophilia with persistent AEC of $\geq 1.5 \times 10^9/L$ and associated with eosinophil-mediated organ damage (Table 110-6). An HES-like disorder that is associated with clonal or phenotypically abnormal T cells is referred to as lymphocytic variant hypereosinophilia (Table 110-6).

Clonal Eosinophilia Examples of clonal eosinophilia include eosinophilia associated with AML, MDS, CML, mastocytosis, and MDS/

TABLE 110-6 Primary Eosinophilia Classification

VARIABLES	EOSINOPHILIA ASSOCIATED WITH <i>PDGFRA</i> , <i>PDGFRB</i> , <i>FGFR1</i> , OR <i>PCM1-JAK2</i> ABNORMALITY	CHRONIC EOSINOPHILIA NOT OTHERWISE SPECIFIED (CEL-NOS)	LYMPHOCYTIC VARIANT HYPEREOSINOPHILIA	HYPEREOSINOPHILIC SYNDROME
Absolute eosinophil count	>600 × 10 ⁹ /L	>1500 × 10 ⁹ /L	>1500 × 10 ⁹ /L	>1500 × 10 ⁹ /L
Peripheral blood blasts >2%	Yes or no	Yes or no	No	No
Bone marrow blasts >5%	Yes or no	Yes or No	No	No
Abnormal karyotype	Yes or no	Yes or no	No	No
<i>PDGFRA</i> , <i>PDGFRB</i> , <i>FGFR1</i> , or <i>PCM1-JAK2</i> abnormality	Yes	No	No	No
<i>BCR-ABL1</i>	No	No	No	No
Abnormal T lymphocyte phenotype or clonal T-cell clones	No	No	Yes	No
Eosinophil-mediated tissue damage	Yes or no	Yes or no	Yes or no	Yes

MPN overlap. Myeloid neoplasm–associated eosinophilia also includes the WHO MPN subcategory of chronic eosinophilic leukemia, not otherwise specified (CEL-NOS) and the WHO myeloid malignancy subcategory referred to as myeloid/lymphoid neoplasms with eosinophilia and rearrangement of platelet-derived growth factor receptor (*PDGFR*) α/β or fibroblast growth factor receptor 1 (*FGFR1*) or with *PCM1-JAK2* (Table 110-4).

The diagnostic workup for clonal eosinophilia that is not associated with morphologically overt myeloid malignancy should start with PB mutation screening for *FIP1L1-PDGFRα* and *PDGFRβ* mutations using fluorescence in situ hybridization (FISH) or reverse transcription polymerase chain reaction. This is crucial since such eosinophilia is easily treated with imatinib. If mutation screening is negative, a BM examination with cytogenetic studies is indicated. In this regard, one must first pay attention to the presence or absence of 5q33, 4q12, 8p11.2, or t(8;9)(p22;p24.1) translocations, which, if present, would suggest *PDGFRβ*-, *PDGFRα*-, or *FGFR1*-rearranged or *PCM1-JAK2*-associated clonal eosinophilia, respectively. The presence of 5q33 or 4q12 translocations predicts favorable response to treatment with imatinib mesylate and presence of t(8;9)(p22;p24.1) predicts a transient response to ruxolitinib, whereas 8p11.2 translocations are associated with aggressive myeloid malignancies that are refractory to current drug therapy.

Chronic Eosinophilic Leukemia, Not Otherwise Specified (CEL-NOS) CEL-NOS is a subset of clonal eosinophilia that is neither molecularly defined nor classified as an alternative clinicopathologically assigned myeloid malignancy. We prefer to use the term strictly in patients with an HES phenotype who also display either a clonal cytogenetic/molecular abnormality or excess blasts in the BM or PB. The WHO defines CEL-NOS as the presence of ≥1.5 × 10⁹/L AEC that is accompanied by either the presence of myeloblast excess (either >2% in the PB or 5–19% in the BM) or evidence of myeloid clonality.

In a recent Mayo Clinic survey of 1416 patients with PB eosinophilia evaluated between 2008 and 2019, 17 patients (1.2%) fulfilled the WHO 2016 criteria for CEL-NOS. Median age was 63 years (range 25–92 years) with the vast majority of patients (88%) presenting with systemic symptoms. Organ involvement was a prominent feature, and involved organs included spleen, cardiac and pulmonary organs, and distal esophagus. Laboratory abnormalities included anemia, leukocytosis, and eosinophilia (median eosinophil count of 6.4 × 10⁹/L; range 2.0–53.1). The most common BM abnormalities included abnormal eosinophils, abnormal and increased megakaryocytes, and fibrosis (18%). Cytogenetic abnormalities occurred in 88% of patients and included trisomy 8, complex karyotype, 13q–, 20q–, and chromosome 1 abnormalities. All seven patients with next-generation sequencing studies harbored one or more mutations, including *ASXL1* (43%) and *IDH1* (29%). Half of patients treated with hydroxyurea-based regimens responded with a persistent decline in eosinophil count for a median duration of 18 months. One-third of patients treated with prednisone responded, with a median duration of response of 13 months. Three

patients were treated with imatinib, of whom two had normalization of eosinophil count. At a median follow-up of 13 months, nine patients had died including three patients who underwent leukemic transformation.

***PDGFR* Mutated Eosinophilia** Both platelet-derived growth factor receptors α (*PDGFRA* located on chromosome 4q12) and β (*PDGFRB* located on chromosome 5q31-q32) are involved in MPN-relevant activating mutations. Clinical phenotype in both instances includes prominent blood eosinophilia and excellent response to imatinib therapy. In regard to *PDGFRA* mutations, the most popular is *FIP1L1-PDGFRα*, a karyotypically occult del(4)(q12), that was described in 2003 as an imatinib-sensitive activating mutation. Functional studies have demonstrated transforming properties in cell lines and the induction of MPN in mice. Cloning of the *FIP1L1-PDGFRα* fusion gene identified a novel molecular mechanism for generating this constitutively active fusion tyrosine kinase, wherein a ~800-kb interstitial deletion within 4q12 fuses the 5' portion of *FIP1L1* to the 3' portion of *PDGFRA*. *FIP1L1-PDGFRα* occurs in a very small subset of patients who present with the phenotypic features of either systemic mastocytosis (SM) or HES, but the presence of the mutation reliably predicts complete hematologic and molecular response to imatinib therapy.

In a recent retrospective survey of 151 patients with *FIP1L1-PDGFRα*–associated eosinophilia (143 males; mean age at diagnosis 49 years), organopathy involved the spleen (44%), skin (32%), lungs (30%), heart (19%), and central nervous system (9%); none of 31 patients initially treated with corticosteroids achieved complete hematologic remission, whereas all 148 patients treated with imatinib achieved complete hematologic responses and also molecular responses, when evaluated. Treatment discontinuation was documented in 46 patients followed by a 57% relapse rate; the 1-, 5-, and 10-year overall survival rates in imatinib-treated patients were 99%, 95%, and 84%, respectively. Other studies have confirmed the possibility of treatment-free remissions in some patients after imatinib discontinuation. Infrequent occurrence of *FIP1L1-PDGFRα* mutated acute myeloid leukemia associated with eosinophilia has also been shown to respond to low-dose imatinib therapy (100 mg/d).

The association between eosinophilic myeloid malignancies and *PDGFRB* rearrangement was first characterized and published in 1994 where fusion of the tyrosine kinase encoding region of *PDGFRB* to the *ets*-like gene *ETV6* (*ETV6-PDGFRB*, t[5;12](q33;p13)) was demonstrated. The fusion protein was transforming to cell lines and resulted in constitutive activation of *PDGFRB* signaling. Since then, several other *PDGFRB* fusion transcripts with similar disease phenotypes have been described, and cell line transformation and MPD induction in mice have been demonstrated. Imatinib therapy was shown to be effective when employed.

***FGFR1* Mutated Eosinophilia** The 8p11 myeloproliferative syndrome (EMS) (also known as human stem cell leukemic/lymphoma syndrome) constitutes a clinical phenotype with features of both lymphoma and eosinophilic MPN and is characterized by a fusion

mutation that involves the gene for fibroblast growth factor receptor-1 (*FGFR1*), which is located on chromosome 8p11. In EMS, both myeloid and lymphoid lineage cells exhibit the 8p11 translocation, thus demonstrating the stem cell origin of the disease. The disease features several 8p11-linked chromosome translocations, and some of the corresponding fusion *FGFR1* mutants have been shown to transform cell lines and induce EMS- or CML-like disease in mice depending on the specific *FGFR1* partner gene (*ZNF198* or *BCR*, respectively). Consistent with this laboratory observation, some patients with *BCR-FGFR1* mutation manifest a more indolent CML-like disease. The mechanism of *FGFR1* activation in EMS is similar to that seen with *PDGFRB*-associated MPD; the tyrosine kinase domain of *FGFR1* is juxtaposed to a dimerization domain from the partner gene. EMS is aggressive and requires combination chemotherapy followed by ASCT.

***PCM1-JAK2*–Associated Myeloid/Lymphoid Neoplasm with Eosinophilia** The 2016 revised WHO document includes a provisional entity under myeloid/lymphoid neoplasms with eosinophilia referred to as “myeloid/lymphoid neoplasms with *PCM1-JAK2*. The entity is characterized by the t(8;9)(p22;p24.1) cytogenetic abnormality and a phenotype that displays marked male predominance, hepatosplenomegaly, eosinophilia, and morphologic features similar to MPN, MDS, or MDS/MPN. Current drug therapy for *PCM1-JAK2*–associated disease is suboptimal, although some affected patients have displayed transient responses to ruxolitinib therapy.

Hypereosinophilic Syndrome Blood eosinophilia that is neither secondary nor clonal is operationally labeled as being “idiopathic.” HES is a subcategory of idiopathic eosinophilia with persistent increase of the AEC to $\geq 1.5 \times 10^9/L$ and presence of eosinophil-mediated organ damage, including cardiomyopathy, gastroenteritis, cutaneous lesions, sinusitis, pneumonitis, neuritis, and vasculitis. In addition, some patients manifest thromboembolic complications, hepatosplenomegaly, and either cytopenia or cytosis.

BM histologic and cytogenetic/molecular studies should be examined before a working diagnosis of HES is made. Additional blood studies that are currently recommended during the evaluation of HES include serum tryptase (an increased level suggests mastocytosis and warrants molecular studies to detect *FIP1L1-PDGFRα*), T-cell immunophenotyping, and T-cell receptor antigen gene rearrangement analysis (a positive test suggests an underlying clonal or phenotypically abnormal T-cell disorder). In addition, initial evaluation in HES should include echocardiogram and measurement of serum troponin levels to screen for myocardial involvement by the disease.

Initial evaluation of the patient with eosinophilia should include tests that facilitate assessment of target organ damage: complete blood count, chest x-ray, echocardiogram, and serum troponin level. Increased level of serum cardiac troponin has been shown to correlate with the presence of cardiomyopathy in HES. Typical echocardiographic findings in HES include ventricular apical thrombus, posterior mitral leaflet or tricuspid valve abnormality, endocardial thickening, dilated left ventricle, and pericardial effusion.

In a recent Mayo Clinic study of 98 consecutive patients with idiopathic eosinophilia, including HES, median age was 53 years (55% males) and overt organ involvement was seen in >80% of the cases, including 54% involving organs other than the skin. The frequencies of cardiac involvement, hepatosplenomegaly, and increased serum tryptase and interleukin 5 (IL-5) levels were 8%, 4%, 24%, and 31%, respectively. The study also revealed that 11% of the affected patients harbored pathogenetic mutations including *TET2*, *ASXL1*, and *KIT*; the presence of such mutations did not appear to influence phenotype, and the number of informative cases was too small to assess prognostic relevance. Instead, the study identified anemia and presence of cardiac involvement or hepatosplenomegaly as risk factors for survival.

Corticosteroids are the cornerstone of therapy in HES. Treatment with oral prednisone is usually started at 1 mg/kg/d and continued for 1–2 weeks before the dose is tapered slowly over the ensuing 2–3 months. If symptoms recur at a prednisone dose level of >10 mg/d, either hydroxyurea or interferon α is used as a steroid-sparing agent. In patients who fail usual therapy as outlined above, mepolizumab

or alemtuzumab might be considered. Mepolizumab is a monoclonal antibody that targets IL-5, which is a well-recognized survival factor for eosinophils. Alemtuzumab targets the CD52 antigen, which has been shown to be expressed by eosinophils but not by neutrophils. In a recently reported, placebo-controlled, phase 3 study, HES patients received subcutaneous mepolizumab (300 mg) every 4 weeks, in addition to their preprotocol therapy, and experienced significantly fewer disease flare ups or treatment discontinuations (28 vs 56% for placebo), without excess adverse events. Mepolizumab was approved by the U.S. Food and Drug Administration (FDA) for use in HES on September 25, 2020. In a smaller phase 2 study, benralizumab (monoclonal antibody targeting the receptor for IL-5; 30 mg given subcutaneously every 4 weeks) was also shown to reduce eosinophil count more efficiently compared to placebo (90 vs 30%).

■ MASTOCYTOSIS

Mast cell disease (MCD) is defined as tissue infiltration by morphologically and immunophenotypically abnormal mast cells. MCD is classified into two broad categories: cutaneous mastocytosis (CM) and systemic mastocytosis (SM). MCD in adults is usually systemic, and the clinical course can be either indolent or aggressive, depending on the respective absence or presence of impaired organ function. Symptoms and signs of MCD include urticaria pigmentosa, mast cell mediator release symptoms (e.g., headache, flushing, lightheadedness, syncope, anaphylaxis, pruritus, urticaria, angioedema, nausea, diarrhea, abdominal cramps), and organ damage (lytic bone lesions, osteoporosis, hepatosplenomegaly, cytopenia). Aggressive SM can be associated with another myeloid malignancy, including MPN, MDS, or MDS/MPN overlap (e.g., CMML), or present as overt mast cell leukemia. In general, life expectancy is near normal in indolent SM but significantly shortened in aggressive SM.

Diagnosis of SM is based on BM examination that shows clusters of morphologically abnormal, spindle-shaped mast cells that are best evaluated by the use of immunohistochemical stains that are specific to mast cells (tryptase, CD117). In addition, mast cell immunophenotyping reveals aberrant CD25 expression by neoplastic mast cells. Other laboratory findings in SM include increased levels of serum tryptase, histamine and urine histamine metabolites, and prostaglandins. SM is associated with *KIT* mutations, usually *KIT* D816V, in the majority of patients. Accordingly, mutation screening for *KIT* D816V is diagnostically useful. However, the ability to detect *KIT* D816V depends on assay sensitivity and mast cell content of the test sample. The 2016 WHO classification of mastocytosis includes (1) CM, (2) SM, and (3) mast cell sarcoma (MCS). SM is further classified into (1) indolent SM (ISM), (2) smoldering SM (SSM), (3) SM with an associated hematologic neoplasm (SM-AHN), (4) aggressive SM (ASM), and (5) mast cell leukemia (MCL).

In a recent Mayo Clinic study of 580 patients (median age 55 years; range 18–88 years) with SM, morphologic subcategories were indolent/smoldering in 291 patients (50%) and advanced in 289 patients (50%), including SM-AHN in 199, ASM in 85, and MCL in 5. Multivariable analysis of clinical variables identified age >60 years, advanced SM, thrombocytopenia $<150 \times 10^9/L$, anemia below sex-adjusted normal, and increased alkaline phosphatase as independent risk factors for survival. In addition, *ASXL1*, *RUNX1*, and *NRAS* mutations were also independently associated with inferior survival. Combined clinical, cytogenetic, and molecular risk factor analysis confirmed the independent prognostic contribution of adverse mutations, advanced SM, thrombocytopenia, increased alkaline phosphatase, and age >60 years. These data were subsequently used to develop clinical and hybrid clinical-molecular risk models. The clinical risk model uses six readily accessible risk variables including age >60 years, platelet count $<150 \times 10^9/L$, anemia, hypoalbuminemia, increased alkaline phosphatase, and morphologic classification as advanced SM. Accordingly, median survival times were not reached, 148, 65, 31, 18, and 5 months in the presence of 1, 2, 3, 4, 5, and 6 of these risk factors, respectively.

Both ISM and ASM patients might experience mast cell mediator release symptoms, which are usually managed by both H_1 and H_2 histamine receptor blockers as well as cromolyn sodium. In addition,

patients with propensity to vasodilatory shock should wear a medical alert bracelet and carry an Epi-Pen self-injector for self-administration of subcutaneous epinephrine. Urticaria pigmentosa shows variable response to both topical and systemic corticosteroid therapy. Cytoaberrant therapy is not recommended for ISM, and instead, such patients are managed with use of H₁ and H₂ blockers, leukotriene antagonists, sodium cromolyn, phototherapy, topical steroids, and osteoporosis prevention with diphosphonates including alendronate and pamidronate. In ASM, either interferon α or cladribine is considered first-line therapy and benefits the majority of patients. Cladribine is administered by 2-h infusion (5 mg/m²) daily for 5 days, repeated monthly for 4–6 cycles; expected overall response is ~50%, including major response in ~38%. In contrast, imatinib is ineffective in the treatment of *PDGFR* unmutated SM. A controlled study of patients with ISM or SSM demonstrated marginal value of masitinib (oral tyrosine kinase inhibitor that inhibits KIT and LYN), with a reported cumulative symptomatic response rate of 18.7% versus 7.4% for placebo. Treatment responses were more impressive in another study that used the multikinase inhibitor midostaurin in patients with the more aggressive forms of SM, with 45% of the patients achieving major response. Most recently, equally impressive responses were seen with the use of another kinase inhibitor, avapritinib (specifically targets KIT D816V), in both ISM and ASM; however, significant drug-related toxicity, including intracranial bleed, cognitive impairment, and moderate to severe cytopenias, has been observed.

DENDRITIC AND HISTIOCYTIC NEOPLASMS

Dendritic cell (DC) and histiocyte/macrophage neoplasms are extremely rare. DCs are antigen-presenting cells, whereas histiocytes/macrophages are antigen-processing. BM myeloid stem cells (CD34+) give rise to monocyte (CD14+, CD68+, CD11c+, CD1a-) and DC (CD14-, CD11c+/-, CD1a+/-) precursors. Monocyte precursors, in turn, give rise to macrophages (CD14+, CD68+, CD11c+, CD163+, lysozyme+) and interstitial DCs (CD68+, CD1a-). DC precursors give rise to Langerhans cell DCs (Birbeck granules, CD1a+, S100+, langerin+) and plasmacytoid DCs (CD68+, CD123+). Follicular DCs (CD21+, CD23+, CD35+) originate from mesenchymal stem cells. Dendritic and histiocytic neoplasms are operationally classified into macrophage/histiocyte-related and DC-related. The former includes histiocytic sarcoma/malignant histiocytosis, and the latter includes Langerhans cell histiocytosis, Langerhans cell sarcoma, interdigitating DC sarcoma, and follicular DC sarcoma.

Histiocytic Sarcoma/ Malignant Histiocytosis Histiocytic sarcoma represents malignant proliferation of mature tissue histiocytes and is often localized. Median age at diagnosis is estimated at 46 years with slight male predilection. Some patients might have history of lymphoma, MDS, or germ cell tumors at time of disease presentation. The three typical disease sites are lymph nodes, skin, and gastrointestinal system. Patients may or may not have systemic symptoms including fever and weight loss, and other symptoms include hepatosplenomegaly, lytic bone lesions, and pancytopenia. Immunophenotype includes presence of histiocytic markers (CD68, lysozyme, CD11c, CD14) and absence of myeloid or lymphoid markers. Prognosis is poor and treatment often ineffective. The term *malignant histiocytosis* (MH) refers to a disseminated disease and systemic symptoms. Lymphoma-like treatment induces complete remissions in some patients, and median survival is estimated at 2 years.

Langerhans Cell Histiocytosis Langerhans cells (LCs) are specialized DCs that reside in mucocutaneous tissue and, upon activation, become specialized for antigen presentation to T cells. LC histiocytosis (LCH; also known as histiocytosis X) represents neoplastic proliferation of LCs (S100+, CD1a+, and Birbeck granules on electron microscopy). LCH incidence is estimated at 5 per million, and the disease typically affects children with a male predilection. Presentation can be either unifocal (eosinophilic granuloma) or multifocal. The former usually affects bones and less frequently lymph nodes, skin, and lung, whereas the latter is more disseminated. Unifocal disease often affects older children and adults, whereas multisystem disease affects infants.

LCH of the lung in adults is characterized by bilateral nodules. Prognosis depends on organs involved. Only 10% of patients progress from unifocal to multiorgan disease. LCH of the lung might improve upon cessation of smoking. Approximately 55% of patients with LCH harbor *BRAF* V600E gain-of-function mutations, which indicates high-risk disease and resistance to first-line therapy; however, responses to targeted therapy with vemurafenib have been reported. Other forms of treatment for LCH include combination chemotherapy and MEK inhibitors in *BRAF* wild-type disease with other MAPK pathway mutations. Unfortunately, such targeted therapy has not secured long-lasting, treatment-free remissions.

Langerhans Cell Sarcoma Langerhans cell sarcoma (LCS) also represents neoplastic proliferation of LCs with overtly malignant morphology. The disease can present de novo or progress from antecedent LCH. There is a female predilection, and median age at diagnosis is estimated at 41 years. Immunophenotype is similar to that seen in LCH, and liver, spleen, lung, and bone are the usual sites of disease. Prognosis is poor and treatment generally ineffective.

Interdigitating Dendritic Cell Sarcoma Interdigitating DC sarcoma (IDCS), also known as reticulum cell sarcoma, represents neoplastic proliferation of IDCs. The disease is extremely rare and affects elderly adults with no sex predilection. Typical presentation is asymptomatic solitary lymphadenopathy. Immunophenotype includes S100 positivity and negativity for vimentin and CD1a. Prognosis ranges from benign local disease to widespread lethal disease.

Follicular Dendritic Cell Sarcoma FDCs reside in B-cell follicles and present antigen to B cells. FDC neoplasms (FDCNs) are usually localized and often affect adults. FDCN might be associated with Castleman's disease in 10–20% of cases, and increased incidence in schizophrenia has been reported. Cervical lymph nodes are the most frequent site of involvement in FDCN, and other sites include maxillary, mediastinal, and retroperitoneal lymph nodes; oral cavity; gastrointestinal system; skin; and breasts. Sites of metastasis include lung and liver. Immunophenotype includes CD21, CD35, and CD23. Clinical course is typically indolent, and treatment includes surgical excision followed by regional radiotherapy and sometimes systemic chemotherapy.

Hemophagocytic Syndrome Hemophagocytic syndrome (HPS), also known as hemophagocytic lymphohistiocytosis (HLH), represents nonneoplastic proliferation and activation of macrophages that induce cytokine-mediated BM suppression and features of intense phagocytosis in BM and liver. HPS may result from genetic (primary) or acquired (secondary) disorders of macrophages. The former entail genetically determined inability to regulate macrophage proliferation and activation and might include alterations in familial HLH genes (*PRFI*, *UNC13D*, *STXBP2*, and *STX11*), granule/pigment abnormality genes (*RAB27A*, *LYST*, and *AP3B1*), or X-linked lymphoproliferative disease genes (*SH2D1A* and *XIAP*). Acquired HPS is often precipitated by viral infections, most notably Epstein-Barr virus. HPS might also accompany certain malignancies such as T-cell lymphoma and autoimmune diseases. Clinical presentation includes fever, severe constitutional symptoms, enlarged lymph nodes, hepatosplenomegaly, neurologic dysfunction, and abnormalities in multiple organ function tests. Diagnosis is accomplished by either detection of HLH-related mutations or meeting five of the following eight conventional criteria: (1) hemophagocytosis in the BM/spleen/lymph nodes; (2) serum ferritin ≥ 500 μ g/L; (3) hypofibrinogenemia (fibrinogen ≤ 1.5 g/L) or hypertriglyceridemia (triglycerides ≥ 3 mmol/L); (4) low NK cell activity; (5) elevated soluble IL-2 receptor (CD25) ≥ 400 U/mL; (6) bi- or tricytopenia (platelets $< 100 \times 10^9$ /L, hemoglobin < 9 g/dL, absolute neutrophil count $< 1 \times 10^9$ /L); (7) splenomegaly palpable > 3 cm below left costal margin; and (8) fever. Clinical course is often fulminant and fatal. Current therapeutic approaches for primary or secondary HLH include the so-called "HLH-94 protocol," which consists of weekly treatments with etoposide and dexamethasone, stem cell transplantation, emapalumab (a monoclonal antibody that binds and neutralizes interferon γ), and the JAK1/2 inhibitor ruxolitinib. Emapalumab was FDA approved in

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of the same species in the amino acid sequences of otherwise similar immunoglobulins. These differences are determined by allelic genes; by definition, they are detected by antibodies made in the same species. *Idiotypes* are the third category of antigenic determinants. They are unique to the molecules produced by a given clone of antibody-producing cells. Idiotypes are formed by the unique structure of the antigen-binding portion of the molecule.

Antibody molecules (Fig. 111-1) are composed of two heavy chains (~50,000 molecular weight [mol wt]) and two light chains (~25,000 mol wt). Each chain has a constant portion (limited amino acid sequence variability) and a variable region (extensive sequence variability). The light and heavy chains are linked by disulfide bonds and are aligned so that their variable regions are adjacent to one another. This variable region forms the antigen recognition site of the antibody molecule; its unique structural features form idiotypes that are reliable markers for a particular clone of cells because each antibody is formed and secreted by a single clone. Because of the mechanics of the gene rearrangements necessary to specify the immunoglobulin variable regions (VDJ joining for the heavy chain, VJ joining for the light chain), a particular clone rearranges only one of the two chromosomes to produce an immunoglobulin molecule of only one light chain isotype and only one allotype (allelic exclusion) (Fig. 111-1). After exposure to antigen, the variable region may become associated with a new heavy chain isotype (class switch). Each clone of cells performs these sequential gene arrangements in a unique way. This results in each clone producing a unique immunoglobulin molecule. In most plasma cells, light chains are synthesized in slight excess, secreted as free light chains, and cleared by the kidney, but <10 mg of such light chains is excreted per day.

Electrophoretic analysis permits separation of components of the serum proteins (Fig. 111-2). The immunoglobulins move heterogeneously in an electric field and form a broad peak in the gamma region, which is usually increased in the sera of patients with plasma cell tumors. There is a sharp spike in this region called an *M component* (M for monoclonal). Less commonly, the M component may appear in the β_1 or α_2 globulin region. The monoclonal antibody must be present at a concentration of at least 5 g/L (0.5 g/dL) to be accurately quantitated by this method. This corresponds to $\sim 10^9$ cells producing the antibody. Confirmation of the type of immunoglobulin and that it is truly monoclonal is determined by immunoelectrophoresis that reveals a single heavy and/or light chain type. Hence, immunoelectrophoresis and electrophoresis provide qualitative and quantitative assessment of the M component, respectively. Once the presence of an M component has been confirmed, the amount of M component in the serum is a reliable measure of the tumor burden, making M component an excellent tumor marker to manage therapy, yet it is not specific enough to be used to screen asymptomatic patients. In addition to the plasma cell disorders, M components may be detected in other lymphoid neoplasms such as chronic lymphocytic leukemia (CLL) and lymphomas of B- or T-cell origin; nonlymphoid neoplasms such as chronic myeloid leukemia, breast cancer, and colon cancer; a variety of nonneoplastic conditions such as cirrhosis, sarcoidosis, parasitic diseases, Gaucher's disease, and pyoderma gangrenosum; and a number of autoimmune conditions, including rheumatoid arthritis, myasthenia gravis, and cold agglutinin disease. Monoclonal proteins are also observed in immunosuppressed patients after organ transplant and, rarely, allogeneic transplant. At least two very rare skin diseases—lichen myxedematosus (also known as papular mucinosis) and necrobiotic xanthogranuloma—are associated with a monoclonal gammopathy. In papular mucinosis, highly cationic IgG is deposited in the dermis of patients. This organ specificity may reflect the specificity of the antibody for some antigenic component of the dermis. Necrobiotic xanthogranuloma is a histiocytic infiltration of the skin, usually of the face, that produces red or yellow nodules that can enlarge to plaques. Approximately 10% progress to myeloma. Five percent of patients with sensory motor neuropathy also have a monoclonal paraprotein.

The nature of the M component is variable in plasma cell disorders. It may be an intact antibody molecule of any heavy chain subclass, or it may be an altered antibody or fragment. Isolated light or heavy chains may be produced. In some plasma cell tumors such as extramedullary

111 Plasma Cell Disorders

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Kenneth C. Anderson



The *plasma cell disorders* are monoclonal neoplasms related to each other by virtue of their development from common progenitors in the late B-lymphocyte lineage. Multiple myeloma (MM), Waldenström's macroglobulinemia, primary amyloidosis (Chap. 112), and the heavy chain diseases comprise this group and may be designated by a variety of synonyms such as *monoclonal gammopathies*, *paraproteinemias*, *plasma cell dyscrasias*, and *dysproteinemias*. Mature B lymphocytes destined to produce IgG bear surface immunoglobulin molecules of both μ and γ heavy chain isotypes with both isotypes having identical idiotypes (variable regions). Under normal circumstances, maturation to antibody-secreting plasma cells and their proliferation is stimulated by exposure to the antigen for which the surface immunoglobulin is specific; however, in the plasma cell disorders, the control over this process is lost. The clinical manifestations of all the plasma cell disorders relate to the expansion of the neoplastic cells, to the secretion of cell products (immunoglobulin molecules or subunits, lymphokines), and to some extent to the host's response to the tumor. Normal development of B lymphocytes is discussed in Chap. 349 and depicted in Fig. 108-2.

Three categories of structural variation are present among immunoglobulin molecules that form antigenic determinants, and these are used to classify immunoglobulins. *Isotypes* are those determinants that distinguish among the main classes of antibodies of a given species and are the same in all normal individuals of that species. Therefore, isotypic determinants are, by definition, recognized by antibodies from a distinct species (heterologous sera) but not by antibodies from the same species (homologous sera). There are five heavy chain isotypes (M, G, A, D, E) and two light chain isotypes (κ , λ). *Allotypes* are distinct determinants that reflect regular small differences between individuals

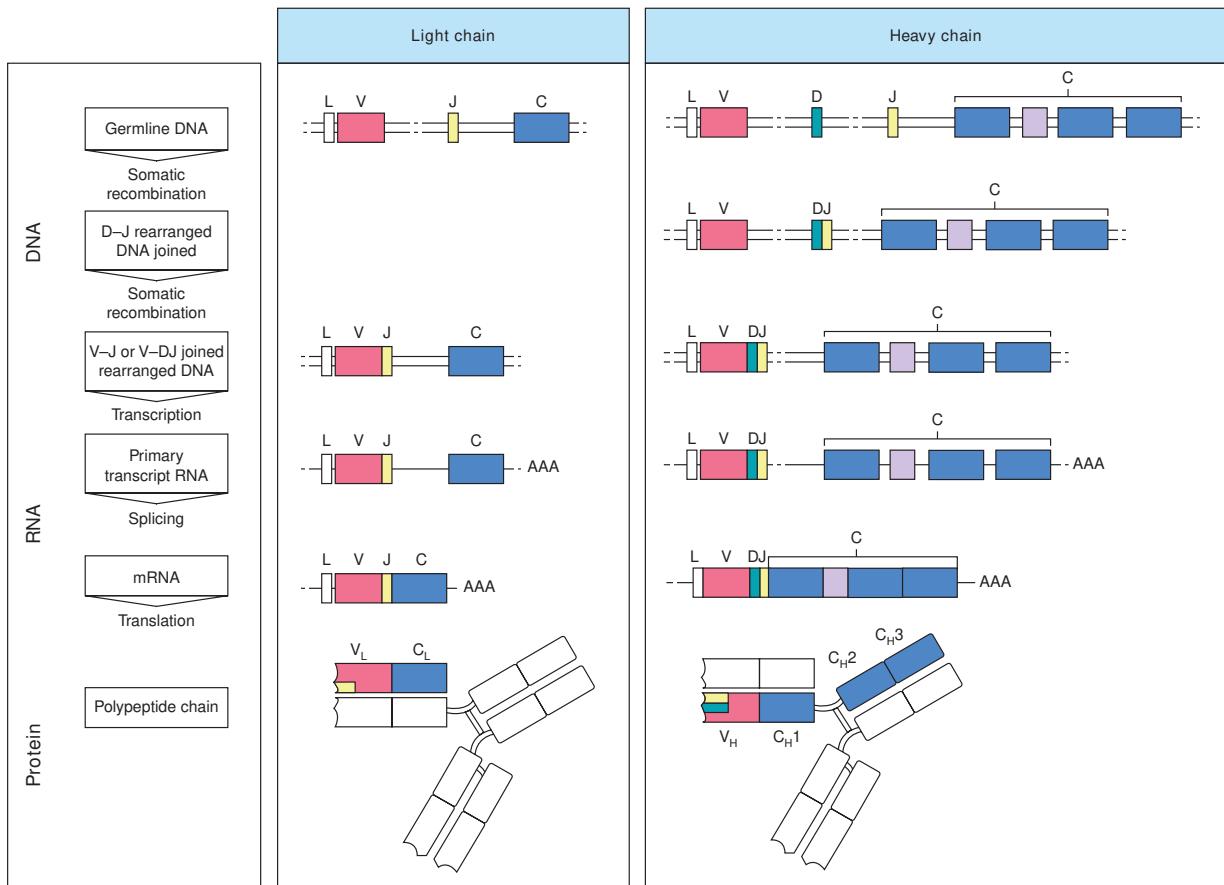
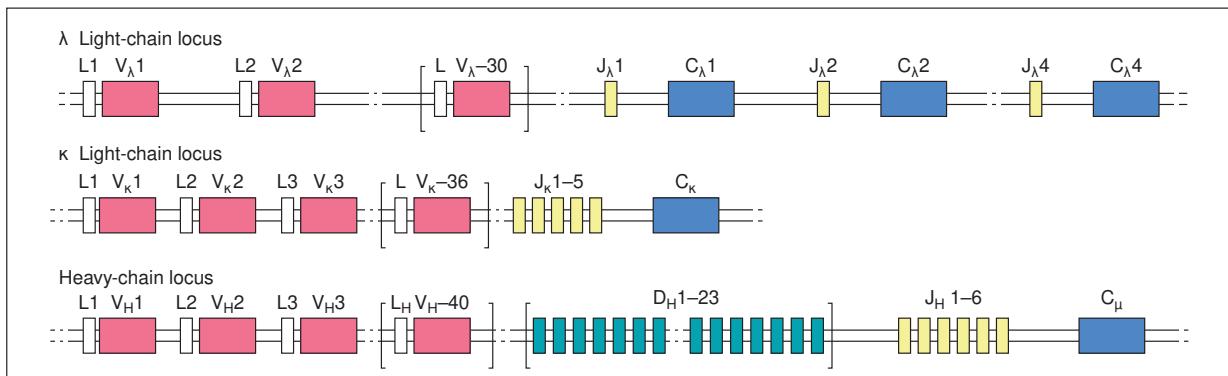


FIGURE 111-1 Immunoglobulin genetics and the relationship of gene segments to the antibody protein. The top portion of the figure is a schematic of the organization of the immunoglobulin genes, λ on chromosome 22, κ on chromosome 2, and the heavy chain locus on chromosome 14. The heavy chain locus is >2 megabases, and some of the D region gene segments are only a few bases long, so the figure depicts the schematic relationship among the segments, not their actual size. The bottom portion of the figure outlines the steps in going from the noncontiguous germline gene segments to an intact antibody molecule. Two recombination events juxtapose the V-D-J (or V-J for light chains) segments. The rearranged gene is transcribed, and RNA splicing cuts out intervening sequences to produce an mRNA, which is then translated into an antibody light or heavy chain. The sites on the antibody that bind to antigen (the so-called CDR3 regions) are encoded by D and J segments for heavy chains and the J segments for light chains. (From Janeway's Immunobiology, 9th ed by Kenneth Murphy and Casey Weaver. Copyright © 2017 by Garland Science, Taylor & Francis Group, LLC. Used by permission of W. W. Norton & Company, Inc.)

or solitary bone plasmacytomas, less than one-third of patients will have an M component. In ~20% of myelomas, only light chains are produced and, in most cases, are secreted in the urine as Bence Jones proteins. The frequency of myelomas of a particular heavy chain class is roughly proportional to the serum concentration, and therefore, IgG myelomas are more common than IgA and IgD myelomas. In ~1% of patients with myeloma, byclonal or triclonal gammopathy is observed.

MULTIPLE MYELOMA

■ DEFINITION

MM represents a malignant proliferation of plasma cells derived from a single clone. The tumor, its products, and the host response to it result in a number of organ dysfunctions and symptoms, including bone pain or fracture, renal failure, susceptibility to infection, anemia,

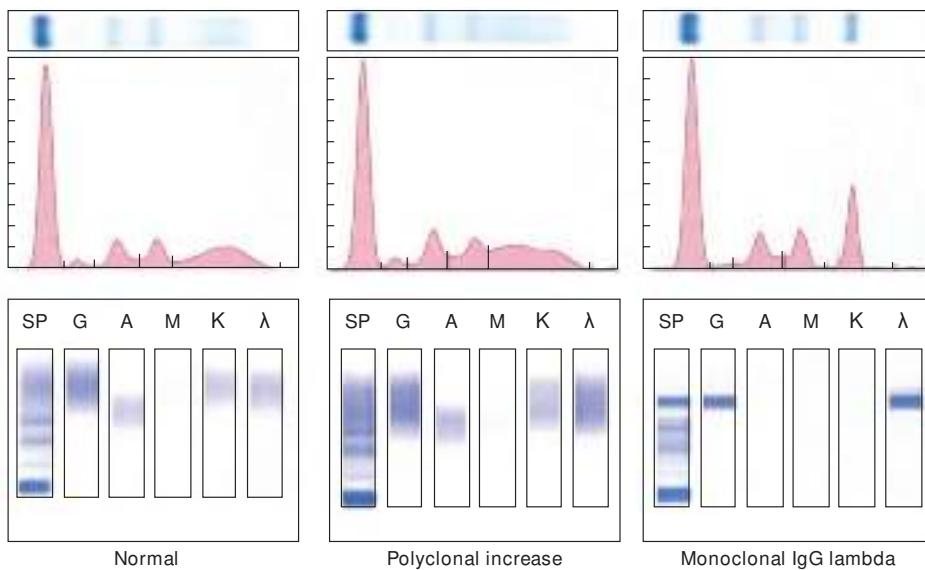


FIGURE 111-2 Representative patterns of serum electrophoresis and immunofixation. The *upper panels* represent agarose gel, middle panels are the densitometric tracing of the gel, and *lower panels* are immunofixation patterns. The panel on the *left* illustrates the normal pattern of serum protein on electrophoresis. Because there are many different immunoglobulins in the serum, their differing mobilities in an electric field produce a broad peak. In conditions associated with increases in polyclonal immunoglobulin, the broad peak is more prominent (*middle panel*). In monoclonal gammopathies, the predominance of a product of a single cell produces a “church spire” sharp peak, usually in the γ globulin region (*right panel*). The immunofixation (*lower panel*) identifies the type of immunoglobulin. For example, normal and polyclonal increases in immunoglobulins produce no distinct bands; however, the *right panel* shows distinct bands in IgG and lambda protein lanes, confirming the presence of IgG lambda monoclonal protein. (Courtesy of Dr. Neal I. Lindeman.)

hypercalcemia, and occasionally clotting abnormalities, neurologic symptoms, and manifestations of hyperviscosity.

Etiology

The cause of myeloma is not known. Myeloma occurred with increased frequency in those exposed to the radiation of nuclear warheads in World War II after a 20-year latency. Myeloma has been seen more commonly than expected among farmers, wood workers, leather workers, and those exposed to petroleum products. A variety of recurrent chromosomal alterations have been found in patients with myeloma: hyperdiploidy (trisomies involving one or more of chromosomes 3, 5, 7, 9, 11, 15, 19, or 21) is observed in half of the patients, while the other half have translocations involving the 14q32 chromosome with variable partners including t(11;14)(q13;q32), t(4;14)(p16;q32), and t(14;16). Other frequent abnormalities include 13q14 deletion, 1q amplification or 1p deletion, and 17p13 deletions. Evidence is strong that errors in switch recombination—the genetic mechanism to change antibody heavy chain isotype—participate in the early transformation process. However, no single common molecular pathogenetic pathway has yet emerged. Genome sequencing studies have failed to identify any recurrent mutation with frequency >20%; N-ras, K-ras, and Braf mutations are most common and, combined, occur in >40% of patients. Evidence of complex clusters of subclonal variants is present at diagnosis, and additional mutations are acquired over time, indicative of genomic evolution that may drive disease progression. Interleukin (IL) 6 may play a role in driving myeloma cell proliferation. It remains difficult to distinguish benign from malignant plasma cells based on morphologic criteria in all but a few cases (Fig. 111-3).

Incidence and Prevalence

In 2021 in the United States, 34,920 new cases of myeloma were estimated to be diagnosed, and 12,410 people were estimated to die from the disease. Myeloma increases in incidence with age. The median age at diagnosis is 69 years; it is uncommon under age 40. Males are more commonly affected than females, and blacks have nearly twice the incidence of whites. In 2018, myeloma accounted for 1.8% of all malignancies, with incidence rates per 100,000 of 6.1 in whites and 13.6 in blacks.

Global Considerations

The incidence of myeloma is highest in blacks and Pacific Islanders; intermediate in Europeans and North American whites; and lowest in people from developing countries including Asia. The higher incidence in more developed countries may result from the combination of a longer life expectancy and more frequent medical surveillance. Incidence of MM in other ethnic groups including native Hawaiians, female Hispanics, American Indians from New Mexico, and Alaskan natives is higher relative to U.S. whites in the same geographic area. Chinese and Japanese populations have a lower incidence than whites. Immunoproliferative small-intestinal disease (IPSID) with α heavy chain disease is most prevalent in the Mediterranean area. Despite these differences in prevalence, the characteristics, response to therapy, and prognosis of myeloma are similar worldwide.

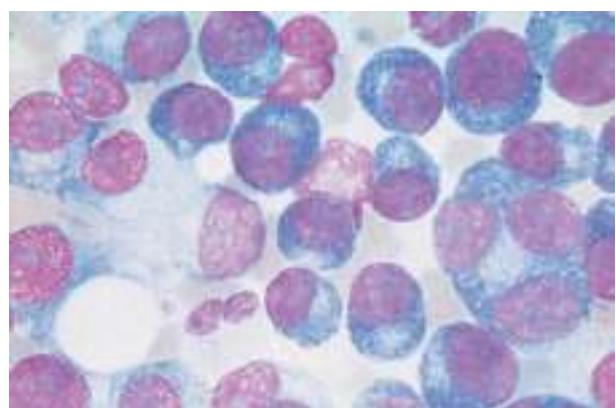


FIGURE 111-3 Multiple myeloma (marrow). The cells bear characteristic morphologic features of plasma cells: round or oval cells with an eccentric nucleus composed of coarsely clumped chromatin, a densely basophilic cytoplasm, and a perinuclear clear zone containing the Golgi apparatus. Binucleate and multinucleate malignant plasma cells can be seen.

PATHOGENESIS AND CLINICAL MANIFESTATIONS
MM cells bind via cell-surface adhesion molecules to bone marrow stromal cells (BMSCs) and extracellular matrix (ECM), which triggers MM cell growth, survival, drug resistance, and migration in the bone marrow milieu (Fig. 111-4). These effects are due both to direct MM cell–BMSC binding via adhesion molecules and to induction of various cytokines, including IL-6, insulin-like growth factor type 1 (IGF-1), vascular endothelial growth factor (VEGF), and stromal cell–derived growth factor (SDF)-1 α . Growth, drug resistance, and migration are mediated via Ras/Raf/mitogen-activated protein kinase, PI3K/Akt, and protein kinase C signaling cascades, respectively. Other cellular elements in the bone marrow microenvironment also significantly impact MM cell growth and survival. The major myeloma supporting interactions are with endothelial cells and osteoclasts. Immune cells such as plasmacytoid dendritic cells (pDC), myeloid-derived suppressor cells (MDSC), and T helper 17 ($T_{H}17$) cells are increased in number and support myeloma growth, while antimyeloma immune responses, especially T helper and cytotoxic cells, B cells, and natural killer T cells, are suppressed.

Bone pain is the most common symptom in myeloma, affecting nearly 70% of patients. Persistent localized pain usually signifies a pathologic fracture. The bone lesions of myeloma are caused by the proliferation of tumor cells, activation of osteoclasts that destroy bone, and suppression of osteoblasts that form new bone. The increased osteoclast activity is mediated by osteoclast activating factors (OAFs) produced by the myeloma cells (mediated by several cytokines, including IL-1, lymphotoxin, vascular endothelial growth factor [VEGF], receptor activator of nuclear factor- κ B [RANK] ligand, macrophage

inhibitory factor [MIP]-1 α , and tumor necrosis factor [TNF]). The bone lesions are lytic in nature (Fig. 111-5) and are rarely associated with osteoblastic new bone formation due to their suppression by dickhoff-1 (DKK-1) produced by myeloma cells. Therefore, radioisotopic bone scanning is less useful in diagnosis than is plain radiography. The bony lysis results in substantial mobilization of calcium from bone, and serious acute and chronic complications of hypercalcemia may dominate the clinical picture (see below). Localized bone lesions may cause the collapse of vertebrae, leading to spinal cord compression. The next most common clinical problem in patients with myeloma is susceptibility to bacterial infections. The most common infections are pneumonias and pyelonephritis, and the most frequent pathogens are *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* in the lungs and *Escherichia coli* and other gram-negative organisms in the urinary tract. In ~25% of patients, recurrent infections are the presenting features, and >75% of patients will have a serious infection at some time in their course. The susceptibility to infection has several contributing causes. First, patients with myeloma have diffuse hypogammaglobulinemia if the M component is excluded. The hypogammaglobulinemia is related to both decreased production and increased destruction of normal antibodies. The large M component results in fractional catabolic rates of 8–16% instead of the normal 2%. Moreover, some patients generate a population of circulating regulatory cells in response to their myeloma that can suppress normal antibody synthesis. These patients have very poor antibody responses, especially to polysaccharide antigens such as those on bacterial cell walls. Various abnormalities in T-cell function are also observed including decreased $T_{H}1$ response, increase in $T_{H}17$ cells producing

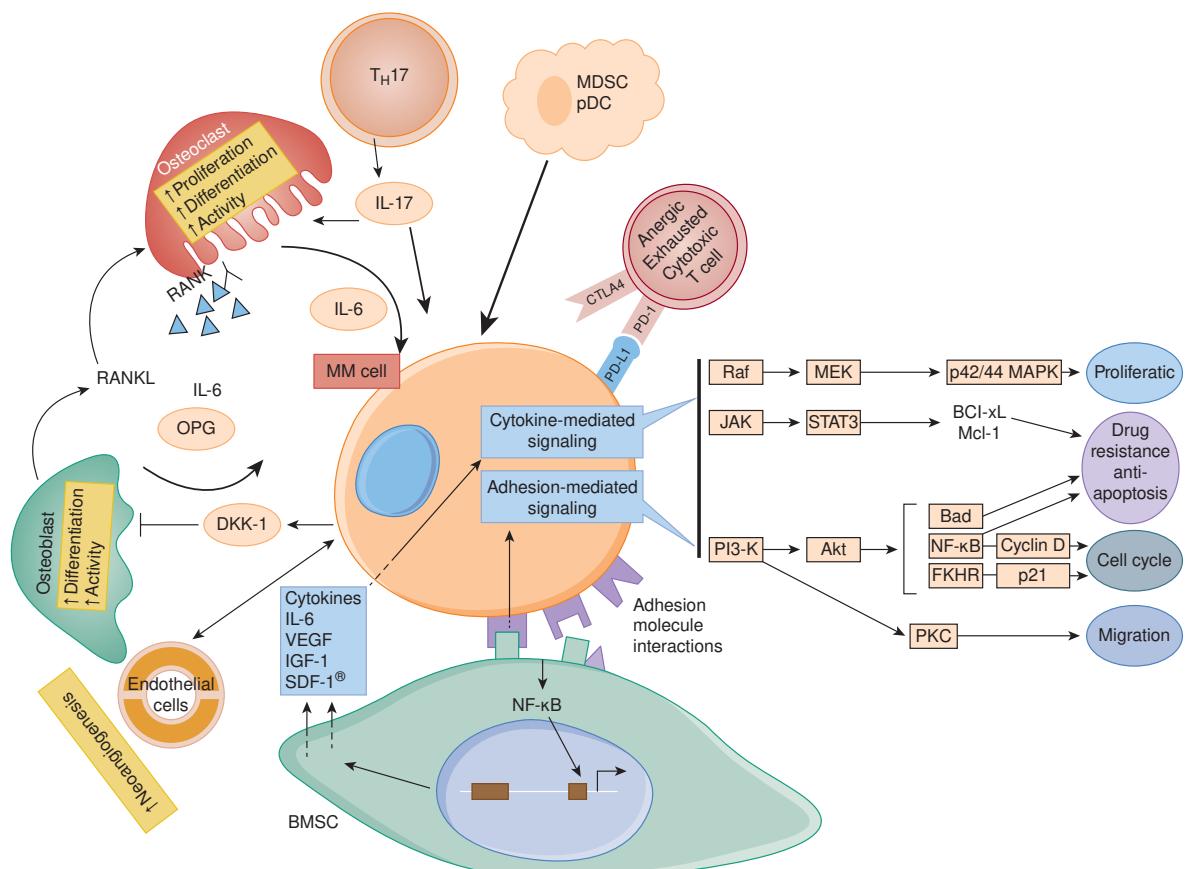


FIGURE 111-4 Pathogenesis of multiple myeloma. Multiple myeloma (MM) cells interact with bone marrow stromal cells (BMSCs) and extracellular matrix proteins via adhesion molecules, triggering adhesion-mediated signaling as well as cytokine production. This triggers cytokine-mediated signaling that provides growth, survival, and antiapoptotic effects as well as development of drug resistance. Additional bidirectional interactions lead to inhibition of osteoblast and increase in osteoclast activity which leads to bone-related issues in myeloma. Similar interactions with immune microenvironment lead to augmentation of tumor promoting immune responses and suppression of tumor protective immune responses, overall allowing myeloma cell growth. (Adapted from G Bianchi, NC Munshi: Blood 125: 3049, 2015.)



A



B

FIGURE 111-5 Bony lesions in multiple myeloma (MM). *A*, The skull demonstrates the typical “punched out” lesions characteristic of MM. The lesion represents a purely osteolytic lesion with little or no osteoblastic activity (above). *B*, PET/CT showing multiple fluorodeoxyglucose (FDG)-avid lesions in skeleton (left panel) with their resolution on achieving complete response (CR) (right panel). (Part A courtesy of Dr. Geraldine Schechter; with permission. Part B courtesy of Dr. Sundar Jagannath; with permission.)

proinflammatory cytokines, and aberrant T regulatory cell function. Granulocyte lysozyme content is low, and granulocyte migration is not as rapid as normal in patients with myeloma, probably the result of a tumor product. There are also a variety of abnormalities in complement functions in myeloma patients. All these factors contribute to the immune deficiency in these patients. Some commonly used therapeutic agents may significantly affect immune function; e.g., dexamethasone suppresses immune responses and increases susceptibility to bacterial and fungal infection, B-cell maturation antigen (BCMA)-targeting chimeric antigen receptor T (CAR-T) cells can eliminate plasma cells inducing hypogammaglobulinemia, and bortezomib predisposes to herpesvirus reactivation.

Renal failure occurs in nearly 25% of myeloma patients, and some renal pathology is noted in >50%. Of many contributing factors, hypercalcemia is the most common cause of renal failure. Glomerular deposits of amyloid, hyperuricemia, recurrent infections, frequent use of nonsteroidal anti-inflammatory agents for pain control, use of iodinated contrast dye for imaging, bisphosphonate use, and occasional infiltration of the kidney by myeloma cells all may contribute to renal dysfunction. However, tubular damage associated with the excretion of light chains is almost always present. Normally, light chains are filtered, reabsorbed in the tubules, and catabolized. With the increase in the amount of light chains presented to the tubule, the tubular cells become overloaded with these proteins, and tubular damage results either directly from light chain toxic effects or indirectly from the release of intracellular lysosomal enzymes. The earliest manifestation of this tubular damage is the adult Fanconi’s syndrome (a type 2 proximal renal tubular acidosis), with loss of glucose and amino acids, as well as defects in the ability of the kidney to acidify and concentrate the urine. The proteinuria is not accompanied by hypertension, and the protein is nearly all light chains. Generally, very little albumin is in the urine because glomerular function is usually normal. When the glomeruli are involved, nonselective proteinuria is also observed. Patients with myeloma also have a decreased anion gap [i.e., $\text{Na}^+ - (\text{Cl}^- + \text{HCO}_3^-)$] because the M component is cationic, resulting in retention of chloride. This is often accompanied by hyponatremia that is felt to be artificial (pseudohyponatremia) because each volume of serum has less water as a result of the increased protein. Renal dysfunction due to light chain deposition disease, light chain cast nephropathy, and amyloidosis is partially reversible with effective therapy. Myeloma patients are susceptible to developing acute renal failure if they become dehydrated.

Normocytic and normochromic anemia occurs in ~80% of myeloma patients. It is usually related to the replacement of normal marrow by expanding tumor cells, to the inhibition of hematopoiesis by factors made by the tumor, to reduced production of erythropoietin by the kidney, and to the effects of long-term therapy. In addition, mild hemolysis may contribute to the anemia. A larger than expected fraction of patients may have megaloblastic anemia due to either folate or vitamin B_{12} deficiency. Granulocytopenia and thrombocytopenia are rare except when therapy-induced. Clotting abnormalities may be seen due to the failure of antibody-coated platelets to function properly; the interaction of the M component with clotting factors I, II, V, VII, or VIII; antibody to clotting factors; or amyloid damage of endothelium. Deep venous thrombosis is also observed with use of thalidomide, lenalidomide, or pomalidomide in combination with dexamethasone. Raynaud’s phenomenon and impaired circulation may result if the M component forms cryoglobulins, and hyperviscosity syndromes may develop depending on the physical properties of the M component (most common with IgM, IgG3, and IgA paraproteins). Hyperviscosity is defined based on the relative viscosity of serum as compared with water. Normal relative serum viscosity is 1.8 (i.e., serum is normally almost twice as viscous as water). Symptoms of hyperviscosity occur at a level greater than 4 centipoise (cP), which is usually reached at paraprotein concentrations of ~40 g/L (4 g/dL) for IgM, 50 g/L (5 g/dL) for IgG3, and 70 g/L (7 g/dL) for IgA; however, depending on chemical and physical properties of the paraprotein molecule, it can occasionally be observed at lower levels.

Although neurologic symptoms occur in a minority of patients, they may have many causes. Hypercalcemia may produce lethargy, weakness, depression, and confusion. Hyperviscosity may lead to headache, fatigue, shortness of breath, exacerbation or precipitation of heart failure, visual disturbances, ataxia, vertigo, retinopathy, somnolence, and coma. Bony damage and collapse may lead to cord compression, radicular pain, and loss of bowel and bladder control. Infiltration of peripheral nerves by amyloid can be a cause of carpal tunnel syndrome and other sensorimotor mono- and polyneuropathies. Neuropathy associated with monoclonal gammopathy of undetermined significance (MGUS) and myeloma is more frequently sensory than motor neuropathy and is associated with IgM more than other isotypes. In >50% of patients with neuropathy, the IgM monoclonal protein is directed against myelin-associated globulin (MAG). Sensory neuropathy is also a side effect of therapy, specifically thalidomide and bortezomib.

Many of the clinical features of myeloma, e.g., cord compression, pathologic fractures, hyperviscosity, sepsis, and hypercalcemia, can present as medical emergencies. Despite the widespread distribution of plasma cells in the body, tumor expansion is dominantly within bone and bone marrow and, for reasons unknown, rarely causes enlargement of spleen, lymph nodes, or gut-associated lymphatic tissue.

■ DIAGNOSIS AND STAGING

The diagnosis of myeloma requires marrow plasmacytosis ($>10\%$), a serum and/or urine M component, and at least one of the myeloma-defining events detailed in **Table 111-1**. Bone marrow plasma cells are CD138+ and either monoclonal kappa or lambda light chain positive. The most important differential diagnosis in patients with myeloma involves their separation from individuals with MGUS or smoldering multiple myeloma (SMM). MGUS is vastly more common than myeloma, occurring in 1% of the population aged >50 years and in up to 10% of individuals aged >75 years. The diagnostic criteria for

MGUS, SMM, and myeloma are described in Table 111-1. Although ~1% of patients per year with MGUS go on to develop myeloma, all cases of myeloma are preceded by MGUS. Non-IgG subtype, abnormal kappa/lambda free light chain ratio, and serum M protein >15 g/L (1.5 g/dL) are associated with higher incidence of progression of MGUS to myeloma. Absence of all three features predicts a 5% chance of progression, whereas higher-risk MGUS with the presence of all three features predicts a 60% chance of progression over 20 years. The features responsible for higher risk of progression from SMM to MM are bone marrow plasmacytosis $>10\%$, abnormal kappa/lambda free light chain ratio, and serum M protein >30 g/L (3 g/dL). Patients with only one of these three features have a 25% chance of progression to MM in 5 years, whereas patients with high-risk SMM with all three features have a 76% chance of progression. Two important variants of myeloma are solitary bone plasmacytoma and solitary extramedullary plasmacytoma. These lesions are associated with an M component in <30% of the cases, they may affect younger individuals, and both are associated

TABLE 111-1 Diagnostic Criteria for Multiple Myeloma, Myeloma Variants, and Monoclonal Gammopathy of Undetermined Significance

Monoclonal Gammopathy of Undetermined Significance (MGUS)

Serum monoclonal protein (non-IgM type) <30 g/L

Clonal bone marrow plasma cells $<10\%$ ^a

Absence of myeloma-defining events or amyloidosis that can be attributed to the plasma cell proliferative disorder

Smoldering Multiple Myeloma (Asymptomatic Myeloma)

Both criteria must be met:

- Serum monoclonal protein (IgG or IgA) ≥ 30 g/L or urinary monoclonal protein ≥ 500 mg per 24 h and/or clonal bone marrow plasma cells 10–60%
- Absence of myeloma-defining events or amyloidosis

Symptomatic Multiple Myeloma

Clonal bone marrow plasma cells or biopsy-proven bony or extramedullary plasmacytoma^a and any one or more of the following myeloma-defining events:

- Evidence of one or more indicators of end-organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
 - Hypercalcemia: serum calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL)
 - Renal insufficiency: creatinine clearance <40 mL/min^b or serum creatinine >177 µmol/L (>2 mg/dL)
 - Anemia: hemoglobin value of >20 g/L below the lower limit of normal, or a hemoglobin value <100 g/L
 - Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT^c
 - Any one or more of the following biomarkers of malignancy:
 - Clonal bone marrow plasma cell percentage^a $\geq 60\%$
 - Involved/uninvolved serum free light chain ratio^d ≥ 100
 - >1 focal lesion on MRI studies^e

Nonsecretory Myeloma

No M protein in serum and/or urine with immunofixation^f

Bone marrow clonal plasmacytosis $\geq 10\%$ or plasmacytoma^a

Myeloma-related organ or tissue impairment (end-organ damage, as described above)

Solitary Plasmacytoma

Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells

Normal bone marrow with no evidence of clonal plasma cells^a

Normal skeletal survey and MRI (or CT) of spine and pelvis (except for the primary solitary lesion)

Absence of end-organ damage such as hypercalcemia, renal insufficiency, anemia, or bone lesions (CRAB) that can be attributed to a lymphoplasma cell proliferative disorder

POEMS Syndrome

All of the following four criteria must be met:

1. Polyneuropathy
2. Monoclonal plasma cell proliferative disorder
3. Any one of the following: (a) sclerotic bone lesions; (b) Castleman's disease; (c) elevated levels of vascular endothelial growth factor (VEGF)
4. Any one of the following: (a) organomegaly (splenomegaly, hepatomegaly, or lymphadenopathy); (b) extravascular volume overload (edema, pleural effusion, or ascites); (c) endocrinopathy (adrenal, thyroid, pituitary, gonadal, parathyroid, and pancreatic); (d) skin changes (hyperpigmentation, hypertrichosis, glomeruloid hemangioma, plethora, acrocytosis, flushing, and white nails); (e) papilledema; (f) thrombocytosis/polycythemia^g

^aClonality should be established by showing κ/λ light chain restriction on flow cytometry, immunohistochemistry, or immunofluorescence. Bone marrow plasma cell percentage should preferably be estimated from a core biopsy specimen; in case of a disparity between the aspirate and core biopsy, the highest value should be used. ^bMeasured or estimated by validated equations. ^cIf bone marrow has $<10\%$ clonal plasma cells, more than one bone lesion is required to distinguish from solitary plasmacytoma with minimal marrow involvement. ^dThese values are based on the serum Freeelite assay (The Binding Site Group, Birmingham, United Kingdom). The involved free light chain must be ≥ 100 mg/L. ^eEach focal lesion must be ≥ 5 mm in size. ^fA small M component may sometimes be present. ^gThese features should have no other attributable causes and have temporal relation with each other.

Abbreviations: PET-CT, ¹⁸F-fluorodeoxyglucose positron emission tomography with computed tomography; POEMS, polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes.

with median survivals of ≥10 years. Solitary bone plasmacytoma is a single lytic bone lesion without marrow plasmacytosis. Extramedullary plasmacytomas usually involve the submucosal lymphoid tissue of the nasopharynx or paranasal sinuses without marrow plasmacytosis. Both tumors are highly responsive to local radiation therapy. If an M component is present, it should disappear after treatment. Solitary bone plasmacytomas may recur in other bony sites or evolve into myeloma. Extramedullary plasmacytomas rarely recur or progress.

Serum protein electrophoresis and measurement of serum immunoglobulins and free light chains are useful for detecting and characterizing M spikes, supplemented by immunoelectrophoresis, which is especially sensitive for identifying low concentrations of M components not detectable by protein electrophoresis. A 24-h urine specimen is necessary to quantitate Bence Jones protein (immunoglobulin light chain) excretion. The serum M component will be IgG in 53% of patients, IgA in 25%, and IgD in 1%; 20% of patients will have only light chains in serum and urine. Dipsticks for detecting proteinuria are not reliable at identifying light chains, and the heat test for detecting Bence Jones protein is falsely negative in ~50% of patients with light chain myeloma. Fewer than 1% of patients have no identifiable M component; these patients usually have light chain myeloma in which renal catabolism has made the light chains undetectable in the urine. In most of these patients, light chains can now be detected by serum free light chain assay. IgD myeloma may also present with light chain disease. About two-thirds of patients with serum M components also have urinary light chains. The light chain isotype may have an impact on disease behavior. Whether this is due to some genetically important determinant of cell proliferation or because lambda light chains are more likely to cause renal damage and form amyloid than are kappa light chains is unclear. The heavy chain isotype may have an impact on patient management as well. About half of patients with IgM paraproteins develop hyperviscosity compared with only 2–4% of patients with IgA and IgG M components. Among IgG myelomas, it is the IgG3 subclass that has the highest tendency to form both concentration- and temperature-dependent aggregates, leading to hyperviscosity and cold agglutination at lower serum concentrations. A standard workup directed at detecting monoclonal plasma cells and myeloma-defining events as well as prognosis is detailed in Table 111-2.

A complete blood count with differential may reveal anemia. Erythrocyte sedimentation rate is elevated. Rare patients (~1%) may have plasma cell leukemia with >2000 plasma cells/µL. This may be seen in disproportionate frequency in IgD (12%) and IgE (25%) myelomas. Serum calcium, urea nitrogen, creatinine, and uric acid levels may be elevated. Serum alkaline phosphatase is usually normal even with extensive bone involvement because of the absence of osteoblastic activity. It is also important to quantitate serum β_2 -microglobulin and albumin (see below).

Chest and bone radiographs may reveal lytic lesions or diffuse osteopenia. Magnetic resonance imaging (MRI) offers a sensitive means to document extent of bone marrow infiltration and cord or root compression in patients with pain syndromes. ^{18}F -fluorodeoxyglucose (^{18}F -FDG) positron emission tomography (PET)/computed tomography (CT) is a valuable tool to assess bone damage and detect extramedullary sites of the disease (Fig. 111-5). The use of ^{18}F -FDG PET/CT is recommended to distinguish between smoldering and active MM and to confirm a suspected diagnosis of solitary plasmacytoma. It is also a valuable tool to evaluate response in patients with oligo- or nonsecretory myeloma.

■ PROGNOSIS

Serum β_2 -microglobulin is the single most powerful predictor of survival and can substitute for staging. β_2 -Microglobulin is the light chain of the class I major histocompatibility antigens (HLA-A, -B, -C) on the surface of every cell. Combination of serum β_2 -microglobulin and albumin levels forms the basis for a three-stage International Staging System (ISS) (Table 111-3) that predicts survival. With the use of high-dose therapy and the newer agents, the Durie-Salmon staging system is unable to predict outcome and is no longer used. High labeling index, circulating plasma cells, performance status, and high levels of lactate dehydrogenase are also associated with poor prognosis.

TABLE 111-2 Standard Investigative Workup in Multiple Myeloma (MM)

Investigations to Evaluate for Clonal Plasma Cells

Bone marrow aspirate and biopsy (fine-needle aspiration of plasmacytoma if indicated)

- Histology
- Clonality by kappa/lambda immunostaining by flow cytometry or immunohistochemistry

Investigations to Evaluate Clonal Paraprotein

Serum protein electrophoresis and immunofixation
Quantitative serum immunoglobulin levels (IgG, IgA, and IgM)
24-h urine protein electrophoresis and immunofixation
Serum free light chain and ratio
Immunofixation for IgD or IgE in select cases

Investigation to Evaluate End-Organ Damage

Hemogram to assess for anemia
Chemistry panel for renal function and calcium
Skeletal survey to evaluate bone lesions
PET/CT or MRI if smoldering MM or solitary plasmacytoma with no other MDE or extramedullary disease

Investigation for Risk Stratification

β_2 -Microglobulin and serum albumin for ISS stage
Fluorescent in situ hybridization for hyperdiploidy, del17p, t(4;14), t(11;14), t(14;16), t(14;20), amp1q34, and del13 on bone marrow sample
LDH

Specialized Investigation in Selected Cases

Abdominal fat pad for amyloid
Serum viscosity if IgM component or high IgA levels or serum M component >7 g/dL
Myd88 and *CXCR4* mutation analysis if IgM component

Abbreviations: ISS, International Staging System; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; PET/CT, positron emission tomography/computed tomography.

Other factors that may influence prognosis are detection of any cytogenetic abnormalities including hypodiploidy by karyotype, fluorescent in situ hybridization (FISH)-identified chromosome 17p deletion, and translocations (t(4;14), (14;16), and t(14;20) and 1q34 amplification. Chromosome 13q deletion, previously thought to predict poor outcome, is not a predictor following the use of newer agents. The ISS system incorporating the cytogenetic changes (Revised ISS) is the most widely used method for assessing prognosis (Table 111-3). Microarray profiling has formed the basis for RNA-based prognostic staging systems. Genome sequencing efforts have allowed for characterization of critical genes, pathways, and clonal heterogeneity in myeloma. The median number of mutations per transcribed genome in myeloma is ~58, and within the whole genome, it is >7000. A very heterogeneous mutational landscape with no unifying mutation has been observed. The most frequently mutated genes are *KRAS* and *NRAS* (~20% each), followed by *TP53*, *DIS3*, *FAM46C*, and *BRAF*, all mutated in 5–10% of patients. All other mutations were observed in <5% of the patients. These results are now being applied to develop new targeted personalized therapies in myeloma.

TREATMENT

Multiple Myeloma

MGUS, SMM, AND SOLITARY PLASMACYTOMA

No specific intervention is indicated for patients with MGUS. Follow-up once a year or less frequently is adequate except in higher-risk MGUS, where serum protein electrophoresis, complete blood count, creatinine, and calcium should be repeated every 6 months. A patient with MGUS and severe polyneuropathy is considered for therapeutic intervention if a causal relationship can be

TABLE 111-3 Risk Stratification in Myeloma

CHROMOSOMAL ABNORMALITIES (CA)		
METHOD	STANDARD RISK (80%) (EXPECTED SURVIVAL 6–7+ YEARS)	HIGH RISK (20%) (EXPECTED SURVIVAL 2–3 YEARS)
Karyotype	No chromosomal aberration	Any abnormality on conventional karyotype
FISH	t(11;14) del(13)	del(17p) t(4;14) t(14;16) t(14;20) amp 1q34
INTERNATIONAL STAGING SYSTEM (ISS)		
	STAGE	MEDIAN SURVIVAL, MONTHS
$\beta_2\text{M} < 3.5$, alb ≥ 3.5	I (28%) ^a	62
$\beta_2\text{M} < 3.5$, alb < 3.5 or $\beta_2\text{M} = 3.5\text{--}5.5$	II (39%)	44
$\beta_2\text{M} > 5.5$	III (33%)	29
REVISED INTERNATIONAL STAGING SYSTEM (R-ISS)		
Stage I: ISS stage 1; standard risk for CA and normal LDH		
Stage II: Patients not meeting criteria for stage I or stage III		
Stage III: ISS stage III and either high risk for CA or high LDH		
Other features suggesting high-risk disease:		
De novo plasma cell leukemia		
Extramedullary disease		
Elevated LDH		
High-risk gene expression profile		

^aPercentage of patients presenting at each stage.

Abbreviations: $\beta_2\text{M}$, serum β_2 -microglobulin in mg/L; alb, serum albumin in g/dL; FISH, fluorescent in situ hybridization; LDH, lactate dehydrogenase.

assumed, especially in the absence of any other potential causes for neuropathy. Therapy can include plasmapheresis and occasionally rituximab in patients with IgM MGUS or myeloma-like therapy in those with IgG or IgA disease. A subset of patients with MGUS develop renal dysfunction usually based on renal damage from the monoclonal antibody. The damage may affect the glomeruli, tubules, or vessels. No consensus exists on management, but lowering the level of the monoclonal antibody with bortezomib has had some advocates.

About 10% of patients have SMM and will have an indolent course demonstrating only slow progression of disease over many years. For patients with SMM, no specific therapeutic intervention is indicated, although early intervention with lenalidomide and dexamethasone may prevent progression from high-risk SMM to active MM. At present, patients with SMM only require antitumor therapy when myeloma-defining events are identified.

Patients with solitary bone plasmacytomas and extramedullary plasmacytomas may be expected to enjoy prolonged disease-free survival after local radiation therapy at a dose of ~40 Gy. Occult marrow involvement may occur at low incidence in patients with solitary bone plasmacytoma. Such patients are usually identified because their serum M component falls slowly or disappears initially after local therapy, only to return after a few months. These patients respond well to systemic therapy.

SYMPOMATIC MM

Patients with symptomatic myeloma require therapeutic intervention. In general, such therapy has two purposes: (1) systemic therapy to control myeloma; and (2) supportive care to control symptoms of the disease, its complications, and adverse effects of therapy. Therapy can significantly prolong survival and improve the quality of life for myeloma patients.

The therapy of myeloma includes an initial induction regimen followed by consolidation and/or maintenance therapy and, on subsequent progression, management of relapsed disease. All agents available for use at various stages of the therapy and their doses, schedules, and combinations are detailed in Table 111-4. Therapy is partly dictated by the patient's age and comorbidities, which may affect a patient's ability to undergo high-dose therapy and transplantation (Fig. 111-6).

Three important classes of agents approved for treatment of newly diagnosed MM are immunomodulatory agents, proteasome inhibitors, and targeted antibodies. Thalidomide, when combined with dexamethasone, achieved responses in two-thirds of newly diagnosed MM patients. Subsequently, lenalidomide, an immunomodulatory derivative of thalidomide, and bortezomib, a proteasome inhibitor, have each been combined with dexamethasone with high response rates (>80%) in newly diagnosed patients with MM. Importantly, their lower toxicity profile with improved efficacy has made them the preferred agents for induction therapy. Efforts to improve the depth and frequency of response have involved using three-drug regimens. The combination of lenalidomide with a proteasome inhibitor (bortezomib or carfilzomib) and dexamethasone achieves close to a 100% response rate and a >30% complete response (CR) rate, making this combination one of the preferred induction regimens in transplant-eligible patients. Other similar three-drug combinations (bortezomib, thalidomide, and dexamethasone or bortezomib, cyclophosphamide, and dexamethasone) also achieve >90% response rate. Addition of a fourth agent, daratumumab, an anti-CD38 antibody, is providing even deeper responses. Usually between four and six cycles of these combination regimens are utilized to achieve initial deep cytoreduction before consideration of high-dose therapy with autologous stem cell transplantation.

In patients who are not transplant candidates due to physiologic age >70 years, significant cardiopulmonary problems, or other comorbid illnesses, the same two- or three-drug combinations described above are considered standard of care as induction therapy with age- and frailty-guided dose and schedule modifications. Modified lenalidomide-bortezomib-dexamethasone (RVD lite) combination achieves high overall response rate (86%) and CR (32%). Intermittent pulses of melphalan, an alkylating agent, with prednisone (MP) are combined with novel agents to achieve superior response and survival outcomes. In patients >65 years old, combining thalidomide with MP (MPT) obtains higher response rates and overall survival compared with MP alone. Similarly, significantly improved response (71 vs 35%) and overall survival (3-year survival 72 vs 59%) were observed with the combination of bortezomib and MP compared with MP alone. Continuous use of the lenalidomide and dexamethasone combination appears to be superior to the MPT regimen, and its combination with the anti-CD38 antibody daratumumab provides even higher overall response (92.9%) and CR rates (46.7%) and improved survival; the combination of lenalidomide, dexamethasone, and daratumumab is a standard-of-care regimen for older adults with myeloma.

HIGH-DOSE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

High-dose therapy (HDT) and consolidation/maintenance are standard practice in the majority of eligible patients. In patients who are transplant candidates, alkylating agents such as melphalan should be avoided because they damage stem cells and compromise the ability to collect stem cells. Similarly, in patients receiving lenalidomide, stem cells should be collected within 6 months because the continued use of lenalidomide may compromise the ability to collect adequate numbers of stem cells. Randomized studies comparing standard-dose therapy to high-dose melphalan therapy with hematopoietic stem cell support have shown that HDT can achieve higher overall response rates, with up to 25–40% additional CRs and prolonged progression-free and overall survival; however, few, if any, patients are cured. Although two successive HDTs (tandem

TABLE 111-4 Standard Therapeutic Agents in Myeloma

CLASS	AGENT	STANDARD DOSAGE AND ADMINISTRATION	COMBINATION	MYELOMA INDICATION
Immunomodulatory drugs (IMiD)	Thalidomide (T)	Oral 50–200 mg qd	TD, VTD	Newly diagnosed and relapsed
	Lenalidomide (R)	Oral 5–25 mg daily × 21 days q 4 weeks	RD, RVD, DaRD, ERD, KRD, IRD	Newly diagnosed, maintenance, and relapsed
	Pomalidomide (P)	Oral 2–4 mg daily × 21 days q 4 weeks	PD	Relapsed
Proteasome inhibitors (PI)	Bortezomib (V)	IV or SC 1.3 mg/m ² days 1, 4, 8, 11 OR days 1, 8, 15	VD, VTD, VRD, DaVD, VCD	Newly diagnosed and relapsed
	Carfilzomib (K)	IV 20–56 mg/m ² days 1, 2, 8, 9, 15, 16 q 4 weeks	KD, KRD, KPD, Da KD, Da KRD, IsaKD	Newly diagnosed and relapsed
	Ixazomib (I)	Oral 4 mg days 1, 8, 15	IRD	Relapsed
Antibodies	Daratumumab (Da)	IV 16 mg/kg per week for 8 weeks then every 2 weeks for 16 weeks and then every 4 weeks thereafter	Dara, DaRD, DaVD, DaPD, DaKD	Newly diagnosed, maintenance, and relapsed
	Elotuzumab (E)	IV 10 mg/kg days 1, 8, 15, and 22 for first two cycles, then on days 1 and 15; along with RD	ERD, EPD	Relapsed
	Isatuximab (Isa)	IV 10 mg/kg weekly for 4 weeks and then every 2 weeks	IsaPD, IsaKD	Relapsed
	Belantamab mafodotin	IV 2.5 mg/kg once every 3 weeks		Relapsed or refractory - 4 prior lines of therapy
Selective inhibitor of nuclear export (SINE)	Selinexor (S)	Oral 80 mg on days 1 and 3 of each week	SVD	Relapsed
Histone deacetylase inhibitor	Panobinostat (Pa)	Oral 20 mg once every other day for 3 doses/week for 2 weeks every 21 days	PaVD	Relapsed
Alkylating agents	Melphalan (M)	Oral 0.25 mg/kg per day for 4 days (with P) every 4–6 weeks	MP, MPT, MPR, MPV, DaMPV, high-dose M	Newly diagnosed and relapsed conditioning
	Cyclophosphamide (C)	IV—300–500 mg/m ² weekly × 2 q 4 weeks Oral—50 mg qd × 21 days	VCD	Newly diagnosed and relapsed
	Bendamustine (B)	IV 70–90 mg days 1, 2 OR days 1, 8 q 4 weeks	BD or BVD	Relapsed
	Mel氟fen (Me)	IV 40 mg day 1 (with D 40 mg on days 1, 8, 15, and 22) q 28 days	MeD	Relapsed or refractory - 4 prior lines of therapy
Cellular therapy	Idecabtagene vicleucel (Ide-cel)	IV 450 × 10 ⁶ cells	None	Relapsed or refractory - 4 prior lines of therapy with prior exposure to PI, IMiD, and anti-CD38 antibody
Glucocorticoid	Dexamethasone (D) Prednisone (P)	Oral 10–40 mg q week Oral 1 mg/kg		All stages

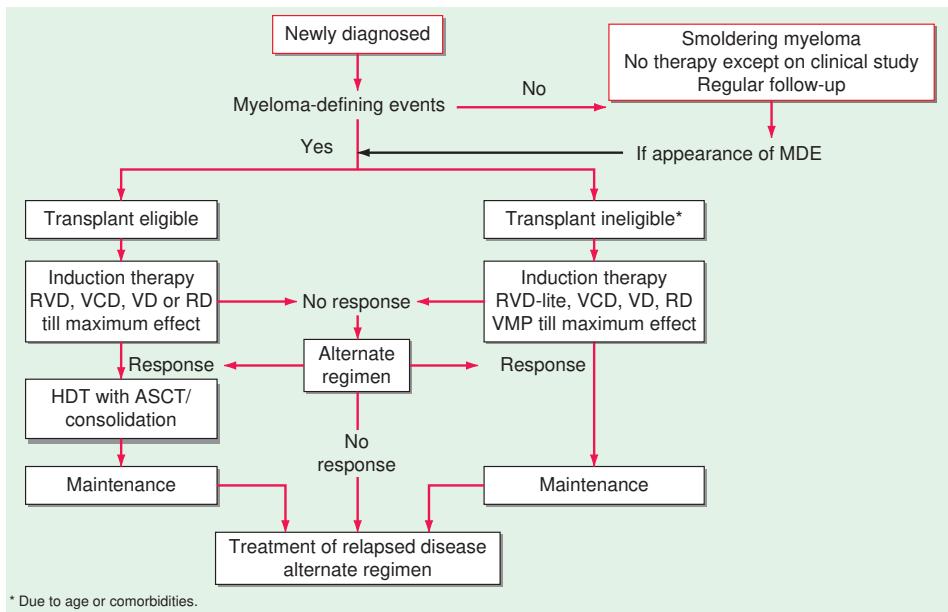


FIGURE 111-6 Treatment algorithm for multiple myeloma. C, cyclophosphamide; D, dexamethasone; M, melphalan; P, prednisone; R, lenalidomide; RVD-lite, weekly regimen; V, bortezomib. *Alternate regimen* indicates combinations including daratumumab, elotuzumab, panobinostat, carfilzomib, ixazomib, pomalidomide, or other agents. ASCT, autologous stem cell transplantation; HDT, high-dose therapy; MDE, myeloma-defining events.

transplantations) are more effective than single HDT, the benefit is only observed in the subset of patients who do not achieve a complete or very good partial response to the first transplantation, which is a rare subset. Moreover, a randomized study failed to show any significant difference in overall survival between early transplantation after induction therapy versus delayed transplantation at relapse. These data allow an option to delay transplantation, especially with the availability of newer agents and combinations. Allogeneic transplants may also produce high response rates, but with significant toxicities. Nonmyeloablative allogeneic transplantation can reduce toxicity but is recommended only under the auspices of a clinical trial to exploit an immune graft-versus-myeloma effect while avoiding attendant toxicity.

Maintenance therapy prolongs remissions following standard-dose regimens as well as HDT. Two phase 3 studies have demonstrated improved progression-free survival, and one study showed prolonged overall survival in patients receiving lenalidomide compared to placebo as maintenance therapy after HDT. In non-transplant candidates, two phase 3 studies showed prolonged progression-free survival with lenalidomide maintenance after MP plus lenalidomide or lenalidomide plus dexamethasone induction therapy. Although concern arises regarding an increased incidence of second primary malignancies in patients receiving lenalidomide maintenance, its benefits in reducing the risk of progressive disease and death from myeloma far outweigh the small increased risk of second cancers. In patients with high-risk cytogenetics, lenalidomide and bortezomib or an oral proteasome inhibitor, ixazomib, show promise as maintenance combination therapy after transplantation.

RELAPSED DISEASE

Relapsed myeloma can be treated with a number of agents including lenalidomide and/or bortezomib, if previously not used. The second-generation proteasome inhibitor carfilzomib and immunomodulatory agent pomalidomide have shown efficacy in relapsed and refractory MM, even MM refractory to lenalidomide and bortezomib. An oral proteasome inhibitor, ixazomib, has also been approved in combination with lenalidomide and dexamethasone as an all-oral regimen for relapsed MM. Three antibodies are approved for treatment of relapsed MM. Daratumumab targeting CD38 achieves high response rates and improved progression-free survival as a single agent with further improvement in response and survival when added to bortezomib and dexamethasone or lenalidomide and dexamethasone. A formulation of daratumumab for subcutaneous administration provides decreased toxicity and improved convenience. Isatuximab, another antibody targeting CD38, achieves high response rates and improved progression-free survival in combination with pomalidomide or carfilzomib and dexamethasone. Elotuzumab, which targets SLAMF7, has shown significant activity in combination with lenalidomide and dexamethasone in relapsed/refractory myeloma but not as a single agent. Panobinostat, a histone deacetylase inhibitor, in combination with bortezomib and dexamethasone has been approved for treatment of relapsed refractory myeloma based on superior response and progression-free survival compared to bortezomib and dexamethasone alone. Two additional newer agents have unique mechanisms of action: selinexor is a first-in-class exportin inhibitor that blocks export of proteins from the cell nucleus, and melflufen is an alkylating agent conjugated to a peptide to improve specific delivery to myeloma cells that express aminopeptidase required for cleaving of the peptide to deliver the drug intracellularly in myeloma cells. Both agents have been approved based on their effectiveness in relapsed/refractory myeloma. Another therapeutic focus has been to target BCMA, which is exclusively expressed on normal plasma cells and myeloma cells. An anti-BCMA antibody-drug conjugate, belantamab, targets BCMA and delivers auristatin to the tumor cells and achieves responses in relapsed/refractory myeloma. The drug has a unique ophthalmologic toxicity that requires close monitoring. Finally, a cellular therapy approved for myeloma is an anti-BCMA CAR transduced

T cell (idecabtagene vicleucel [Ide-cel]), which is approved beyond fourth-line therapy. In patients with advanced myeloma with a median of six prior lines of treatment, 73% of patients receiving Ide-cel responded, and a CR rate of 33% was observed. Cytokine release syndrome and neurotoxicity remain primary toxicities requiring close monitoring and aggressive management. BCMA is also the target for a number of investigational agents including other CAR-T cell approaches as well as bispecific antibodies combining anti-BCMA with anti-CD3 antibody. Incorporation of the large number of active agents at various stages of treatment, including in newly diagnosed patients, is improving survival as well as quality of life.

THERAPY ENDPOINT

Improvement in the serum M component may lag behind the symptomatic improvement due to longer serum half-life (~3 weeks) of the immunoglobulin. The fall in M component depends on the rate of tumor kill and the fractional catabolic rate of immunoglobulin. Serum and urine light chains with a functional half-life of ~6 h may fall much quicker within the first week of treatment. Because urine light chain levels may relate to renal tubular function, they are not a reliable measure of tumor cell kill in patients with renal dysfunction. Achieving CR, defined as disappearance of serum and urine monoclonal protein with normal bone marrow by light microscopy, has been a standard goal of therapy. However, sequencing or multicolor flow cytometry-based assessment of minimal residual disease (MRD) in bone marrow to measure the presence of one myeloma cell in a million cells is being considered as an important new endpoint, especially in newly diagnosed patients. Absence of MRD at this sensitivity predicts for both longer progression-free survival and longer overall survival. Although patients may not achieve complete remission, clinical responses may last for long periods of time in small numbers of patients.

The median overall survival of patients with myeloma is 8+ years, with subsets of younger patients surviving >10 years. The major causes of death are progressive myeloma, renal failure, sepsis, or therapy-related myelodysplasia. Nearly a quarter of patients die of myocardial infarction, chronic lung disease, diabetes, or stroke, which are all intercurrent illnesses related more to the age of the patient group than to the tumor.

SUPPORTIVE THERAPY

Herpes zoster prophylaxis is indicated if bortezomib is used, and neuropathy attendant to bortezomib can be decreased both by its subcutaneous administration and by administration on a weekly schedule. Lenalidomide use requires prophylaxis for deep-vein thrombosis (DVT) with either aspirin or, if patients are at a greater risk of DVT, warfarin, low-molecular-weight heparin, or direct-acting anticoagulants. Patients receiving anti-BCMA CAR-T cell therapy may need supplementation with intravenous γ globulin due to induction of prolonged hypogammaglobulinemia.

Supportive care directed at the anticipated complications of the disease may be as important as primary antitumor therapy. Hypercalcemia generally responds well to bisphosphonates, glucocorticoid therapy, hydration, and natriuresis and rarely requires calcitonin as well. Bisphosphonates (e.g., pamidronate 90 mg or zoledronate 4 mg initially once a month for 12–24 months and later every 2–3 months) reduce osteoclastic bone resorption and preserve performance status and quality of life, decrease bone-related complications, and may also have antitumor effects. Osteonecrosis of the jaw and renal dysfunction can occur in a minority of patients receiving bisphosphonate therapy. Denosumab is an alternative agent administered intravenously at 120 mg monthly and achieves a similar level of effect as bisphosphonates to prevent bone-related complications in myeloma. Treatments aimed at strengthening the skeleton such as fluorides, calcium, and vitamin D, with or without androgens, have been suggested but are not of proven efficacy. Kyphoplasty or vertebroplasty should be considered in patients with painful collapsed vertebra. Iatrogenic worsening of renal function

may be prevented by maintaining a high fluid intake to prevent dehydration and enhance excretion of light chains and calcium. In the event of acute renal failure, plasmapheresis is ~10 times more effective at clearing light chains than peritoneal dialysis; however, its role in reversing renal failure remains controversial. Importantly, reducing the protein load by effective antitumor therapy with agents such as bortezomib may result in improvement in renal function in over half of the patients. Use of lenalidomide in renal failure is possible but requires dose modification because it is renally excreted. Urinary tract infections should be watched for and treated early. Plasmapheresis may be the treatment of choice for hyperviscosity syndromes. Although the pneumococcus is a dreaded pathogen in myeloma patients, pneumococcal polysaccharide vaccines may not elicit an antibody response. The pneumococcal conjugate vaccines are more protective. Prophylactic administration of intravenous γ globulin preparations is used in the setting of recurrent serious infections. Chronic oral antibiotic prophylaxis is not warranted. Patients developing neurologic symptoms in the lower extremities, severe localized back pain, or problems with bowel and bladder control may need emergency MRI and local radiation therapy and glucocorticoids if cord compression is identified. In patients in whom neurologic deficit is increasing or substantial, emergent surgical decompression may be necessary. Most bone lesions respond to analgesics and systemic therapy, but certain painful lesions may respond more promptly to localized radiation. The anemia associated with myeloma may respond to erythropoietin along with hematins (iron, folate, cobalamin). The pathogenesis of the anemia should be established and specific therapy instituted, whenever possible.

WALDENSTRÖM'S MACROGLOBULINEMIA

In 1948, Waldenström described a malignancy of lymphoplasmacytoid cells that secreted IgM. In contrast to myeloma, the disease was associated with lymphadenopathy and hepatosplenomegaly, but the major clinical manifestation was hyperviscosity syndrome. The disease resembles the related diseases CLL, myeloma, and lymphocytic lymphoma. It originates from a post-germinal center B cell that has undergone somatic mutations and antigenic selection in the lymphoid follicle and has the characteristics of an IgM-bearing memory B cell. Waldenström's macroglobulinemia (WM) and IgM myeloma follow a similar clinical course, but therapeutic options are different. The diagnosis of IgM myeloma is usually reserved for patients with lytic bone lesions and predominant infiltration with CD138+ plasma cells in the bone marrow. Such patients are at greater risk of pathologic fractures than patients with WM.

A familial occurrence is common in WM, but its molecular bases are yet unclear. A distinct *MYD88* L265P somatic mutation is present in >90% of patients with WM and the majority of IgM MGUS. Other commonly occurring mutations include *CXCR4* (30–40%), *ARID1A* (17%), and *CD79B* (8–15%). Presence of *MYD88* mutation status is now used as a diagnostic test to discriminate WM from marginal zone lymphomas (MZLs), IgM-secreting myeloma, and CLL with plasma-cell differentiation. This mutation also explains the molecular pathogenesis of the disease with involvement of Toll-like receptor (TLR) and interleukin 1 receptor (IL-1R) signaling leading to activation of IL-1R-associated kinase (IRAK) 4 and IRAK1 followed by nuclear factor- κ B (NF- κ B) activation. *MYD88* mutation also triggers Bruton's tyrosine kinase (BTK) and hemopoietic cell kinase (HCK)-mediated growth and survival signaling, which are now important therapeutic targets in WM. *CXCR4* mutations induce AKT and extracellular regulated kinase 1/2 (ERK1/2) signaling. This pathway can lead to development of drug resistance in the presence of its ligand CXCL12.

The disease is similar to myeloma in being slightly more common in men and occurring with increased incidence with increasing age (median age 64 years). The IgM in some patients with macroglobulinemia may have specificity for myelin-associated glycoprotein (MAG), a protein that has been associated with demyelinating disease of the peripheral nervous system and may be lost earlier and to a

greater extent than the better-known myelin basic protein in patients with multiple sclerosis. Sometimes patients with macroglobulinemia develop a peripheral neuropathy, and half of these patients are positive for anti-MAG antibody. The neuropathy may precede the appearance of the neoplasm. The whole process may begin with a viral infection that may elicit an antibody response that cross-reacts with a normal tissue component.

Like myeloma, the disease involves the bone marrow, but unlike myeloma, it does not cause bone lesions or hypercalcemia. Bone marrow shows >10% infiltration with lymphoplasmacytic cells (surface IgM+, CD19+, CD20+, and CD22+, rarely CD5+, but CD10- and CD23-) with an increase in number of mast cells. Like myeloma, an M component is present in the serum in excess of 30 g/L (3 g/dL), but unlike myeloma, the size of the IgM paraprotein results in little renal excretion, and only ~20% of patients excrete light chains. Therefore, renal disease is not common. The light chain isotype is kappa in 80% of the cases. Patients present with weakness, fatigue, and recurrent infections similar to myeloma patients, but epistaxis, visual disturbances, and neurologic symptoms such as peripheral neuropathy, dizziness, headache, and transient paresis are much more common in macroglobulinemia. Presence of *MYD88* and *CXCR4* mutations also affects disease presentation. Presence of *CXCR4* mutations is associated with higher bone marrow disease burden and higher incidence of hyperviscosity. Patients with wild-type *MYD88* show lower bone marrow disease burden.

Physical examination reveals adenopathy and hepatosplenomegaly, and ophthalmoscopic examination may reveal vascular segmentation and dilation of the retinal veins characteristic of hyperviscosity states. Patients may have a normocytic, normochromic anemia, but rouleaux formation and a positive Coombs test are much more common than in myeloma. Malignant lymphocytes are usually present in the peripheral blood. About 10% of macroglobulins are cryoglobulins. These are pure M components and are not the mixed cryoglobulins seen in rheumatoid arthritis and other autoimmune diseases. Mixed cryoglobulins are composed of IgM or IgA complexed with IgG, for which they are specific. In both cases, Raynaud's phenomenon and serious vascular symptoms precipitated by the cold may occur, but mixed cryoglobulins are not commonly associated with malignancy. Patients suspected of having a cryoglobulin based on history and physical examination should have their blood drawn into a warm syringe and delivered to the laboratory in a container of warm water to avoid errors in quantitating the cryoglobulin.

TREATMENT

Waldenström's Macroglobulinemia

A diagnosis of WM requires lymphoplasmacytic infiltrate of any level in the bone marrow and an IgM monoclonal paraprotein of any size. Treatment is usually not initiated unless the disease is symptomatic or increasing anemia, hyperviscosity, lymphadenopathy, or hepatosplenomegaly is present. Control of serious hyperviscosity symptoms such as an altered state of consciousness or paresis can be achieved acutely by plasmapheresis because 80% of the IgM paraprotein is intravascular. The median survival of affected individuals is ~50 months. However, many patients with WM have indolent disease that does not require therapy. Pretreatment parameters including older age, male sex, general symptoms, and cytopenias define a high-risk population. BTK inhibitors (ibrutinib), alkylating drugs (bendamustine and cyclophosphamide), and proteasome inhibitors (bortezomib, carfilzomib, and ixazomib), alone or more frequently in combination with rituximab, are considered as first-line therapy for symptomatic patients with WM. Ibrutinib targets the constitutively activated BTK. In patients with one prior line of therapy, the overall response to ibrutinib was 91%. Best responses to ibrutinib are observed in patients with mutated *MYD88* and wild-type *CXCR4* status, while delayed and lower response rates to ibrutinib are observed in patients with mutated *CXCR4*. At first relapse, in patients with an initial durable response,

either the previous regimen or another primary therapy regimen can be used. The therapeutic choice is dependent upon the genomic features, drug availability, and the patient's clinical profile.

Rituximab can produce an IgM flare, so either plasmapheresis should be used before rituximab or its use should be initially withheld in patients with high IgM levels. Fludarabine (25 mg/m² per d for 5 days every 4 weeks) and cladribine (0.1 mg/kg per d for 7 days every 4 weeks) are also highly effective single agents. With identification of the *MYD88* mutation, novel BTK inhibitors (acalabrutinib, zanubrutinib, and tirabrutinib), inhibitors targeting IRAK1/4, and the BCL2 antagonist venetoclax are being explored for the treatment of WM. Although HDT plus autologous transplantation is an option, its use has declined due to the availability of other effective agents.

POEMS SYNDROME

The features of this syndrome are polyneuropathy, organomegaly, endocrinopathy, *M*-protein, and skin changes (POEMS). Diagnostic criteria are described in Table 111-1. Patients usually have a severe, progressive sensorimotor polyneuropathy associated with sclerotic bone lesions from myeloma. Polyneuropathy occurs in ~1.4% of myelomas, but the POEMS syndrome is only a rare subset of that group. Unlike typical myeloma, hepatomegaly and lymphadenopathy occur in about two-thirds of patients, and splenomegaly is seen in one-third. The lymphadenopathy frequently resembles Castleman's disease histologically, a condition that has been linked to IL-6 overproduction. The endocrine manifestations include amenorrhea in women and impotence and gynecomastia in men. Hyperprolactinemia due to loss of normal inhibitory control by the hypothalamus may be associated with other central nervous system manifestations such as papilledema and elevated cerebrospinal fluid pressure and protein. Type 2 diabetes mellitus occurs in about one-third of patients. Hypothyroidism and adrenal insufficiency are occasionally noted. Skin changes are diverse: hyperpigmentation, hypertrichosis, skin thickening, and digital clubbing. Other manifestations include peripheral edema, ascites, pleural effusions, fever, and thrombocytosis. Not all the components of POEMS syndrome may be present initially.

The pathogenesis of the disease is unclear, but high circulating levels of the proinflammatory cytokines IL-1, IL-6, VEGF, and TNF have been documented, and levels of the inhibitory cytokine transforming growth factor β are lower than expected. Treatment of the myeloma may result in an improvement in the other disease manifestations.

Patients are often treated similarly to those with myeloma. Plasmapheresis does not appear to be of benefit in POEMS syndrome. Patients presenting with isolated sclerotic lesions may have resolution of neuropathic symptoms after local therapy for plasmacytoma with radiotherapy. Similar to MM, novel agents and HDT with autologous stem cell transplantation have been pursued in selected patients and have been associated with prolonged progression-free survival.

HEAVY CHAIN DISEASES

The heavy chain diseases are rare lymphoplasmacytic malignancies. Their clinical manifestations vary with the heavy chain isotype. Patients have absence of light chain and secrete a defective heavy chain that usually has an intact Fc fragment and a deletion in the Fd region. Gamma, alpha, and mu heavy chain diseases have been described, but no reports of delta or epsilon heavy chain diseases have appeared. Molecular biologic analysis of these tumors has revealed structural genetic defects that may account for the aberrant chain secreted.

GAMMA HEAVY CHAIN DISEASE

FRANKLIN'S DISEASE

This disease affects individuals of widely different age groups and countries of origin. It is characterized by lymphadenopathy, fever, anemia, malaise, hepatosplenomegaly, and weakness. It is frequently associated with autoimmune diseases, especially rheumatoid arthritis. Its most distinctive symptom is palatal edema, resulting from involvement of nodes in Waldeyer's ring, and this may progress to produce

respiratory compromise. The diagnosis depends on the demonstration of an anomalous serum M component (often <20 g/L [<2 g/dL]) that reacts with anti-IgG but not anti-light chain reagents. The M component is typically present in both serum and urine. Most of the paraproteins have been of the γ_1 subclass, but other subclasses have been seen. The patients may have thrombocytopenia, eosinophilia, and nondiagnostic bone marrow that may show increased numbers of lymphocytes or plasma cells that do not stain for light chain. Patients usually have a rapid downhill course and die of infection; however, some patients have survived 5 years with chemotherapy. Therapy is indicated when symptomatic and involves chemotherapeutic combinations used in low-grade lymphoma. Rituximab has also been reported to show efficacy.

■ ALPHA HEAVY CHAIN DISEASE SELIGMANN'S DISEASE

This is the most common of the heavy chain diseases. It is closely related to a malignancy known as Mediterranean lymphoma, a disease that affects young persons in parts of the world where intestinal parasites are common, such as the Mediterranean, Asia, and South America. The disease is characterized by an infiltration of the lamina propria of the small intestine with lymphoplasmacytoid cells that secrete truncated alpha chains. Demonstrating alpha heavy chains is difficult because the alpha chains tend to polymerize and appear as a smear instead of a sharp peak on electrophoretic profiles. Despite the polymerization, hyperviscosity is not a common problem in alpha heavy chain disease. Without J chain–facilitated dimerization, viscosity does not increase dramatically. Light chains are absent from serum and urine. The patients present with chronic diarrhea, weight loss, and malabsorption and have extensive mesenteric and paraaortic adenopathy. Respiratory tract involvement occurs rarely. Patients may vary widely in their clinical course. Some may develop diffuse aggressive histologies of malignant lymphoma. Chemotherapy may produce long-term remissions. Rare patients appear to have responded to antibiotic therapy, raising the question of the etiologic role of antigenic stimulation, perhaps by some chronic intestinal infection. Chemotherapy plus antibiotics may be more effective than chemotherapy alone. IPSID is recognized as an infectious pathogen–associated human lymphoma associated with *Campylobacter jejuni*. It involves mainly the proximal small intestine, resulting in malabsorption, diarrhea, and abdominal pain. IPSID is associated with excessive plasma cell differentiation and produces truncated alpha heavy chain proteins lacking the light chains as well as the first constant domain. Early-stage IPSID responds to antibiotics (30–70% complete remission). Most untreated IPSID patients progress to lymphoplasmacytic and immunoblastic lymphoma. Patients not responding to antibiotic therapy are considered for treatment with combination chemotherapy used to treat low-grade lymphoma.

■ MU HEAVY CHAIN DISEASE

The secretion of isolated mu heavy chains into the serum appears to occur in a very rare subset of patients with CLL. The only features that may distinguish patients with mu heavy chain disease are the presence of vacuoles in the malignant lymphocytes and the excretion of kappa light chains in the urine. The diagnosis requires ultracentrifugation or gel filtration to confirm the nonreactivity of the paraprotein with the light chain reagents because some intact macroglobulins fail to interact with these serums. The tumor cells seem to have a defect in the assembly of light and heavy chains because they appear to contain both in their cytoplasm. Such patients are not treated differently from other patients with CLL (Chap. 107).

■ FURTHER READING

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and to eliminate proteins that misfold. However, genetic mutation, incorrect processing, and other factors may favor misfolding, with consequent loss of normal protein function and intracellular or extracellular aggregation. Many diseases, ranging from cystic fibrosis to Alzheimer's disease, are now known to involve protein misfolding. In the amyloidoses, the aggregates are typically extracellular, and the misfolded protein subunits assume a common antiparallel, β -pleated sheet-rich structural conformation that leads to the formation of higher-order oligomers and then fibrils with unique staining properties. The term *amyloid* was coined around 1854 by the pathologist Rudolf Virchow, who thought that these deposits resembled starch (Latin *amylum*) under the microscope.

Amyloid diseases, defined by the biochemical nature of the protein composing the fibril deposits, are classified according to whether they are systemic or localized, whether they are acquired or inherited, and their clinical patterns (Table 112-1). The standard nomenclature is AX, where A indicates amyloidosis and X represents the protein present in the fibril. This chapter focuses primarily on the systemic forms. AL refers to amyloid composed of immunoglobulin light chains (LCs); this disorder, formerly termed *primary systemic amyloidosis*, arises from a clonal B-cell or plasma cell disorder and can be associated with myeloma or lymphoma. ATTR, the most prevalent of the *familial amyloidoses*, refers to amyloid derived from wild-type or mutated transthyretin (TTR), the transport protein for thyroid hormone and retinol-binding protein. AA amyloid is composed of the acute-phase reactant protein serum amyloid A (SAA) and occurs in the setting of chronic inflammatory or infectious diseases; for this reason, this type was formerly known as *secondary amyloidosis*. $A\beta_M$ amyloid results from misfolded β_2 -microglobulin, occurring in individuals with long-standing renal disease who have undergone dialysis, typically for years. $A\beta$, the most common form of localized amyloidosis, is found in the brain of patients with Alzheimer's disease after abnormal proteolytic processing and aggregation of polypeptides derived from the amyloid precursor protein.

Diagnosis and treatment of the amyloidoses rest upon the histopathologic identification of amyloid deposits and immunohistochemical, biochemical, or genetic determination of amyloid type (Fig. 112-1). In the systemic amyloidoses, the clinically involved

112 Amyloidosis

John L. Berk, Vaishali Sanchorawala



GENERAL PRINCIPLES

Amyloidosis is the term for a group of protein misfolding disorders characterized by the extracellular deposition of insoluble polymeric protein fibrils in tissues and organs. A robust cellular machinery exists to chaperone proteins during the process of synthesis and secretion, to ensure that they achieve correct tertiary conformation and function,

TABLE 112-1 Amyloid Precursor Proteins and Their Clinical Syndromes

DESIGNATION	PRECURSOR	CLINICAL SYNDROME	CLINICAL INVOLVEMENT
Systemic Amyloidoses			
AL	Immunoglobulin light chain	Primary or myeloma-associated ^a	Any
AH	Immunoglobulin heavy chain	Rare variant of primary or myeloma-associated	Any
AA	Serum amyloid A protein	Secondary; reactive ^b	Renal, heart, other
$A\beta_M$	β_2 -Microglobulin	Hemodialysis-associated	Synovial tissue, bone
ATTR	Transthyretin	Familial (mutant) Age-related (wild type)	Cardiac, peripheral and autonomic nerves, soft tissues, spine, bladder
AApoAI	Apolipoprotein AI	Familial	Hepatic, renal
AApoAII	Apolipoprotein AII	Familial	Renal
AGel	Gelsolin	Familial	Cornea, cranial nerves, skin, renal
AFb	Fibrinogen α α	Familial	Renal, vascular
ALys	Lysozyme	Familial	Renal, hepatic
ALECT2	Leukocyte chemotactic factor 2	Undefined	Renal
Localized Amyloidoses			
$A\beta$	Amyloid β protein	Alzheimer's disease; Down's syndrome	Central nervous system
ACys	Cystatin C	Cerebral amyloid angiopathy	Central nervous system, vascular
APrP	Prion protein	Spongiform encephalopathies	Central nervous system
AIAPP	Islet amyloid polypeptide (amylin)	Diabetes-associated	Pancreas
ACal	Calcitonin	Medullary carcinoma of the thyroid	Thyroid
AANF	Atrial natriuretic factor	Atrial fibrillation	Cardiac atria
APro	Prolactin	Endocrinopathy	Pituitary
ASgl	Semenogelin I	Age-related; incidental autopsy or biopsy finding	Seminal vesicles

^aLocalized AL deposits can occur in skin, conjunctiva, urinary bladder, and the tracheobronchial tree. ^bSecondary to chronic inflammation or infection or to a hereditary periodic fever syndrome such as familial Mediterranean fever.

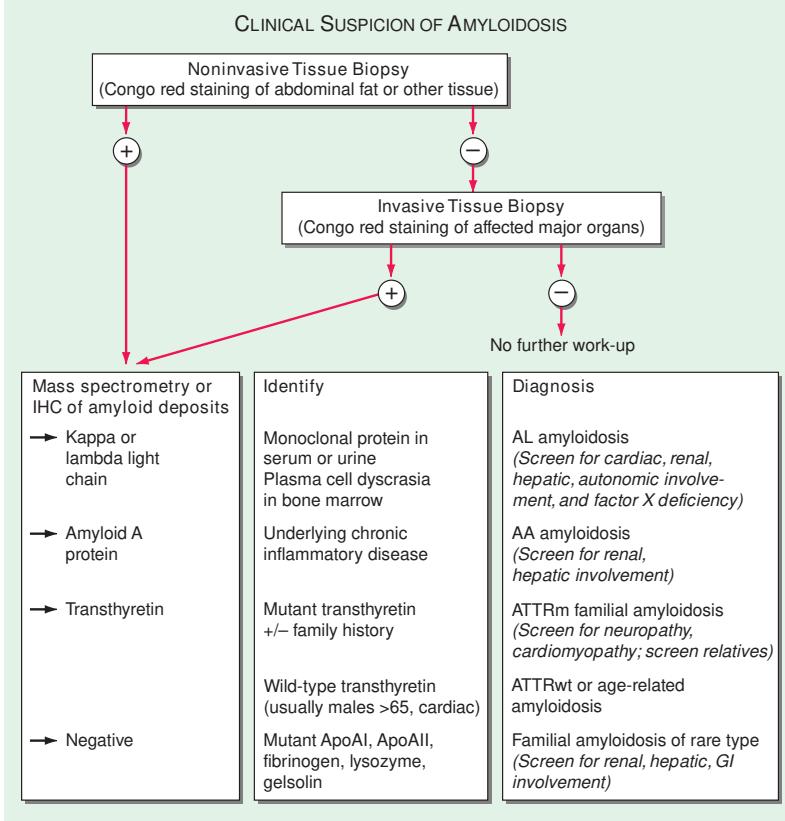


FIGURE 112-1 Algorithm for the diagnosis of amyloidosis and determination of type. Clinical suspicion: unexplained nephropathy, cardiomyopathy, neuropathy, enteropathy, arthropathy, and macroglossia. ApoAI, apolipoprotein AI; ApoAII, apolipoprotein AII; GI, gastrointestinal; IHC, immunohistochemistry.

organs can be biopsied, but amyloid deposits may be found in any tissue of the body. Historically, blood vessels of the gingiva or rectal mucosa were often examined, but the most easily accessible tissue—positive in more than 80% of patients with systemic amyloidosis—is abdominal fat. After local anesthesia, fat is aspirated with a 16-gauge needle from the subcutaneous layer of the abdominal wall. Fat globules expelled onto a glass slide can be stained for amyloid, thus avoiding a surgical procedure. If this material is negative, more invasive biopsies of the kidney, heart, liver, tongue, or gastrointestinal tract can be considered in patients in whom amyloidosis is suspected. The regular β -sheet structure of amyloid deposits exhibits a unique “apple green” birefringence by polarized light microscopy when stained with Congo red dye; other regular protein structures (e.g., collagen) appear white under these conditions. The 10-nm-diameter fibrils can also be visualized by electron microscopy of paraformaldehyde-fixed tissue. Once amyloid is found, the precursor protein type must be determined by immunohistochemistry, immunoelectron microscopy, or extraction and biochemical analysis employing mass spectrometry; gene sequencing is used to identify mutants causing hereditary amyloidosis. The patient’s history, physical findings, and clinical presentation, including age and ethnic origin, organ system involvement, underlying diseases, and family history, may provide helpful clues as to the type of amyloidosis. However, there can be considerable overlap in clinical presentations, and accurate typing is essential to guide appropriate therapy.

The mechanisms of fibril formation and tissue toxicity remain controversial. The “amyloid hypothesis,” as it is currently understood, proposes that precursor proteins undergo a process of reversible unfolding or misfolding; misfolded proteins form oligomeric aggregates, higher-order polymers, and then fibrils that deposit in tissues. Accumulating evidence suggests that the oligomeric intermediates may constitute the most toxic species. Oligomers are more capable than large fibrils of interacting

with cells and inducing formation of reactive oxygen species and stress signaling. Ultimately, the fibrillar tissue deposits are likely to interfere with normal organ function. A more sophisticated understanding of the mechanisms leading to amyloid formation and cell and tissue dysfunction will continue to provide new targets for therapies.

The clinical syndromes of the amyloidoses are associated with relatively nonspecific alterations in routine laboratory tests. Blood counts are usually normal, although the erythrocyte sedimentation rate is frequently elevated. Patients with glomerular kidney involvement generally have proteinuria, often in the nephrotic range, leading to hypoalbuminemia that may be severe; patients with serum albumin levels <2 g/dL generally have pedal edema or anasarca. Amyloid cardiomyopathy is characterized by concentric ventricular hypertrophy and diastolic dysfunction associated with elevation of brain natriuretic peptide (BNP) or N-terminal pro-brain natriuretic peptide (NT-proBNP) as well as troponin. These cardiac biomarkers can be used for disease staging, prognostication, and disease activity monitoring in patients with AL amyloidosis. Notably, renal insufficiency can falsely elevate levels of these biomarkers. Recently, biomarkers of cardiac remodeling—that is, matrix metalloproteinases and tissue inhibitors of metalloproteinases—have been found to be altered in the serum of patients with amyloid cardiomyopathy. Electrocardiographic and echocardiographic features of amyloid cardiomyopathy are described below. Patients with liver involvement, even when advanced, usually develop cholestasis with an elevated alkaline phosphatase concentration with minimal alteration of the aminotransferases and preservation

of synthetic function. In AL amyloidosis, endocrine organs may be infiltrated with fibrils, and hypothyroidism, hypoadrenalinism, or even hypopituitarism can occur. Although none of these findings is specific for amyloidosis, the presence of abnormalities in multiple organ systems should raise suspicions of the diagnosis.

■ AL AMYLOIDOSIS

Etiology and Incidence AL amyloidosis is most frequently caused by a clonal expansion of bone marrow plasma cells that secrete a monoclonal immunoglobulin LC depositing as amyloid fibrils in tissues. Whether the clonal plasma cells produce a LC that misfolds and leads to AL amyloidosis or an LC that folds properly, allowing the cells to inexorably expand over time and develop into multiple myeloma (**Chap. 111**), may depend upon primary sequence of the clonal LC or other genetic or epigenetic factors. AL amyloidosis can occur with multiple myeloma or other B lymphoproliferative diseases, including non-Hodgkin’s lymphoma (**Chap. 108**) and Waldenström’s macroglobulinemia (**Chap. 111**). AL amyloidosis is the most common type of systemic amyloidosis diagnosed in North America. Its incidence has been estimated at 4.5 cases/100,000 population; however, ascertainment continues to be inadequate, and the true incidence may be much higher. AL amyloidosis, like other plasma cell disorders, usually occurs after age 40 and is often progressive and fatal if untreated.

Pathology and Clinical Features Amyloid deposits are usually widespread in AL amyloidosis and can be present in the interstitium of any organ outside the central nervous system. The amyloid fibril deposits are composed of full-length 23-kDa monoclonal immunoglobulin LCs as well as fragments. Accessory molecules co-deposited with LC fibrils (as well as with other amyloid fibrils) include serum amyloid P component, apolipoproteins e and A-IV, glycosaminoglycans, and

metal ions. Although all kappa and lambda LC subtypes have been identified in AL amyloid fibrils, lambda subtypes predominate. The lambda 6 subtype appears to have unique structural properties that predispose it to fibril formation, often in the kidney.

AL amyloidosis is often a rapidly progressive disease that presents as a pleiotropic set of clinical syndromes, recognition of which is key for initiation of the appropriate workup. Nonspecific symptoms of fatigue and weight loss are common; however, the diagnosis is rarely considered until symptoms referable to a specific organ develop. The kidneys are the most frequently involved organ and are affected in 70–80% of patients. Renal amyloidosis usually manifests as proteinuria, often in the nephrotic range and associated with hypoalbuminemia, secondary hypercholesterolemia and hypertriglyceridemia, and edema or anasarca. In some patients, interstitial rather than glomerular amyloid deposition can produce azotemia without proteinuria. The heart is the second most commonly affected organ (50–60% of patients), and cardiac involvement is the leading cause of death from AL amyloidosis. Early on, the electrocardiogram may show low voltage in the limb leads with a pseudo-infarct pattern. Echocardiographic features of disease include concentrically thickened ventricles and diastolic dysfunction with an abnormal global longitudinal strain pattern; a “sparkly” appearance has been described but is often not seen with modern high-resolution echocardiographic techniques. Poor atrial contractility occurs even in sinus rhythm, and patients with cardiac amyloidosis are at risk for development of atrial thrombi and stroke. Cardiac MRI can show increased wall thickness, and characteristic delayed enhancement of the subendocardium has been described following injection of gadolinium contrast. Nervous system symptoms include peripheral sensorimotor neuropathy and/or autonomic dysfunction manifesting as gastrointestinal motility disturbances (early satiety, diarrhea, constipation), dry eyes and mouth, impotence, orthostatic hypotension, and/or neurogenic bladder. Macroglossia (Fig. 112-2A), a pathognomonic sign of AL amyloidosis, is seen in only ~10% of patients. Liver involvement causes cholestasis and hepatomegaly. The spleen is frequently involved, and there may be functional hypersplenism in the absence of significant splenomegaly. Many patients experience “easy bruising” due to amyloid deposits in capillaries or deficiency of clotting factor X due to binding to amyloid fibrils; cutaneous ecchymoses appear, particularly around the eyes, producing another uncommon but pathognomonic finding, the “raccoon-eye” sign (Fig. 112-2B). Other findings include nail dystrophy (Fig. 112-2C), alopecia, and amyloid arthropathy with thickening of synovial membranes in the wrists and shoulders. The presence of a multisystemic illness or general fatigue along with any of these clinical syndromes should prompt a workup for amyloidosis.

Diagnosis Identification of an underlying clonal plasma cell or B lymphoproliferative process and a clonal LC are key to the diagnosis of AL amyloidosis. Serum protein electrophoresis and urine protein electrophoresis, although of value in multiple myeloma, are *not* useful screening tests if AL amyloidosis is suspected because the clonal LC or whole immunoglobulin often is not present in sufficient amounts to produce a monoclonal “M-spike” in the serum or LC (Bence Jones) protein in the urine. However, more than 90% of patients with AL amyloidosis have serum or urine monoclonal LC or whole immunoglobulin

detectable by immunofixation electrophoresis of serum (SIFE) or urine (UIFE) (Fig. 112-3A) or by nephelometric measurement of serum “free” LCs (i.e., LCs circulating in monomeric form rather than in an immunoglobulin tetramer with heavy chain). Examining the ratio as well as the absolute amount of serum-free LCs is essential, as renal insufficiency reduces LC clearance, nonspecifically elevating both isotypes. In addition, an increased percentage of plasma cells in the bone marrow—typically 5–30% of nucleated cells—is found in ~90% of patients. Kappa or lambda clonality should be demonstrated by flow cytometry, immunohistochemistry, or *in situ* hybridization for LC mRNA (Fig. 112-3B).

A monoclonal serum protein by itself is not diagnostic of amyloidosis, since monoclonal gammopathy of uncertain significance is common in older patients (Chap. 111). However, when monoclonal gammopathy of uncertain significance is found in patients with biopsy-proven amyloidosis, the AL type should be ruled out. Similarly, patients thought to have “smoldering myeloma” because of a modest elevation of bone-marrow plasma cells should be screened for AL amyloidosis if they have signs or symptoms of renal, cardiac, or neurologic disease. Accurate tissue amyloid typing is essential for appropriate treatment. Immunohistochemical staining of the amyloid deposits is useful if they selectively bind one LC antibody in preference to the other; some AL deposits bind antibodies nonspecifically. Immunoelectron microscopy is more reliable; laser capture microdissection and tandem mass spectrometry-based typing of the amyloid precursor protein has become the diagnostic standard. In ambiguous cases, other forms of amyloidosis should be thoroughly excluded with appropriate genetic and other testing.

Staging System and Risk Stratification The current staging systems for systemic AL amyloidosis are based on the biomarkers of plasma cell dyscrasia and cardiac and renal involvement. The Mayo 2004 staging system is based on the levels of NT-proBNP and cardiac troponins and was modified by European investigators to identify and classify very-high-risk patients. This cardiac staging system is the most widely used to determine patient management. This staging system was modified (Mayo 2012) to include clonal burden, assessed by dFLC (difference between involved and unininvolved circulating free light chain) concentration, which has independent ability to predict survival. Boston University investigators introduced a staging system incorporating BNP and troponin I that also is able to predict survival. Patients with AL amyloidosis with a very low (<50 mg/L) dFLC level have a significantly better outcome irrespective of cardiac stage. A renal staging system based on 24-h urine protein excretion and estimated glomerular filtration rate (eGFR) predicting the progression to dialysis at 2 years has also been developed and validated. Several other biomarkers have been shown to predict outcomes and survival but have not been incorporated in staging systems yet.

TREATMENT

AL Amyloidosis

Extensive multisystemic involvement typifies AL amyloidosis, and the median survival period without treatment is usually only ~1–2 years from the time of diagnosis. Current therapies target the



FIGURE 112-2 Clinical signs of AL amyloidosis. A. Macroglossia. B. Periorbital ecchymoses. C. Fingernail dystrophy.

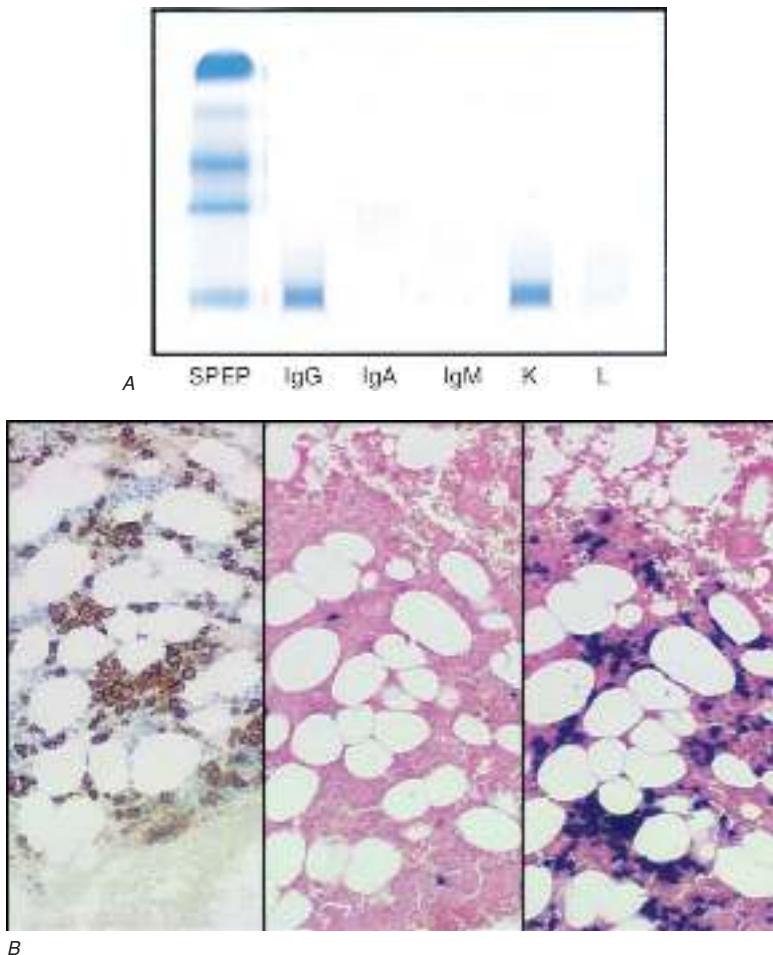


FIGURE 112-3 Laboratory features of AL amyloidosis. *A*, Serum immunofixation electrophoresis reveals an IgG κ monoclonal protein in this example; serum protein electrophoresis is often normal. *B*, Bone marrow biopsy sections stained by immunohistochemistry with antibody to CD138 (syndecan, highly expressed on plasma cells) (*left*) or by *in situ* hybridization with fluorescein-tagged probes (Ventana Medical Systems) binding to κ mRNA (*center*) and λ mRNA (*right*) in plasma cells. (Photomicrograph courtesy of C. O'Hara; with permission.)

clonal bone marrow plasma cells, using approaches employed for multiple myeloma. Treatment with oral melphalan and prednisone can decrease the plasma cell burden but rarely leads to complete hematologic remission, meaningful organ responses, or improved survival and is no longer widely used. The substitution of dexamethasone for prednisone produces a higher response rate and more durable remissions, although dexamethasone is not always well tolerated by patients with significant edema or cardiac disease. High-dose intravenous (IV) melphalan followed by autologous stem cell transplantation (HDM/SCT) produces complete hematologic responses in ~40% of treated patients, as determined by loss of clonal plasma cells in the bone marrow and disappearance of the amyloidogenic monoclonal LC, as determined by SIFE/UIFE and free LC quantitation. Six to 12 months after achieving a hematologic response, improvements in organ function and quality of life may occur. Hematologic responses appear to be more durable after HDM/SCT than in multiple myeloma, with remissions continuing in some patients beyond 15 years without additional treatment. Unfortunately, only ~20–30% of all AL amyloidosis patients are suitable for aggressive treatment, and even at specialized treatment centers, transplantation-related mortality rates are higher than those for other hematologic diseases because of impaired organ function at initial presentation. Amyloid cardiomyopathy, poor nutritional and performance status, and multiorgan disease contribute to excess morbidity and mortality. A bleeding diathesis

resulting from adsorption of clotting factor X to amyloid fibrils also increases mortality rates; however, this syndrome occurs in only 5–10% of patients. A randomized multicenter trial conducted in France compared oral melphalan and dexamethasone with HDM/SCT and failed to show a benefit of dose-intensive treatment, although the transplantation-related mortality rate in this study was very high. It has become clear that careful selection of patients and expert peritransplantation management are essential in reducing transplantation-related mortality.

For patients with AL amyloidosis and impaired cardiac function or arrhythmias due to involvement of the myocardium, the median survival period is only ~6 months without treatment. In these patients, cardiac transplantation can be performed and followed by HDM/SCT to eliminate the noxious LC clone and prevent amyloid deposition in the transplanted heart or other organs.

The best therapy for those who are transplant ineligible varies between centers and countries. A regimen of oral chemotherapy with melphalan and dexamethasone (MDex) had been the standard for patients not eligible for HDM/SCT for more than a decade. Regimens using bortezomib (a proteasome inhibitor) are now considered the standard of care in most patients with AL amyloidosis not eligible for SCT. There is a fine balance between chosen treatment regimens and toxicities, and patient characteristics should be considered when choosing a regimen; for example, treatment with bortezomib plus MDex can overcome the effects of both gain

of 1q21 (which confers a poorer outcome with oral melphalan) and t(11;14) (which confers a poorer outcome with bortezomib). Transplant-ineligible patients in whom bortezomib is contraindicated due to preexisting peripheral neuropathy can be treated with MDex or combinations based on immunomodulatory drugs (e.g., lenalidomide). High-risk patients represent ~20% of all individuals with AL amyloidosis and are a challenge owing to advanced cardiac stage (IIIb) or severe heart failure (New York Heart Association class III or IV).

Newer agents, such as the oral proteasome inhibitor ixazomib and the humanized anti-CD38 monoclonal antibody daratumumab, have also been evaluated in patients with relapsed or refractory disease. Anti-fibril small molecules and humanized monoclonal antibodies are also being tested. Clinical trials are essential in improving therapy for this rare disease.

Supportive care is important for patients with any type of amyloidosis. For nephrotic syndrome, diuretics and supportive stockings can ameliorate edema; angiotensin-converting enzyme inhibitors should be used with caution and have not been shown to slow renal disease progression. Effective diuresis can be facilitated with albumin infusions to raise intravascular oncotic pressure. Congestive heart failure due to amyloid cardiomyopathy is best treated with diuretics; it is important to note that digitalis, calcium channel blockers, and beta blockers are relatively contraindicated as they can interact with amyloid fibrils and produce heart block and worsening heart failure. Amiodarone has been used for atrial and ventricular arrhythmias. Automatic implantable defibrillators appear to have reduced effectiveness due to the thickened myocardium, but they may benefit some patients. Atrial ablation is an effective approach for atrial fibrillation. For conduction abnormalities, ventricular pacing may be indicated. Atrial contractile dysfunction is common in amyloid cardiomyopathy and associated with increased thromboembolic complications, prompting considerations of anti-coagulation even in the absence of atrial fibrillation. Autonomic neuropathy can be treated with α agonists such as midodrine to support postural blood pressure; gastrointestinal dysfunction may respond to motility or bulk agents. Nutritional supplementation, either oral or parenteral, is also important.

In localized AL amyloidosis, amyloid deposits can be produced by clonal plasma cells infiltrating local sites in the airways, bladder, skin, or lymph nodes (Table 112-1). These deposits may respond to surgical intervention or elimination of the responsible plasma cell clone by low-dose radiation therapy (typically only 20 Gy); systemic treatment generally is not appropriate. Patients should be referred to a center familiar with management of these rare manifestations of amyloidosis.

■ AA AMYLOIDOSIS

Etiology and Incidence AA amyloidosis can occur in association with almost any chronic inflammatory state (e.g., rheumatoid arthritis, inflammatory bowel disease, ankylosing spondylitis, familial Mediterranean fever [Chap. 369], or other periodic fever syndromes) or chronic infections such as tuberculosis, osteomyelitis, or subacute bacterial endocarditis. In the United States and Europe, AA amyloidosis has become less common, occurring in fewer than 2% of patients with these diseases, presumably because of advances in anti-inflammatory and antimicrobial therapies. It has also been described in association with Castleman's disease, lymphomas, and renal cell carcinoma, emphasizing the diagnostic importance of CT scanning to look for such tumors as well as serologic and microbiologic studies. In up to 30% of patients, AA amyloidosis can also be seen without any identifiable underlying disease. AA is the most frequent systemic amyloidosis that occurs in children.

Pathology and Clinical Features Organ involvement in AA amyloidosis usually begins in the kidneys. Hepatomegaly, splenomegaly, and autonomic neuropathy can also occur as the disease progresses; cardiomyopathy is a late manifestation in ~25% of patients. The symptoms and signs of AA disease cannot be reliably distinguished from

those of AL amyloidosis. AA amyloid fibrils are usually composed of an 8-kDa, 76-amino-acid *N*-terminal portion of the 12-kDa precursor protein SAA. This acute-phase apoprotein is synthesized in the liver and transported by high-density lipoprotein (HDL3) in the plasma. Several years of an underlying inflammatory disease causing chronic elevation of SAA levels usually precede fibril formation, although infections can lead to AA amyloid deposition more rapidly.

TREATMENT

AA Amyloidosis

Primary therapy for AA amyloidosis consists of treatment of the underlying inflammatory or infectious disease. Treatment that suppresses or eliminates the inflammatory state or infection decreases the SAA concentration, slowing the rate of amyloid fibril formation. For familial Mediterranean fever, colchicine at a dose of 1.2–1.8 mg/d is the standard treatment. However, colchicine has not been helpful for AA amyloidosis of other causes or for other amyloidoses. Tumor necrosis factor and interleukin 1 and interleukin 6 antagonists can effectively interrupt cytokine signaling that drives many inflammatory syndromes, inhibiting hepatic SAA production and limiting AA amyloid deposition. Development of a fibril-specific agent (eprodise) that interferes with the interaction of serum amyloid A protein and glycosaminoglycans to prevent or disrupt fibril formation failed in phase 3 trials.

■ ATTR AND AF AMYLOIDOSIS

The familial amyloidoses are autosomal dominant diseases in which mutated or variant plasma proteins misfold or aggregate to form beta-sheet rich amyloid deposits. These diseases are rare, with an estimated case incidence of <1/100,000 population in the United States, although founder effects in remote areas of Portugal, Sweden, and Japan produced a higher local prevalence of disease. The most prevalent form of hereditary amyloidosis arises from mutation of the abundant liver-derived plasma protein transthyretin (TTR, also known as *prealbumin*) and is termed hATTR amyloid. More than 130 TTR mutations typically conferring one-amino-acid substitutions have been described, with most inducing clinical ATTR amyloid disease. Toxic TTR oligomers and ATTR amyloid deposits target peripheral and autonomic nervous systems and the heart. One TTR variant, V122I, occurs in nearly 4% of the African-American and Afro-Caribbean populations and is associated with late-onset cardiac amyloidosis. The actual incidence and penetrance of disease in the African-American population is the subject of ongoing research, but considerations of V122I ATTR amyloidosis is warranted in African-American patients who present with concentric cardiac hypertrophy and evidence of diastolic heart failure, particularly in the absence of a history of hypertension or valvular disease. Other familial amyloidoses, caused by variant apolipoproteins AI or AII, gelsolin, fibrinogen α , or lysozyme, are reported with lower prevalence worldwide. New amyloidogenic serum proteins continue to be identified periodically, including leukocyte chemotactic factor LECT2, which is a cause of renal amyloidosis in Hispanic and Pakistani populations. Although the clustering of ALECT2 cases suggests heritability, no LECT2 gene-coding sequence variations have been identified.

Normal (wild-type) transthyretin can also misfold and aggregate to form ATTR amyloid, typically expressed in men beginning in the seventh decade with increasing prevalence with age. Formerly termed senile systemic amyloidosis, ATTRwt amyloid is reported at autopsy in 25% of hearts from patients who are 80 years and older. Although it is unclear why a wild-type protein becomes amyloidogenic, aging inefficiencies of intracellular quality-assurance mechanisms (termed the unfolded protein response) likely predispose to secretion of proteins prone to misaggregation. Due to the numbers of aging men globally, ATTRwt is the most prevalent and rapidly growing form of amyloidosis in the world today.

Clinical Features and Diagnosis hATTR amyloidosis has a varied presentation predicted by the specific TTR mutation. Consequently,

kindreds typically express similar disease timing and clinical course. Apparent sporadic presentations (no recognized family history) often reflect incomplete penetrance of the TTR mutation and not a spontaneous event. hATTR amyloidosis presents as familial amyloidotic polyneuropathy (nerve damage) or familial amyloidotic cardiomyopathy (heart damage). Peripheral neuropathy begins as a length-dependent small-fiber sensorimotor neuropathy first exhibited in the feet with ascending progression to the upper extremities. Autonomic neuropathy manifests as smooth muscle dysmotility (dysphagia, diarrhea, urinary retention), vascular dysregulation (orthostatic hypotension, erectile dysfunction), and anhidrosis. Soft tissue disease (carpal tunnel syndrome, tendonopathy, and spinal stenosis) commonly precedes nerve or heart manifestations of disease by 1–2 decades, particularly in ATTRwt amyloid patients who frequently report bicipital, patellar, or Achilles tendon rupture. Less common expressions of hATTR include vitreous opacities and leptomeningeal amyloid deposition from variant protein produced by the retinal epithelium and choroid plexus, respectively. ATTR amyloid involvement of the heart is clinically better tolerated than AL amyloid cardiomyopathy as reflected by the time from heart failure presentation to death in untreated cases of ATTR (median 42–48 months) versus AL (median 6 months) amyloidosis and the dramatically greater burden of disease by echocardiographic measures at symptomatic presentation.

Typical syndromes associated with other forms of AF disease include renal amyloidosis with mutant fibrinogen, lysozyme, or apolipoproteins; hepatic amyloidosis with apolipoprotein AI; and amyloidosis of cranial neuropathy with corneal lattice dystrophy pathognomonic of gelsolin amyloidosis. Patients with AF amyloidosis can present with clinical syndromes that mimic those of patients with AL disease. Rarely, AF carriers can develop AL disease or AF patients may have monoclonal gammopathy without AL. Thus, it is important to screen both for plasma cell disorders and for mutations in patients with amyloidosis. Although mass spectrometry often detects amino acid sequence variations, it is not designed to definitively identify specific protein variations; DNA sequencing is the diagnostic standard for AF mutations.

TREATMENT

ATTR Amyloidosis

Untreated, the survival period after onset of ATTR disease is 5–15 years. At present, three therapeutic strategies are used for ATTR amyloidosis: (1) orthotopic liver transplantation (OLT) to replace the factory of the mutated protein (only applicable to hATTR); (2) stabilization of circulating TTR tetramers, preventing TTR monomer release and amyloid fibril formation; and (3) TTR gene silencing (RNA interference or anti-sense oligonucleotide agents), suppressing hepatic TTR production to eliminate ATTR fibril formation. After 30 years of experience, OLT is largely limited to patients with hATTR amyloid and early peripheral neuropathy (V30M ATTR), as most patients with non-V30M TTR mutations suffer post-transplant progressive amyloid disease due to wild-type TTR from the allograft liver depositing on preexisting amyloid present in the heart and nerves. TTR tetramer stabilization successfully inhibits progressive ATTR amyloid nerve and heart disease as demonstrated by a phase 3 randomized controlled trial—the Diflunisal Trial (hATTR)—and the Transthyretin Amyloidosis Cardiomyopathy Clinical Trial (ATTR-ACT), respectively. Diflunisal, a repurposed generic nonsteroidal anti-inflammatory, and tafamidis, a proprietary thyroxine mimetic, bind TTR tetramers at the thyroxine binding site, minimizing release of the amyloidogenic TTR monomer and slowing progression of nerve and heart disease. Tafamidis, the first U.S. Food and Drug Administration-approved treatment for ATTR amyloid cardiomyopathy, extends survival and slows the decline in walking capacities and quality of life but does not appear to induce improvement in heart thickening or function. TTR gene silencers more reliably stop neurologic disease progression and, in 35–60% of treated patients with hATTR amyloid, improve sensory nerve deficits, a novel finding. Further, preliminary data suggest

TTR gene silencers may promote heart remodeling and improve systolic function.

The therapeutic future of ATTR amyloid patients is bright. Phase 3 randomized controlled clinical trials examining the safety and effectiveness of TTR gene silencers in patients with ATTR amyloid cardiomyopathy are underway, as are studies to determine the impact of second-generation TTR gene silencers on ATTR amyloid neuropathy and cardiomyopathy. TTR gene editing to prevent mRNA production or correct DNA mutations is the next frontier. Finally, as survival improves for patients with ATTR amyloid, therapies that cross the blood-brain barrier to address leptomeningeal (brain) and vitreous (eye) amyloid deposition arising from the choroid plexus and retinal epithelium, respectively, will be challenges to achieve.

A₂M AMYLOIDOSIS

A₂M amyloid is composed of β_2 -microglobulin, the invariant chain of class I human leukocyte antigens, and produces rheumatologic manifestations in patients undergoing long-term hemodialysis and, rarely, in patients with a hereditary form of disease. β_2 -Microglobulin is excreted by the kidney, and levels become elevated in end-stage renal disease. The molecular mass of β_2 M is 11.8 kDa—above the cutoff of some dialysis membranes. The incidence of this disease appears to be declining with the use of newer membranes in high-flow dialysis techniques. A₂M amyloidosis usually presents as carpal tunnel syndrome, persistent joint effusions, spondyloarthropathy, or cystic bone lesions. Carpal tunnel syndrome is often the first symptom. In the past, persistent joint effusions accompanied by mild discomfort were found in up to 50% of patients who had undergone dialysis for >12 years. Involvement is bilateral, and large joints (shoulders, knees, wrists, and hips) are most frequently affected. The synovial fluid is noninflammatory, and β_2 M amyloid can be found if the sediment is stained with Congo red. Although less common, visceral β_2 M amyloid deposits do occasionally occur in the gastrointestinal tract, heart, tendons, and subcutaneous tissues of the buttocks. There are no proven specific therapies for A₂M amyloidosis, but cessation of dialysis after renal allografting may lead to symptomatic improvement.

THERAPEUTIC FRONTIERS

To date, treatment strategies have focused on limiting formation of amyloidogenic proteins. Disruption of existing amyloid by targeting ubiquitous components of the tissue deposits offers theoretical means to improving major end-organ function; however, clinical trial validation remains elusive.

SUMMARY

A diagnosis of amyloidosis should be considered in patients with unexplained nephropathy, cardiomyopathy (particularly with diastolic dysfunction), neuropathy (either peripheral or autonomic), enteropathy, or the pathognomonic soft tissue findings of macroglossia or periorbital ecchymoses. Pathologic identification of amyloid fibrils can be made with Congo red staining of aspirated abdominal fat or of an involved-organ biopsy specimen. Accurate typing by a combination of immunologic, biochemical, and genetic testing is essential in selecting appropriate therapy (Fig. 112-1). Systemic amyloidosis should be considered a treatable condition, as anti-plasma cell chemotherapy is highly effective in AL disease and targeted therapies are being developed for AA and ATTR disease. The combination of precursor and end-organ amyloid therapeutics potentially provide not only disease control but also functional and quality of life improvements for patients with amyloidosis. Tertiary referral centers can provide specialized diagnostic techniques and access to clinical trials for patients with these rare diseases.

FURTHER READING

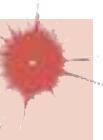
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Transfusion Therapy and Biology

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Jacques Chiaroni



Transfusion encompasses the use of blood components (BCs) to prevent or treat anemia, hemorrhage, and bleeding disorders. Occasionally, BCs may be used to treat infection or relapse of malignant blood diseases after allogeneic hematopoietic transplantation. BCs comprise mainly red blood cell concentrates (RBCCs), platelet concentrates (PCs), and plasma for transfusion use (as opposed to plasma for fractionation into medicinal products such as albumin and immunoglobulin). Alongside transfusion safety, ensuring BC quality, assessing in vivo efficacy, and promoting evidence-based transfusion practices are critical aspects of transfusion medicine.

Blood collection and donor medicine do not fall within the scope of this chapter. Although the processes used are particularly safe, blood donations can cause adverse reactions, among which are fainting reactions and iron deficiency. These risks require preventive approaches and appropriate treatment when needed.

BLOOD COMPONENTS

BC collection and manufacturing processes are described in [Table 113-1](#). Most common BCs are collected as whole blood or directly as components by apheresis. The vast majority of BCs are homologous. Autologous BCs, sometimes collected ahead of planned surgery, are now exceptional as they present little to no evidence-based advantage over homologous BCs. Nevertheless, such donation may still be of benefit in the presence of a rare blood group phenotype.

All BCs comply with common quality and performance standards and guidelines. Quality assurance encompasses well-defined processing steps and stringent BC quality controls as defined by health authorities. Tracing of all manufacturing steps as well as hemovigilance-based reporting of adverse events and incidents associated with blood collection, BC processing, and transfusion are highly recommended.

With the obvious exception of granulocyte concentrates and mononuclear cells, the majority of BCs are now leukocyte-reduced, and universal prestorage leukocyte reduction has been recommended. These BCs contain $<1-5 \cdot 10^6$ donor leukocytes and are associated with reduced incidence febrile nonhemolytic transfusion reactions (FNHTRs), infections with intracellular pathogens such as cytomegalovirus (CMV), alloimmunization, and immunomodulation.

BCs may undergo additional processing steps. These may include irradiation to prevent graft-versus-host disease (GVHD) in immunosuppressed patients, pathogen reduction to further reduce the risk of transfusion-transmitted infections, plasma reduction in patients with

severe allergic reactions to BCs, or the manufacturing of pediatric units for young children and neonates.

BC constituents undergo centrifugation and filtration and are placed in contact with needles, plastic tubing and bags, as well anticoagulant molecules and various additive solutions. BCs are subjected to gas exchanges that are significantly different from aerobic breathing and are maintained at temperatures that are not physiologic, such as 22°C or 4°C. Any of these elements may contribute to so-called “storage lesions” that may occur at any time during BC processing and storage. Some of these lesions have proven to be reversible in the recipient after transfusion, while others may be irreversible. The clinical impacts of such lesions are under investigation. Storage lesions may also account for a number of adverse transfusion reactions, although there is currently no consensus on this issue.

Furthermore, plasma present in BCs contains donor antibodies (Abs). When directed toward antigens (Ags) present in the recipient, such as blood group or tissue (human leukocyte antigen [HLA]) Ags, such Abs may result in adverse events. RBCCs bring only a limited amount of donor plasma (10–30 mL), unlike PCs and obviously plasma. The use of platelet additive solution can replace two-thirds of plasma in PCs, while still leaving the equivalent of one plasma unit of 200 mL per transfused PC.

BLOOD GROUP ANTIGENS AND ANTIBODIES

Red blood cells, as well as other blood constituents such as platelets and neutrophils, express allogeneic determinants. Transfusion may therefore result in alloimmunization and the production of Abs directed against allogeneic determinants. These alloantibodies (alloAbs) comprise anti-red blood cell (RBC) Abs, anti-HLA, anti-human platelet Ag (HPA) Abs, and anti-human neutrophil Ag (HNA) Abs. Anti-RBC immunization may result in hemolysis, while anti-HLA or anti-HPA Abs may result in other transfusion complications such as fever and platelet transfusion refractoriness. Furthermore, anti-HLA and anti-HNA immunization in the donor may result in a severe lung disorder called transfusion-related acute lung injury (TRALI). The Abs against red cell Ags may be IgM or IgG immunoglobulin classes. Some IgG or IgM can activate complement, and some IgG, crossing the placental barrier, may induce hemolytic disease of the fetus and newborn.

Erythrocyte blood groups refer to antigenic molecules that are expressed on the surface of RBC and other cells, genetically transmitted, and recognized by specific Abs. The polymorphism of such molecules explains their immunizing potential in situations such as transfusion, pregnancy, and transplantation. Blood groups can also interact with the environment and with infectious pathogens, leading to individual susceptibilities. For example, malaria is less severe in type O than non-O patients. Conversely, group O is associated with increased susceptibility to *Helicobacter pylori*. Currently, ~380 different blood group Ags have been described, classified within ~43 different systems. Blood group Ags belong to two broad categories based on their biochemical nature: carbohydrate blood groups and protein blood groups. RBC Ags may be the target of autoantibodies (autoAbs) generating autoimmune hemolytic anemia. Some of them, mostly IgG, are active at 37°C, called “warm autoAbs,” and are most often directed against Rh Ags, while others, most often IgM, are active at 4°C, called “cold autoAbs,” and may be directed against ABO, I, i, P, and other Ags.

Carbohydrate blood groups are headed by the ABO system which comprises two main Ags, A and B, encoded by two alleles, which are the A and B alleles, respectively. In addition to these active alleles, there is an inactive allele: O. Depending on the genotype, four different phenotypes are produced ([Table 113-2](#)). Other carbohydrate systems (H, PIPK, Lewis, I, and GLOB) share many characteristics with the ABO system. The main common feature is biochemical. Indeed, given their carbohydrate nature, Ags of the ABO system are considered to be “secondary products” of genes. The A allele encodes the A enzyme, which binds the A-type sugar (GalNAc) A to the H substrate (expressed by action of the H enzyme encoded by the H allele, which happens to be inactive in the Bombay phenotype); sugars are attached to protein substrates on the surface of the RBC and so forth.

TABLE 113-1 Blood Components: Collection and Manufacturing Processes

BLOOD COLLECTION	INITIAL PROCESSING	BLOOD COMPONENT	ADDITIONAL COMPONENT PROCESSING (OPTIONAL TO MANDATORY)	RATIONALE	VOLUME AND CONTENT	STORAGE CONDITIONS AND DURATION
Whole blood	<p>Separation into RBCCs and platelet-rich plasma (PRP) by slow centrifugation, followed by high-speed centrifugation of the PRP to yield one unit of platelets (most often subsequently pooled) and one unit of plasma.</p> <p>Or</p> <p>Separation into a PRBC, a plasma, and a "buffy coat" containing leukocytes and platelets by high-speed centrifugation, followed by pooling and slow-speed centrifugation of the buffy coat to produce a pooled platelet unit. Alternatively, the buffy coat may undergo high-speed centrifugation to produce a granulocyte unit that will be subsequently pooled.</p>	<p>RBCC from whole blood or from apheresis</p> <p>Platelets from whole blood (individual units or pools of 4–6 units of ABO identical units) or from apheresis</p>	<p>Deleukocytation to $<1\text{--}5.10^6$ leukocytes per unit: initial whole blood filtration or RBC elective filtration (highly recommended; mandatory in several international jurisdictions)</p> <p>Irradiation: X-ray or gamma, $\sim 25\text{--}35$ Gy; most often units no older than 28 days after collection</p> <p>Plasma reduction</p> <p>Pediatric preparation</p> <p>Cryopreservation (glycerol)</p> <p>Deleukocytation ($<1\text{--}5.10^6$ leukocytes per unit): initial whole blood filtration or platelet elective filtration (highly recommended, mandatory in several international jurisdictions)</p> <p>Pathogen reduction: Most often nucleic acid cross-linker and/or UV illumination</p>	<p>Reduction of posttransfusion fever and chills</p> <p>Reduction of intracellular pathogens (including CMV infections)</p> <p>Reduction of alloimmunization</p> <p>GVHD prevention in immunosuppressed patients or intrafamilial transfusions</p> <p>Prevention of allergic reactions in patients with prior severe reactions</p> <p>Adjustment to low-weight recipients</p> <p>Most often to ensure availability of RBCCs with a rare blood group for immunized "public-negative" recipients or recipients with complex alloimmunizations^a</p> <p>Reduction of posttransfusion fever and chills</p> <p>Plasma orientation toward fractionation</p> <p>Reduction of posttransfusion fever and chills</p> <p>Reduction of intracellular pathogens (including CMV infections)</p> <p>Reduction of alloimmunization</p> <p>Reduction of transfusion-transmitted infections</p> <p>Prevention of GVHD</p>	<p>250–300 mL (including additive solution, no more than 40–50 mL of plasma)</p> <p>Hemoglobin: 22–40 g/dL</p> <p>Hematocrit: 50–70%</p> <p>Hemolysis $\leq 0.8\%$ at issuing</p> <p>From 100 to 700 mL $\geq 2 \cdot 10^{11}$ platelets Ph ≥ 6.4</p>	<p>4 \pm 2°C</p> <p>Duration depends on the additive solution: 25–42 days; some solutions aim to extend shelf life to 56 days</p> <p>After irradiation: 24 h</p> <p>After plasma reduction: 24 h to 10 days depending on reduction methodology</p> <p>N2 or -80°C electric freeze drying</p> <p>N2: unlimited; -80°C: 30 years</p> <p>7 days after thawing in suitable additive solutions, 24 h if no additive solution</p> <p>At 20–24°C and under permanent motion: 3–7 days</p> <p>Or</p> <p>At 4°C without motion: up to 14–21 days (experimental)</p> <p>If irradiated: <24 h</p>
Apheresis	Various apheresis devices allow for the collection of BCs either as individual BCs such as plasma or platelets (possibly double, such as double RBCCs) or combined BCs, such as platelets and plasma, or RBCC, platelets, and plasma.		<p>Volume reduction</p> <p>Irradiation: X-ray or gamma, $\sim 25\text{--}35$ Gy; in general, on bags no older than 3 days after collection</p> <p>Pediatric</p>	<p>Prevention of allergic reactions in patients with prior severe reactions</p> <p>Prevention of GVHD</p> <p>Volume and content adjustment</p>		

(Continued)

TABLE 113-1 Blood Components: Collection and Manufacturing Processes (Continued)

BLOOD COLLECTION	INITIAL PROCESSING	BLOOD COMPONENT	ADDITIONAL COMPONENT PROCESSING (OPTIONAL TO MANDATORY)	RATIONALE	VOLUME AND CONTENT	STORAGE CONDITIONS AND DURATION
		Plasma from whole blood or from apheresis	Cryopreservation (DMSO) Cryopreservation at -18°C (most often) Deleukocytation (<1–5.10 ⁶ leukocytes per product): Initial whole blood filtration and/or plasma elective filtration Pathogen reduction: Nucleic acid cross-linker and/or UV illumination or solvent detergent treatment (most often on pooled products) Lyophilization	To ensure continuous availability in remote locations To ensure availability of platelets with rare HPA groups Shelf life extension Reduction of posttransfusion fever and chills Reduction of intracellular pathogens (including CMV) Reduction of alloimmunization Reduction of transfusion-transmitted infections To facilitate transportation and storage, as well as immediate availability, in remote locations	200–300 mL Coagulation factors, including fibrinogen (≥2 g/L), factor VIII (≥0.5 IU/mL), protein C and S, antithrombin	6 h after thawing (depending on cryopreservation procedure, may be resuspended in plasma) 1–2 years if cryopreserved Up to 28 days if kept unfrozen
		Granulocyte concentrates from whole blood (pools of up to x ABO identical units) or from apheresis ^b	Irradiation (mandatory)	Prevention of GVHD	≤650 mL ≤2.10 ¹⁰ granulocytes	Room temperature ≤24 h after the end of collection
		Whole blood	Deleukocytation with a platelet-sparing device	Reduction of posttransfusion fever and chills Reduction of intracellular pathogens (including CMV) Reduction of alloimmunization	~520 mL (including additive solution)	At 2–4°C Up to 25 days
		Peripheral blood mononuclear cells (apheresis)	May undergo cryopreservation (N2)	Increased practicability Repeated administration	Number of cells adjusted for a predetermined number of T lymphocytes 10 ⁶ –10 ⁷ CD3+ cells/recipient kg	N2: unlimited Never frozen or thawed: <6 h
		Cryoprecipitate (collected after thawing and centrifugation of plasma)	Resuspension in plasma (10–15 mL) and cryopreservation	N/A	Cold-insoluble plasma proteins (fibrinogen, factor VIII, von Willebrand factor)	12 months After thawing, may be stored at 20–24°C for up to 6 h

^aAntigen frequency below 1 to 4% (1/1000) of the population and contraindication for using regular blood units, depending on country-specific regulations. ^bGranulocyte collection by apheresis requires donor pre-administration of steroids and/or hematopoietic growth factor and exposure to heparin and HES during the apheresis procedure.

Abbreviations: BC, blood component; CMV, cytomegalovirus; DMSO, dimethyl sulfoxide; GVHD, graft-versus-host disease; Hb, hemoglobin; HPA, human platelet antigen; N2, nitrogen gas; N/A, not applicable; RBC, red blood cell; RBCC, red blood cell concentrate; UV, ultraviolet.

TABLE 113-2 ABO Blood Groups and Antibodies: Transfusion Compatibility

GENOTYPE(S)	ENZYME(S)/IMMUNODOMINANT SUGAR(S)	PHENOTYPE	NATURAL ANTIBODIES	TRANSFUSION COMPATIBILITY REQUIREMENTS		
				RBCC	PC ^a	PLASMA
A/A or A/O	"A" transferase/N-acetylgalactosamine (GalNac)	A	Anti-B	A or O	A, O ^b , B ^b , or AB ^b	A or A,B
B/B or B/O	"B" transferase/galactose (Gal)	B	Anti-A	B or O	B, O, A ^b or AB ^b	B or A,B
A/B	"A" transferase and "B" transferase GalNac and Gal	A,B	None	A,B or A or B or O	A,B, O ^b , or A ^b or B ^b	A,B
O/O	Inactive Unconverted H antigen	O	Anti-A and Anti-B	O	O, A, B, or A,B	A or B or A,B or O

^aOrder of priority. ^bWithout high-titer anti-A and/or anti-B antibody.

Abbreviations: PC, platelet concentrate; RBCC, red blood cell concentrate.

Carbohydrate Ags are ubiquitously distributed in the body. The ABO Ags, expressed on endothelial cells, are genuine "tissue" groups and may be involved in graft rejection. These Ags are not specific to humans but are shared by many species including viruses and bacteria. The presence of A and B Ags in the environment and, in particular, on the bacteria of the microbiota explains the synthesis of so-called "natural" or "regular" Abs, aside from any transfusion or pregnancy. Such Abs have a major hemolytic capacity as they bind complement and activate its cascade up to the membrane attack complex. This imposes donor-recipient stringent compatibility rules for RBCCs and

whole blood transfusion and, albeit less stringently, for plasma and PC transfusion.

Protein blood groups are headed by the Rh system (formerly termed "Rhesus" or "Rh") for RBCs (Table 113-3). As these Ags are specific to humans, the occurrence of immunization can only occur upon allogeneic stimulation. The elicited Abs are called "immune" and "irregular" because their appearance following immunization is inconstant. These Abs directed against Ags of RBC groups other than ABO must be detected before RBCC cell transfusion or transplantation and during pregnancy. Of the 43 RBC group systems described, five

TABLE 113-3 Red Blood Cell (RBC) Group Systems and Antibodies: Clinical Significance and Transfusion Recommendations

ISBT NO./SYSTEM	SYMBOL/GENE(S)	ANTIGENS (NO.)	MAIN ANTIBODIES (ANTI-)	HEMOLYSIS CHARACTERISTICS		RBCC TRANSFUSION RECOMMENDATIONS
				TRANSFUSION	HDFN	
1/ABO	ABO/ABO	4	A, B	None to severe; immediate and/or delayed	None to moderate (rarely severe)	Ab-negative RBCC
2/MNS	MNS/GYPA, GYPB, (GYPE)	49	M	None (except in extremely rare cases if active at 37°C)	None (except in extremely rare cases if active at 37°C)	Compatible RBCC (negative DAT at 37°C) Ag-negative red cells in the case of sickle cell disease
			N	None (may be clinically significant in the case of the rare N-S-s-U- phenotype)	None	Compatible RBCC (negative IAT at 37°C) Ag-negative RBCC in the case of N-S-s-U- phenotype
			S, s U	None to moderate (rare) Mild to severe	None to severe (rare) Mild to severe (one reported case requiring an intrauterine transfusion)	Ag-negative RBCC Ag-negative RBCC
3/P1PK	P1PK/A4GALT	3	P1 P1, Pk, P (Tj ^a)	None to moderate; delayed (rare) None to severe	None None to severe	Compatible RBCC (negative DAT at 37°C) Ag-negative RBCC
4/Rh	RH/RHD, RHCE	55	D, C, E, c, e	Mild to severe; immediate or delayed	Mild to severe	Ag-negative RBCC
6/Kell	KEL/KEL	36	K	Mild to severe; delayed	Mild to severe (rare)	Ag-negative RBCC
7/Lewis	LE/FUT3	6	Le ^a , Le ^b	None (rare cases of hemolytic reactions)	None	Compatible RBCC (negative DIAT at 37°C)
8/Duffy	FY/ACKR1	5	Fy ^a , Fy ^b Fy3, Fy5	Mild to severe (rare); immediate/delayed Mild to moderate; immediate (rare)/delayed	Mild to severe (rare) Mild (rare) (no data for anti-Fy5)	Ag-negative RBCC Ag-negative RBCC
9/Kidd	JK/SLC14A1	3	Jk ^a , Jk ^b Jk3	None to severe; immediate or delayed None to severe; immediate or delayed	Mild to moderate (rare) None to mild	Ag-negative RBCC Ag-negative RBCC
18/H	H/FUT1	1	H (Bombay)	None to severe; immediate/delayed	Not none	Ag-negative RBCC
20/Globoside	GLOB/ B3GALNT1	2	P	None to severe	None to mild	Ag-negative RBCC

Abbreviations: Ab, antibody; Ag, antigen; DAT, direct antiglobulin test (Direct Coombs test); HDFN, hemolytic disease of the fetus and newborn; IAT, indirect antiglobulin test (Indirect Coombs test); ISBT, International Society of Blood Transfusion; RBCC, red blood cell concentrate.

(Rh, Kell, Duffy, Kidd, and MNS) are routinely investigated due to the clinical significance of Abs and their frequency. Testing for all five types ensures routine transfusion compatibility of 95%.

The Rh system comprises nearly 56 Abs, the most immunogenic of which is the RhD Ag (RH1). The Rh system has two *RH'D* and *RH'CE* genes located on chromosome 1. The *RH'D* gene codes for the RhD protein expressing the D Ag (RH1) present in 85%, 93%, and >99% of individuals of Caucasian, African, and Asian ancestry, respectively. The *RH'CE* gene codes for RhCE proteins expressing C (RH2) and/or c (RH4), and E (RH3) and/or e (RH4) Abs. The presence of the D Ag confers Rh “positivity,” while its absence confers Rh negativity. The *RH'D* and *RH'CE* genes determine eight main haplotypes (*Dce*, *DcE*, *dce*, *dCe*, *dcE*, and *dCE*) whose frequencies differ considerably among different geographical populations. The high diversity of the Rh Abs includes weak or partial expression. Identifying individuals (especially young females of childbearing potential and multitransfused patients) with a weak or partial RhD Ag is important to adequately select RhD-positive or -negative RBCs. Molecular biology is now routinely applied to resolve such situations.

The Kell system comprises 36 Abs, one of which is routinely determined: the K antigen (KEL1); 9% and 2% of individuals of Caucasian and African ancestry are K positive (KEL1), respectively, whereas 91% and 98%, respectively, are K negative (KEL-1). The immunogenicity of Kell is third behind the ABO and Rh systems. The Kell protein is linked to another blood group protein called Kx. The rare absence of this protein (controlled by a gene on X) is associated with a weak KEL Ag, acanthocytosis, shortened RBC survival, and a progressive form of muscular dystrophy that includes cardiac defects. This rare condition is called the McLeod phenotype.

The Duffy system (FY) comprises five Abs, two of which are routinely tested: the Fy^a Ag (FY1), coded by the *Fy^a* allele, and the Fy^b Ag (FY2), coded by the *Fy^b* allele. Depending on the combination of alleles, three common phenotypes are expected: Fy (a+b+), which has the two alleles *Fy^a* and *Fy^b*; Fy (a+b-), which has only the *Fy^a* allele in a double dose; and Fy (a-b+), which has only a double dose of the Fyb allele. A particular phenotype characterized by the absence of the Fy^a and Fy^b Abs, the Fy(a-b-) phenotype, is exclusive (with some exceptions) to individuals of African ancestry where it can reach frequencies of 70–100% depending on the population. It is linked to the presence of a double dose of a silent *FY'* allele. This distribution may be related to the fact that the Fy Abs serve as receptors for *Plasmodium vivax* and therefore the Fy(a-b-) phenotype. However, these individuals may develop Abs against two high-frequency Abs (FY3 and FY5) after transfusion or pregnancy. They may also have low granulocyte counts that come to the attention of physicians, but the condition is not associated with any disease.

The Kidd system (JK) comprises three Abs, two of which are routinely tested: the Jk^a Ag (JK1), coded by the *Jk^a* allele, and the Jk^b Ag (JK2), coded by the *Jk^b* allele. Depending on the combinations of alleles, three common phenotypes are seen: Jk(a+b+) displaying the two alleles *Jk^a* and *Jk^b*, Jk(a+b-) displaying only the *Jk^a* allele in a double dose, and Jk(a-b+) displaying only a double dose of the *Jk^b* allele. A particular phenotype is characterized by the absence of the Jk^a and Jk^b Abs: the Jk(a-b-) phenotype found in Polynesian populations. It is linked to the presence of a double dose of a silent *JK'* allele. These people may develop Abs against the high-frequency anti-JK3 Ag after transfusion or pregnancy.

The MNS system comprises 49 Abs, four of which are routinely tested. Two genes (*GYP*_A, *GYP*_B) encode two pairs of so-called “antithetical” Abs. The M (MNS1) and N (MNS2) pair Abs encoded by the *M* and *N* alleles, respectively, are branched on the glycophorin A molecule. Their combination will determine whether or not they are present. M+ and N+ subjects have both alleles; an M+, N- subject is homozygous for the *M* allele; and an M-, N+ subject is homozygous for the *N* allele. The same holds true for the other pair of Abs, S (MNS3) and s (MNS4) expressed on glycophorin B. Therefore, an M+, N-, S-, s+ subject (in international nomenclature, this is written as MNS:1,-2,-3,5) will be homozygous for the *M* and *s* alleles. A rare phenotype,

S-s-, found exclusively in individuals of African ancestry, can develop an Ab against the high-frequency U Ag (MNS:5) after transfusion or pregnancy.

RARE RBC PHENOTYPES

Some patients present with rare genotype/phenotype assortments and their RBCs display so-called private Abs or, conversely, lack public Abs (i.e., widely shared Abs) toward which the patient may develop an immune response when exposed to these Abs. Public-negative immunized individuals are virtually impossible to transfuse using conventional blood bank resources and require access to designated blood banks that have access to rare blood programs. Their primary responsibility is to identify and collect blood from donors exhibiting particular Ag displays on their RBCs or platelets that are uncommon in the given jurisdiction. Specific ethnic populations may be targeted, as some may display genotype specificities, such as the Bombay group in southwestern Indians. Several hemoglobinopathies, such as sickle cell disease, are more common in individuals of African ancestry. Such patients may display RBC phenotypes that are uncommon in countries in the Northern Hemisphere, resulting in difficulties adequately identifying donors to match the need, as a last resort, for highly valued cryopreserved BCs.

CLINICAL INDICATIONS AND EFFICACY ASSESSMENT OF BLOOD COMPONENTS

BCs are life-saving therapies but also scarce resources. Furthermore, transfusion may result in well-identified adverse reactions as well as more ill-defined adverse events, including inflammation and therapeutic inefficacy. As highlighted in so-called patient blood management programs, transfusion should be considered within a multidisciplinary approach that includes optimization of hematopoiesis, minimization of blood loss during surgical interventions, and optimization of tolerance to anemia. Clinical indications of BCs as well as means to assess therapeutic efficacy are detailed in Table 113-4.

ADVERSE REACTIONS TO BLOOD COMPONENTS

Adverse reactions to transfused BCs are most commonly non-life-threatening, although serious reactions can present with mild symptoms and signs. Transfused patients should be closely monitored for warning signs suggestive of adverse reactions, as described in Table 113-5. When an adverse reaction is suspected, the transfusion must be stopped while the recipient's clinical status is assessed and supportive care is initiated as needed. An average of 35 transfusion-associated fatalities with possible to definite imputability were reported yearly to the U.S. Food and Drug Administration (FDA) between 2014 and 2018 among ~14 million transfused BCs. Most frequent causes of death were transfusion-associated circulatory overload (TACO) (32%), followed by TRALI (26%), hemolysis (18%), and sepsis (14%).

Adverse reactions to BCs may result in immune and nonimmune mechanisms. Immune-mediated reactions are often due to recipient or donor alloimmunization and the presence of preformed recipient or donor Abs. Nonimmune causes of reactions are from the physical or chemical properties of BCs or from pathogens present in the BC.

IMMUNE MEDIATED ADVERSE REACTIONS

Hemolytic Transfusion Adverse Reactions Immune-mediated acute hemolysis occurs when the recipient preformed Abs lyse transfused donor RBCs and may occur during or 24 h after transfusion. The anti-A or anti-B Abs are responsible for the majority of the most severe reactions, which can be fatal. However, alloAbs directed against other RBC Abs (i.e., Rh, Kell, and Duffy) are also responsible for severe hemolytic reactions. Such dramatic reactions are usually caused by a failure in product or patient identification, erroneous blood grouping, or unidentified anti-RBC alloimmunization in the recipient. Hemolysis, most often of lesser severity, may also occur upon transfusion of BCs containing incompatible plasma with a large amount of alloAbs directed against the recipient's RBCs. This may typically occur after

TABLE 113-4 Blood Components: Clinical Use

COMPONENT		THERAPEUTIC INDICATION	GOAL	DONOR/RECIPIENT COMPATIBILITY	DOSAGE	EFFICACY EVALUATION
Red blood cell concentrate (RBCC)	Transfusion	Anemia and/or tissue ischemia (treatment or prevention) Hb below a given threshold (to be considered in relation with clinical symptoms): <7 g/dL for patients hemodynamically stable, except for patients undergoing orthopedic surgery, cardiac surgery, or with preexisting cardiovascular disease (<8 g/dL) as well as for patients with acute coronary disease (<9–10 g/dL). Such thresholds do not apply to neonates and patients with severe thrombocytopenia and chronic transfusion-dependent anemia. Not recommended: nutritional anemia (iron, vitamin B ₁₂ , or folate deficiency)	Improve systemic and tissue oxygenation	ABO compatible (cellular) and ABO identical when achievable. RhD compatibility is required in young and childbearing females, and whenever possible if multitransfused RhC/c/E/e; Kell-compatible RBCCs are required in frequently transfused patients. Additional compatibility may be required depending on the clinical setting and screening results.	1 unit at a time (250–350 mL, including additive solution), repeated per clinical status and Hb level	Reduction of anemia-related symptoms, clinical improvement Increased Hb (+1 g/dL) and hematocrit (+3%)
	RBC exchange	Anemia/sickle cell crisis in hemoglobinopathies (sickle cell disease, thalassemia)	Replace altered RBCs with donor RBCs and compensate for hemolysis, prevention of sickle cell occlusive crisis		25–30 mL/kg	Sickle cell disease: reduced percentage of HbS
Platelet concentrates (PCs) (from pooled whole blood-derived platelets or single donor apheresis), maintained at room temperature (most often) or at 4°C		Thrombocytopenia-related bleeding disorders: treatment (cold or room temperature PC) or prevention (room temperature PC) Platelet level below a given threshold: ≤5000/µL in the absence of fever or infection, ≤10,000/µL to 20,000/µL if fever or infection; ≤50,000/µL if surgery, DIC, endoscopy, invasive procedures; ≤80,000/µL if neurosurgery or eye surgery Acute hypovolemic coagulopathy (see below) Not recommended: immune thrombocytopenia, thrombotic microangiopathy, heparin-induced thrombocytopenia	Correct impaired primary hemostasis, including vessel healing Cold stored platelets, despite lower <i>in vivo</i> survival, have maintained and possibly improved hemostatic capacity compared with room temperature stored platelets	ABO identical preferable; if not, ABO compatible (cellular) with low-titer anti-A/B Ab; RhD compatible preferred in premenopausal women HLA compatible (negative lymphocyte crossmatch) or HLA identical in case of refractoriness related to the presence of anti-HLA Ab HPA compatible in thrombocytopenic neonates to HPA immunized mother (fetal neonatal alloimmune thrombocytopenia)	0.5–0.7 × 10 ¹⁰ platelets/kg (apheresis or pooled whole blood-derived PCs)	Prevention and/or resolution of bleeding Corrected count increment ^a ≥10 × 10 ⁹ /L within 1 h and ≥7.5 × 10 ⁹ /L within 24 h after transfusion (not applicable to cold/cryopreserved platelets)
Plasma (thawed frozen, never frozen and maintained at 4°C or at room temperature, freeze-dried)	Transfusion	Coagulation factor-related bleeding disorders Acute hypovolemic coagulopathy (see below)	Correct impaired hemostasis by providing missing elements of coagulation or fibrinolysis cascade, as well as elements to heal injured vessel endothelium	ABO compatible (plasma)	10–15 mL/kg	Reduced bleeding disorder
	Plasma exchange (plasma or combined plasma and albumin)	Infectious disease treatment (convalescent plasma containing pathogen-specific Abs): Argentina hemorrhagic fever, viral respiratory infections (experimental) Pathogenic Ab removal and supplementation of lacking enzyme (e.g., thrombotic thrombocytopenic microangiopathy or Guillain-Barre syndrome) Pathogenic Ab removal (e.g., anti-HLA Ab prior to kidney transplantation)	Provide Abs against relevant pathogens Deplete pathogenic elements in the blood (auto-antibodies such as anti-ADAMTS-13 Ab in case of TTP, excess cholesterol, etc.); plasma may also bring anti-inflammatory and/or immunomodulatory factors such as immunoglobulin	ABO compatible (plasma)	Not determined 45–60 mL/kg	Infection resolution Improved disease-specific symptomatology (i.e., apyrexia and platelet recovery in case of TTP) Reduced antibody levels (e.g., anti-HLA antibodies prior to organ transplantation)

(Continued)

TABLE 113-4 Blood Components: Clinical Use (Continued)

COMPONENT	THERAPEUTIC INDICATION	GOAL	DONOR/RECIPIENT COMPATIBILITY	DOSAGE	EFFICACY EVALUATION
Whole blood	Acute hypovolemic coagulopathy requiring massive transfusion	Balanced provision of blood components maintained at 4°C and without an additive solution and related dilution	ABO-identical or group O with low-titer anti-A/B Ab	Repeated per clinical status	Normovolemia; bleeding resolution
Multicomponent (RBCC, PC, and plasma)	Acute hypovolemic coagulopathy requiring massive transfusion	Appropriate ratio is under investigation; a ratio of 1 RBCC/1 plasma/0.25 PC (platelet content of a whole blood) is currently favored	Standard RBCC, PC, and plasma compatibility	1 RBCC/1 plasma/0.25 PC ratio, repeated per clinical status	Normovolemia; bleeding resolution
Granulocyte concentrates (apheresis or a pool of whole blood-derived granulocytes)	Severe refractory bacterial or fungal infection in patients with neutropenia (<100/ μ L) or with dysfunctional granulocytes (CGD) (mainly soft tissues and lung). Neutropenia can be acquired (chemotherapy) or congenital. Usefulness of granulocyte transfusions is debated. Formal proof of efficacy is lacking.	Correct impaired granulocyte function in relation to granulocytopenia or granulocyte dysfunction	ABO compatible	1–2 $\times 10^{10}$, repeated per clinical status	Infection resolution (or stabilization until recovery from neutropenia)
Donor mononuclear cells	Relapse of malignant hemopathy after allogeneic hematopoietic cell transplantation	Graft-versus-leukemia effect (and graft enhancement effect)	N/A	10 ⁵ –10 ⁷ T lymphocytes/kg	Disease specific (remission)
Cryoprecipitate	Acute bleeding coagulopathy, type II (dysfunctional factor) or type III (absent factor) Von Willebrand disease, hemophilia A in the absence of factor VIII concentrates	Provision of fibrinogen, factor VIII, von Willebrand factor, and factor XIII	ABO compatibility is not required	10–15 mL/unit, pool of 4–5 units	Increased plasma fibrinogen (0.3–1 g/L)

^aCCI calculation:

$$\text{CCI} = \frac{\text{Postransfusion count } (\text{}/\mu\text{L}) - \text{pretransfusion count } (\text{}/\mu\text{L})}{\text{Number of platelets transfused} \times 10^{11}} \times \text{Body surface area } (\text{m}^2)$$

Abbreviations: Ab, antibody; CCI, corrected count increment; CGD, chronic granulomatous disease; DIC, disseminated intravascular coagulation; Hb, hemoglobin; HLA, human leukocyte antigen; N/A, not applicable; RBC, red blood cell; TTP, thrombotic thrombocytopenic purpura.

TABLE 113-5 Transfusion Adverse Reactions: Main Warning Signs

Fever ($\geq 38^{\circ}\text{C}$)	+1–2°C within 4 h +1–2°C within 15 min +/-: • Chills • Dyspnea • Hypotension • Digestive disorders • Disseminated intravascular coagulation • Hemoglobinuria $>2^{\circ}\text{C}$ or $\geq 39^{\circ}\text{C}$	FNHTR Anti-HLA immunization and cognate Ag in the blood product TRALI (with dyspnea at the forefront) Transfusion-transmitted bacterial infection Hemolysis
Hypotension (≥ 30 mmHg decrease in systolic blood pressure)		Hemolytic shock Anaphylactic shock Septic shock TRALI (with dyspnea at the forefront)
Dyspnea		TRALI (within 6 h of transfusion) TACO (within 6 h of transfusion) Severe allergy (immediate; within 4 h)
Hemoglobinuria		Intravascular hemolysis • Immunologic • Mechanical • Toxic • Thermic
Rash	<2/3 of the body within 2–3 h >2/3 of the body during or within 2–3 h >2/3 of the body within 5 min Associated with dyspnea and shock	Minor allergy Severe allergy Anaphylaxis
Icterus		Delayed hemolysis
New alloantibody		Alloimmunization
Rash, diarrhea, and fever occurring 2 days to 6 weeks after transfusion		GVHD
Gum bleeding, purpura 5–12 days after transfusion		Posttransfusion purpura
Cardiac, hepatic, and/or renal insufficiency in frequently transfused patients		Posttransfusion iron overload
Top-down investigation after a blood donor is subsequently found to be infected Bottom-up investigation after another recipient of a same blood donation is found to be infected Infectious symptoms within 6 months		Transfusion-transmitted infection

Abbreviations: Ag, antigen; FNHTR, febrile nonhemolytic transfusion reaction; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; TACO, transfusion-associated circulatory overload; TRALI, transfusion-related acute lung injury.

transfusion of a PC containing ABO-incompatible plasma. Estimated frequencies of acute and chronic hemolytic adverse reactions are 1–10 and 5–40 per 10^5 transfused BCs, respectively.

Mechanisms of transfusion hemolytic reactions are described in **Figure 113-1**.

Prevention of hemolytic reactions relies on pretransfusion testing of potential recipients. Testing will include determination of the ABO RhD phenotype (and anti-ABO Abs) as well as additional typing for the other main Rh Ags (CcEe): K Ag of the Kell system and, more rarely, Duffy, Kidd and Ss Ags, depending on the clinical setting. These determinations are most often performed by serology. However, molecular typing is increasingly being used to predict RBC phenotype and facilitate the selection of a compatible component. Special care must be taken to verify the patient's identity and apply adequate tube labeling. A double ABO determination performed separately may be considered, especially in the absence of a systematic crossmatch.

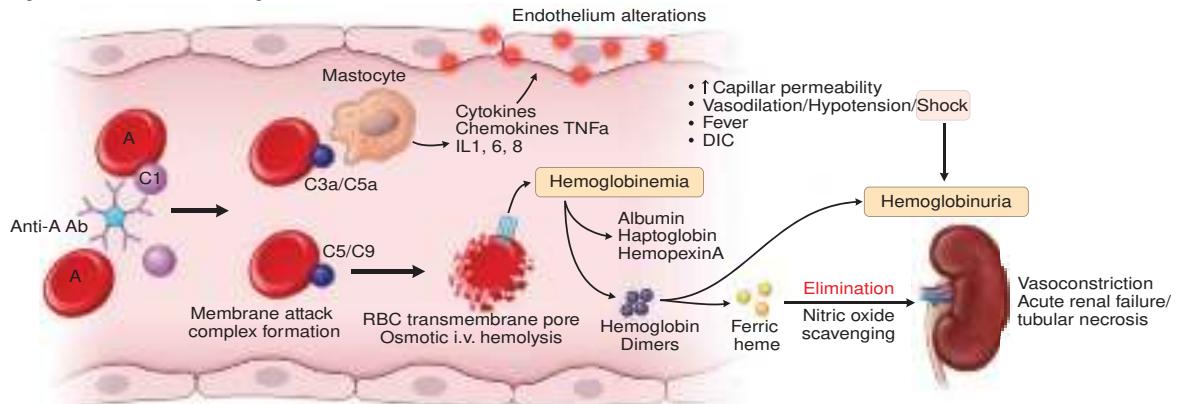
Testing will also include the screening and identification of alloAbs directed against RBC Ags other than ABO. This screen is performed by mixing patient serum with type O RBCs expressing Ags from most blood group systems and whose extended phenotype is known. The specificity of the alloAb is identified by correlating the presence or absence of Ag with the induced—or not—agglutination. Special attention should be paid to patients receiving monoclonal Ab treatment that

may bind to erythrocytes in vivo (such as anti-CD38 IgG treatment for multiple myeloma) and therefore interfere with alloAb screening. Such interference may be offset by sample dithiothreitol pretreatment.

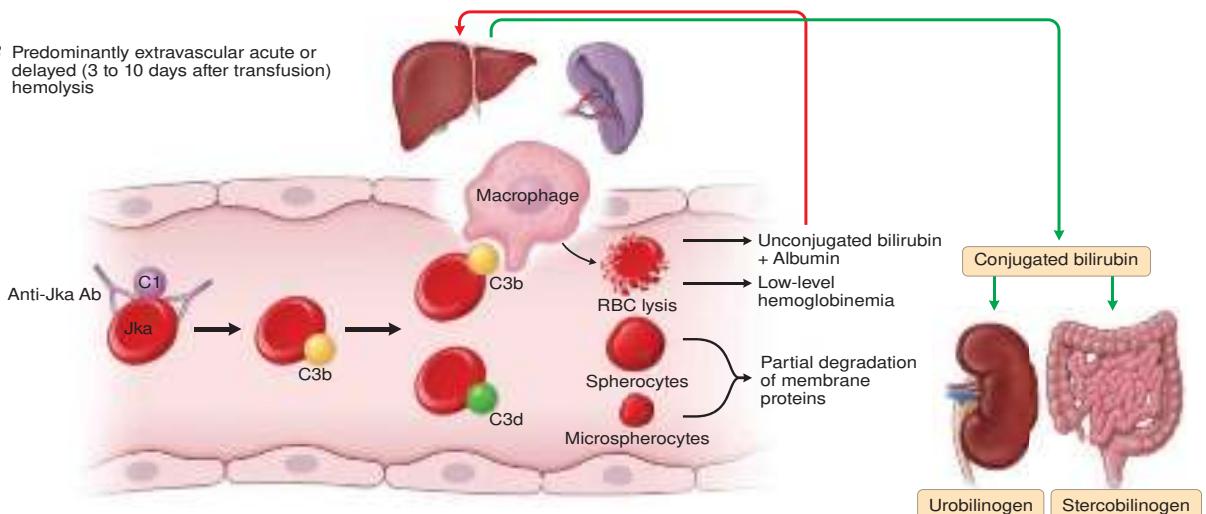
Crossmatching between the recipient plasma/serum and the sample of selected RBCs may be performed, especially when the recipient is alloimmunized against RBC or is frequently transfused, as well as in specific clinical settings such as sickle cell disease, even if the Ab screening is negative.

The selection of a compatible BC should take into account pretransfusion testing as well as the recipient's clinical status. In the case of D (Rh1)-negative patients, every attempt must be made to provide Rh-negative BC to prevent anti-D alloimmunization. In an emergency situation, D-positive RBCC can be safely transfused to a D-negative patient who lacks anti-D. However, an estimated 20–22% of RBCC recipients will become alloimmunized and produce anti-D Abs after transfusion with D-positive RBCs (this frequency is higher in healthy individuals). Such alloimmunization can occur after PC transfusion, although at a much lower frequency (~1%). Whenever possible, females with childbearing potential (to include prepubertal girls) should be transfused with D- and K (KEL1)-compatible RBCCs and D-compatible PCs to prevent alloimmunization and protect a future fetus/newborn from an alloimmune-mediated hemolytic disease. D-negative females with childbearing potential who are transfused

A Predominantly intravascular acute hemolysis occurring during or within 24 hours following transfusion



B Predominantly extravascular acute or delayed (3 to 10 days after transfusion) hemolysis



C Predominantly extravascular acute or delayed (3 to 10 days after transfusion) hemolysis

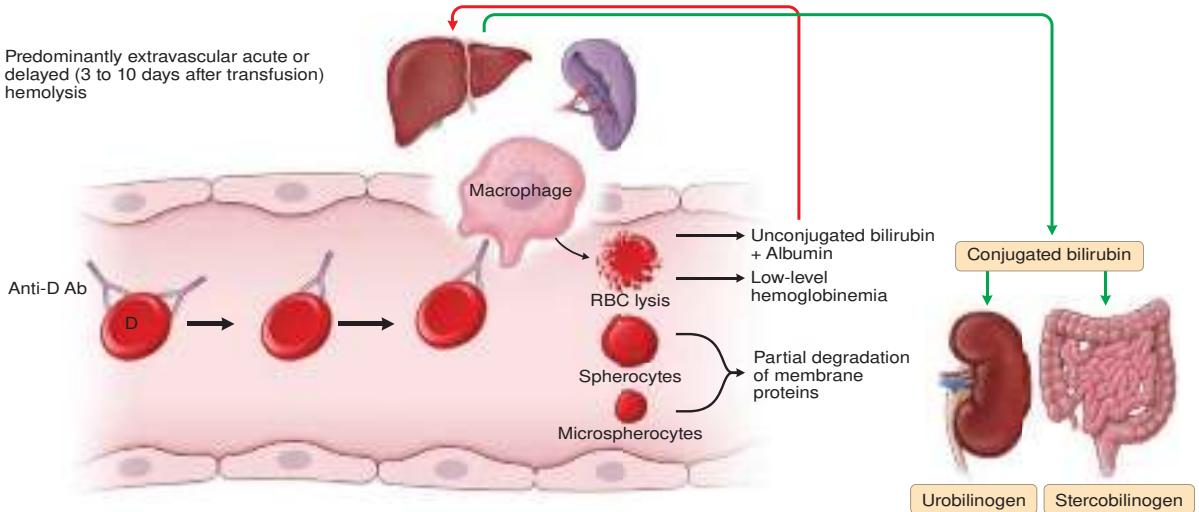


FIGURE 113-1 Mechanisms of transfusion hemolytic reactions. **A.** Acute responses will involve preexisting antibodies (Abs), naturally occurring anti-A/anti-B IgM or IgG directed against other RBC Ab and resulting from prior sensitization. Upon interaction with cognate antigen (Ag) on transfused red blood cells (RBCs), recipient allogeenic Ab (alloAb), mostly natural anti-A/anti-B IgM, may fix and activate complement up to C5/C9. Formation of membrane attack complex (MAC) will create pores in transfused RBCs with resulting intravascular hemolysis, release of toxic moieties including free hemoglobin responsible for end-organ damage including renal failure, and tissue factors contributing to occurrence of disseminated intravascular coagulation (DIC). **B.** Alternatively, complement activation may be incomplete, as typically observed in a delayed hemolytic transfusion reaction involving neofomed allogeenic IgG. In such cases, complement activation up to C3 results in C3b-mediated opsonization of RBCs, extravascular hemolysis, and clearance through immunophagocytosis. Anemia and jaundice will be the primary clinical manifestations. **C.** Lastly, alloAb may not fix complement while ensuring antibody-dependent cellular cytotoxicity (ADCC)-mediated phagocytosis of targeted RBC. (Adapted from SR Panch et al: Hemolytic transfusion reactions. *N Engl J Med* 381:150, 2019.)

with BCs containing Rh-positive RBCs should receive anti-D Ig to prevent alloimmunization.

Hemolysis, most often of lesser severity, may also occur after transfer of alloAbs directed against the recipient's RBC Ags. Such ABO "plasmatic" incompatibility, called "minor ABO incompatibility," will occur mainly with PC transfusions, where platelets are suspended in ~100–300 mL of plasma (depending on whether part of the plasma is substituted by additive solution). BCs containing plasma with high-titer anti-A/B Ab may induce a hemolytic reaction. When the transfusion of ABO-identical (vs ABO-compatible) PCs is feasible, PCs provided by donors with low-titer anti-A/B only should be preferred. "High-titer" PCs should be restricted to group O recipients. While there is no universal definition of high-titer Abs, a threshold titer of 1/64 (as assessed by hemagglutination) may be appropriate. It should be noted that the use of an additive solution in PCs substantially mitigates this risk. Lastly, ABO plasmatic incompatibility can lead to the formation of immune complexes with soluble A and/or B Ags and ensuing inflammation and platelet activation.

Acute hemolytic reactions may present with hypotension, tachypnea, tachycardia, fever (+1–2°C), chills, chest and back pain, hemoglobinuria, and hemoglobinemia. In the most severe cases, DIC, acute renal failure, shock, and death may occur.

Delayed hemolytic reactions, with icterus and persisting or worsening anemia as the main clinical manifestations, result from an anamnestic response. Such reactions may occur in patients previously

sensitized to RBC Ags who have a negative alloAb screen at the time of transfusion due to low Ab levels. The alloAb is detectable 1–2 weeks after the transfusion.

Diagnosis of transfusion-associated hemolysis relies on persistent and/or worsening anemia, depleted plasma haptoglobin levels, hemoglobinuria and hemoglobinuria, as well as elevated plasma lactate dehydrogenase and unconjugated bilirubin. The direct antiglobulin test (DAT, or direct Coombs test) that detects immunoglobulin, and possibly complement (C3d), on the surface of the recipient's RBC will most often be positive (Fig. 113-2). Similarly, a positive indirect antiglobulin test (IAT, or indirect Coombs test) that detects anti-RBC alloAb in the serum will also be positive. An elution of the Ab on the surface of the RBC may allow for the identification of the culprit alloAb.

The management of an immune-mediated acute hemolytic transfusion reaction is mainly supportive. Prompt interruption of the transfusion, biological workup, and a thorough clerical check to prevent a possible second misidentified transfusion are crucial initial steps. Vigorous hydration with isotonic saline and diuretics to maintain urine output is recommended. Although often self-limiting, acute hemolysis may also require forced alkaline diuresis, correction of electrolyte abnormalities, and pressor support as needed. In patients with DIC and severe bleeding, PC, plasma, and cryoprecipitate or fibrinogen may be required. When transfusion of incompatible RBCCs is unavoidable, prophylaxis with steroids (100 mg of hydrocortisone) just before the transfusion and repeated 24 h later and polyvalent immunoglobulin

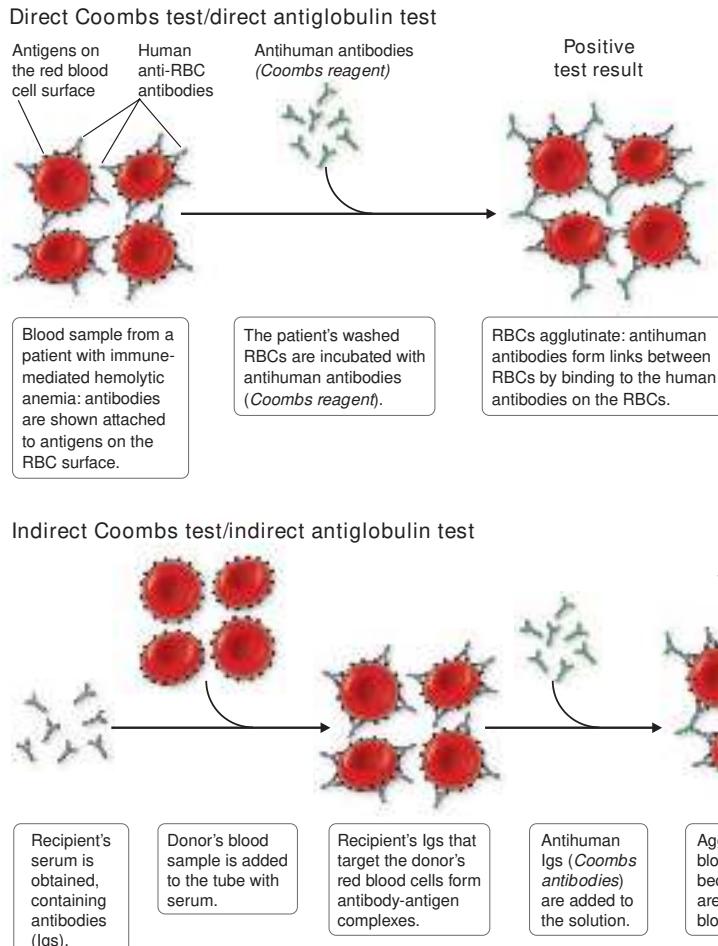


FIGURE 113-2 Direct and indirect Coombs test. The direct Coombs (antiglobulin) test detects the presence of antibodies (or complement) on the surface of erythrocytes. The indirect Coombs (antiglobulin) test detects antibodies in the serum that may bind to donor erythrocytes. Igs, immunoglobulins; RBC, red blood cell. (Adapted from http://upload.wikimedia.org/wikipedia/commons/1/1c/coombs_test_schematic.png.)

(1.2–2.0 g/kg per day over 2–3 days, initiated just before the transfusion) have been successfully used to prevent or minimize acute and delayed hemolysis.

Immune-mediated hemolysis may also occur after allogeneic hematopoietic transplantation (most often involving a peripheral blood stem cell graft) or, more seldomly, solid organ transplantation. Minor ABO incompatibility, with subsequent red cell destruction in the recipient, is the most common cause of clinically significant hemolysis in such cases. Viable donor B lymphocytes, called “passenger lymphocytes,” transferred passively with the graft, may produce alloAbs (including anti-D or anti-A1 in an A2 donor) that target recipient red cells. Such hemolysis has been reported to develop 5–14 days after transplantation. Reduced-intensity conditioning regimens and cyclosporine as prophylaxis against GVHD or rejection have been associated with increased risk. Transfusing RBCs compatible with the graft donor and the use of GVHD prophylaxis able to target B cells (e.g., methotrexate) have significantly reduced the incidence of passenger lymphocyte syndrome. Allogeneic hematopoietic transplantation may also result in acute hemolysis due to incompatible donor-derived red cell (and precursor) destruction by the recipient alloAbs (i.e., major ABO incompatibility). Prolonged pure red cell aplasia may occur in such a situation. Graft deserythrocytation will reduce the risk of early acute hemolysis.

Polyvalent immunoglobulin may contain high titers of anti-A (mostly) and/or anti-B Abs and induce acute hemolysis, most often of limited severity. Such hemolysis is particularly described in group A or AB children receiving high-dose immunoglobulin, notably for Kawasaki's disease, as well as in adults treated for thrombotic thrombocytopenic purpura. A similar mechanism may lead to hemolysis after anti-D immunoglobulin treatment for immune thrombocytopenia in RhD-positive patients.

Nonimmune mechanisms of transfusion-associated hemolysis include thermal (overheated or cold BCs), osmotic (concurrent hypo-osmotic perfusion), and mechanical (pressure related to high-flow transfusion filtering during cell saver processing) mechanisms.

Autoimmune and drug-induced hemolytic anemias may be exacerbated by transfusion and can therefore mimic hemolytic transfusion reactions. Transfusion of RBCs with enzymatic defects may mimic immune-mediated hemolysis as well. Notably, severe hemolytic reactions in patients receiving long-term transfusions for hemoglobinopathies (mainly sickle cell disease) can precipitate bystander hemolysis, in addition to clearing transfused red cells. The mechanisms of this hyperhemolytic transfusion reaction may be a mediated RBC hemolysis-related systemic inflammatory response and resulting lysis of red cell precursors by macrophages. This process may be immediate or delayed, with hemoglobin levels falling below the pretransfusion values, often to life-threatening levels. Further RBCC transfusion typically exacerbates ongoing hemolysis, with the exogenous (transfused) allogeneic Ags probably triggering further nonspecific hemolysis.

Febrile Nonhemolytic Transfusion Reaction The most frequent reaction associated with the transfusion of cellular BCs is FNHTR. This reaction is characterized by chills and rigors and a $\geq 1^{\circ}\text{C}$ rise in body temperature and is caused by proinflammatory cytokines in the BC or by recipient Abs directed against donor cell Ags present in the BC. FNHTR is diagnosed when other causes of fever, notably infection and hemolysis, have been excluded in the transfused patient. Leukocyte reduction, especially prestorage, can prevent the occurrence of FNHTR. Moreover, the use of additive solutions decreases FNHTR frequency associated with PC transfusion. Premedication with antipyretics has generally proven ineffective at decreasing the rate of such reactions and may mask relevant clinical symptoms.

Allergic Reactions Most allergic transfusion reactions are mild and include rash, pruritus, urticaria, and localized edema. More rarely, allergic reactions may be severe to life-threatening with an anaphylactic reaction that can involve bronchospasm, respiratory distress, hypotension, nausea, vomiting, and shock. Frequencies of mild and severe allergic reactions are ~100 and ~5 per 105 BCs, respectively.

Allergic reactions are related to plasma proteins found in transfused components. Mild reactions may be treated by temporarily stopping the transfusion and administering antihistamine drugs. Patients with a history of allergic transfusion reaction may be premedicated with an antihistamine, although there is no consensus on this issue. Cellular components can be washed to remove residual plasma for extremely sensitized patients. Most of the allergic presentation may not depend on preformed Abs and may be attributable to soluble mediators triggering histamine and serotonin release from platelets and leukocytes. An anaphylactic reaction may occur after the transfusion of only a few milliliters of the BC. Treatment includes stopping the transfusion, maintaining vascular access, and administering adrenaline (0.3–0.5 mg subcutaneously). Additional treatment with steroids, antihistamine drugs, and bronchodilators may also be required.

Patients who are IgA deficient (<1% of the population) may be sensitized to this immunoglobulin isotype and may be at risk of anaphylactic reactions associated with plasma transfusion. As a precaution, individuals with severe IgA deficiency should therefore receive, where available, IgA-deficient plasma and washed cellular BCs. Patients who have anaphylactic or repeated allergic reactions to BCs should be tested for IgA deficiency. It should be noted that the importance, or even the reality, of such a transfusion-related allergic risk is currently debated.

Graft-Versus-Host Disease GVHD is an extremely rare adverse reaction caused by transfusion, although it is a frequent complication of allogeneic hematopoietic transplantation. Transfusion-related GVHD is mediated by engrafted donor T lymphocytes in a recipient unable to reject such allogenic lymphocytes (as in severely immunosuppressed patients or patients homozygous for an HLA haplotype shared with the donor). Such donor T lymphocytes interact with host HLA Ags and mount an immune response, which is manifested clinically by the development, 5–10 days after transfusion, of cytopenia, fever, a characteristic skin rash, diarrhea, and liver function abnormalities. Transfusion-associated GVHD is highly resistant to treatment with immunosuppressive therapies as well as ablative therapy followed by allogeneic bone marrow transplantation and is fatal in >90% of cases. Prevention in at-risk patients relies on the irradiation of cellular BCs (minimum of 25 Gy) or treating BCs with pathogen reduction technology that will deplete all living cells in the component. At-risk patients include patients with inherited immune deficiency, patients undergoing autologous or allogeneic hematopoietic transplantation, patients treated with immunosuppressive drugs such as purine or pyrimidine analogues, anti-CD52 Ab or antithymocyte globulin, fetuses receiving intrauterine transfusions, and recipients of BCs provided by a blood relative. Because granulocyte concentrates contain a large number of lymphocytes, they should always be irradiated.

Transfusion-Related Acute Lung Injury TRALI is characterized by the occurrence or worsening of hypoxia and noncardiogenic pulmonary edema with bilateral interstitial infiltrates on chest x-ray during or within 6 h after transfusion, although delayed cases may occur up to 72 h later. Frequency of TRALI is BC dependent and ranges, on average, from 0.5 to 10 per 10^6 BCs. TRALI may be difficult to distinguish from other causes of hypoxia, such as circulatory overload, and is among the most common causes of transfusion-related fatalities. Treatment is supportive only. TRALI usually results from the transfusion of donor plasma that contains high-titer anti-HLA class II Abs that bind recipient cognate Ag. Anti-HLA class I and anti-human neutrophil antigen (HNA) Abs may also be involved. TRALI mediated by cytokines and chemokines in the absence of an HLA-mediated interaction may occur also. Leukocytes, especially when primed by either a bacterial moiety such as lipopolysaccharide or a cytokine/chemokine, aggregate in the pulmonary vasculature and release inflammatory mediators. The transfusion of plasma and PCs from male donors and nulliparous or parous female donors without anti-HLA Abs has significantly reduced the risk of TRALI where implemented. Recipient factors associated with an increased risk of TRALI include smoking, chronic alcohol use, shock, liver surgery (transplantation), cancer surgery, mechanical ventilation, and positive fluid balance.

Posttransfusion Purpura This rare reaction ($\sim 1/10^5$ BCs) is defined as a thrombocytopenia-related bleeding disorder developing 5–12 days after PC (and more rarely RBCC) transfusion, predominantly in women. Platelet-specific alloAbs are found in the recipient, most frequently anti-HPA-1a in HPA-1a-negative alloimmunized individuals. The delayed thrombocytopenia is due to a secondary increased production of alloAbs. The mechanisms for the destruction of the patient's own platelets remain unclear. Management is mostly supportive but may require polyvalent immunoglobulin, steroids, or plasma exchange. Additional platelet transfusions may worsen the thrombocytopenia or be associated with poor increments. Prevention of recurrence includes use of washed BCs or BCs from HPA-compatible donors.

Alloimmunization/Platelet Refractoriness A recipient may become alloimmunized to a number of Ags on cellular blood elements and plasma proteins. AlloAbs to RBC Ags are detected during pretransfusion testing, and their presence may delay finding Ag-negative crossmatch-compatible products for transfusion. Women of childbearing age who are sensitized to RBC Ags (i.e., D, c, E, Kell, or Duffy) are at risk of bearing a fetus with hemolytic disease of the fetus or newborn. Ag matching is the only pretransfusion selection test to prevent RBC alloimmunization, which is found to occur with a frequency of $\sim 100/10^5$ RBCC transfusions. Alloimmunization to Ags on leukocytes and platelets, most often anti-HLA Abs, can result in refractoriness to PC transfusions (as defined by a low increase in platelet count after transfusion). Once alloimmunization has developed, HLA-compatible (crossmatched) PCs should be preferred if available. If not, repeated PCs at shortened intervals may be considered. Use of leukocyte-reduced cellular BCs will reduce the incidence of immunization. Transfusion refractoriness may also result from an anti-HPA alloimmunization, although less commonly. Recipient factors associated with platelet refractoriness include fever, splenomegaly, bleeding, DIC, and medications such as amphotericin B. Notably, cold-stored (and cryopreserved) PCs have been found to have preserved hemostatic function in acutely bleeding patients despite poor platelet increments.

Immunomodulation Transfusion of allogeneic blood may be associated with immunosuppression, as evidenced early on by the beneficial effect of pretransplant transfusion on kidney graft survival. The intensity of such an effect is debated and, if present, is most probably attenuated by the use of leukoreduced BCs. Transfusion-related immunomodulation is indeed thought to be mainly mediated by donor leukocytes, whether transfused to the recipient or undergoing apoptosis during storage. However, leukoreduced RBCCs or PCs still release immunomodulatory mediators during storage. These mediators, along with the transfused RBCs or platelets, may exert various, possibly opposing, immune effects *in vivo*, including immunosuppression and inflammation.

■ NONIMMUNOLOGIC TRANSFUSION ADVERSE REACTIONS

Fluid Overload TACO is a common and underrecognized transfusion adverse reaction. Estimated frequencies vary from ~ 10 to 1000 per 10^5 BCs. TACO is now the main cause of death from transfusion since the TRALI risk has been mitigated. Risk factors include older age, renal failure, preexisting fluid overload, cardiac dysfunction, administration of a large volume of BCs, and an excessive rate of transfusion in relation to the patient's hemodynamic tolerance. TACO results in dyspnea, hypoxia, bilateral and predominantly alveolar infiltrates on chest x-ray, frequent systolic hypertension, and elevated brain natriuretic peptide. Fever may also exist. Prevention involves identifying at-risk patients, close monitoring, a slow transfusion rate (1 RBCC over 3–4 h), and use of diuretics in hemodynamically stable patients with a history of TACO. Treatment requires stopping the transfusion and administering oxygen and diuretics.

Massive Transfusion-Associated Reactions/Electrolyte and Cold Toxicity Reactions Reactions related to massive transfusion,

i.e., transfusion of 50% of the patient's total blood volume over 3 h or >5 –10 units of RBCCs (plus associated BCs), include citrate toxicity, hypothermia, hyperkalemia, and dilutional coagulopathy. Citrate, which is commonly used to anticoagulate BCs, chelates calcium. Hypocalcemia, manifested by circumoral paresthesia, and changes in cardiac function may result from multiple rapid transfusions. Although citrate is quickly metabolized to bicarbonate, calcium infusion (through a separate line) may be required. Rapid transfusion of BCs still at 4°C can result in hypothermia and cardiac dysrhythmias. Use of an inline warmer will prevent this complication. RBC leakage during storage, longer storage, and irradiation increase the concentration of potassium in the unit. Neonates and patients with renal failure or other comorbidities (e.g., hyperglycemia or hypocalcemia) are at risk of hyperkalemia and resulting acute cardiac toxicity. Treatment includes insulin, glucose, calcium gluconate, and furosemide, and prevention includes the use of washed or plasma-reduced RBCCs or a storage age of <7 –10 days and the avoidance of RBCCs stored for >24 h after irradiation.

Iron Overload Each unit of RBCs contains 200–250 mg of iron. In frequently transfused recipients, iron accumulation that is left untreated will affect endocrine, hepatic, and cardiac function. Death may occur from cardiac failure or arrhythmia. Iron overload can be assessed by means of serum ferritin measurements, magnetic resonance imaging, and liver biopsy. Prevention and treatment of this frequently underreported transfusion adverse event rely on careful monitoring and iron chelation.

Hypotensive Reactions Acute hypotensive transfusion reactions are defined as an abrupt drop in blood pressure of >30 mmHg early after the start of transfusion and resolving quickly once the transfusion is stopped, without further intervention. Respiratory, gastrointestinal, or mild allergic reactions may also be present. Estimated frequency is 1 – $10/10^5$ BCs. These reactions may result from the generation of vasoactive kinins in the BCs and are more likely to occur in hypertensive patients taking angiotensin-converting enzyme (ACE) inhibitors who are therefore less able to metabolize bradykinin. Upon resolution, the same blood product should not be restarted. Switching from an ACE inhibitor to an alternative drug should be considered for patients requiring further transfusions.

Adverse Transfusion Reactions of Uncertain Imputability Necrotizing enterocolitis, which is common in preterm and very-low-birth-weight neonates, has been infrequently described with close temporal association with RBC transfusion. However, the causality of any association remains to be further ascertained, as does the efficacy of withholding feeds during transfusion to prevent such a complication. Posterior reversible encephalopathy syndrome is a rare syndrome characterized by acute reversible neurologic symptoms related to subcortical vasogenic brain edema. It has been described within 10 days after RBCC transfusion, mainly in women with severe (and long-standing) anemia. The prognosis is most often favorable, although irreversible neurologic disturbance has been described. Prevention may include avoiding rapid correction of chronic severe anemia. Again, causality remains to be established.

■ INFECTIOUS ADVERSE REACTIONS

Donor screening involves the selection of healthy donors without high-risk lifestyles, medical conditions, or exposure to transmissible pathogens. Tests are performed on donated blood to detect the presence of infectious agents by testing for relevant Abs or by directly detecting infectious agents most often by nucleic acid amplification testing. The increasing sensitivity of testing methods has progressively narrowed the “window” period early on after infection during which a low-titer undetectable virus may be present in the blood and result in a transfusion-transmitted infection.

Transfusion-transmitted bacterial infection remains a significant concern, notably with PCs stored at room temperature, which allows for bacterial proliferation and results in an increased risk during storage. However, some gram-negative bacteria such as *Yersinia* can grow

TABLE 113-6 Infectious Transfusion Adverse Events

PATHOGEN		DONATION PREVALENCE (/10 ⁶ BLOOD DONATIONS)	PREVENTION MEASURES (IN ADDITION TO DONOR DEFERRAL)	INFECTION PREVALENCE IN RECIPIENTS (/10 ⁶ BLOOD PRODUCTS TRANSFUSED)
Bacteria	Pyogenic bacteria	PC: 10–20	Asepsis, diversion of the initial 10–30 mL of blood, bacterial detection, pathogen reduction (for PC)	Sepsis: PC: 5–30; with bacterial detection: 2–20; with pathogen reduction: <0.5 RBCC: <0.2
	<i>Treponema pallidum</i> (syphilis)	~1 ^a	Serology ^{b,c}	<0.1
Virus	HIV-1/2	~0.1	Serology, NAT (+/− p24 Ag) ^{b,c}	0.1–1 ^d
	HBV	~0.5	Serology, NAT ^{b,c}	<0.5 (3 without NAT) ^d
	HCV	0.2–1.2	Serology, NAT ^{b,c}	<0.1–1 ^d
	HTLV-1/2	0.05–0.1 ^a	Serology, BCdeleukocytation ^{b,c}	0.1–0.3 ^d
	HEV	0–10 (in endemic regions)	NAT	Endemic regions: <0.1 with NAT; a transmission rate from infected donors of ~50% has been reported
	CMV	Undetermined	Serology, BCdeleukocytation ^{b,c}	<0.1 in deleukocyted BCs
	Parvovirus B19	~0.5 with viral DNA >10 ⁶ IU/mL, ^e up to 100 overall	NAT	Most adults are immune to parvovirus B19; up to 0.12% in seronegative adults has been reported
	West Nile virus	Up to 3 in high season endemic regions ^a	NAT ^b	High season endemic regions: <1 with NAT
Parasite	<i>Plasmodium</i> (Malaria)	~4 (40–50 in donors from endemic regions) ^a	Serology (NAT may be soon available)	<0.1 in non endemic regions
	<i>Babesia</i>	~90 (in endemic regions) ^a	Serology (NAT implementation is underway)	ND (0.04% donors may be within the serology window period)
	<i>Trypanosoma cruzi</i> (Chagas disease)	~0.14 in donors/mothers from endemic regions ^a	Serology	ND

^aAs assessed based on seropositivity, i.e., including a varying percentage of individuals not harboring the pathogen in their blood. ^bPrevention measures may also include pathogen reduction (for PC and plasma). ^cPrevention measures may also include a quarantine of the (cryopreserved) BC pending a negative serology on a subsequent donation (for plasma). ^dEstimated residual risk. ^eTransfusion risk deemed as absent below this threshold.

Note. Other pathogens associated with transfusion-transmitted infections at a very low frequency include arboviruses other than West Nile (dengue, Zika virus), hepatitis A, human herpesvirus-8, Japanese encephalitis virus, tick-borne encephalitis virus complex, and the prion responsible for variant Creutzfeldt-Jakob disease (4 cases in the United Kingdom, in the context of the bovine spongiform encephalopathy epidemic, before implementation of systematic deleukocytation).

Abbreviations: Ag, antigen; BC, blood component; CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HTLV, human T-cell leukemia virus; NAT, nucleic acid detection test; ND, not determined; PC platelet concentrate; RBCC, red blood cell concentrate.

at 4°C and therefore may be implicated in infections related to RBCC transfusion. Recipients of contaminated BCs may develop abrupt (during transfusion and up to several hours after) fever and chills, which can deteriorate to septic shock, DIC, and death. Endotoxin formed within the BC may be implicated. After sampling for bacterial culture, broad-spectrum antibiotics should be promptly initiated.

Pathogen reduction of platelets and plasma, and perhaps soon of RBCs as well, offers an additional means of reducing transfusion infection risks. Although effective for a wide range of pathogens, such processes are most often ineffective for bacterial spores and nonenveloped viruses such as hepatitis A virus (HAV), parvovirus B19, and hepatitis E virus (HEV). Postdonation information provided by the donor (i.e., fever occurring within 24 h after donation) may allow the involved blood products to be quarantined and provide an additional safety measure.

Transfusion-transmitted infections are increasingly rare. However, new or previously unidentified infectious risks may occur, as highlighted by the emergence of the transfusion-associated West Nile virus infection and babesiosis in early 2000 in the United States, as well as transfusion-associated hepatitis E in early 2010 in Europe. Such occurrences require active surveillance programs and the appropriate implementation of mitigation measures such as additional testing, pathogen reduction, and travel-related deferral criteria. Along with West Nile virus, a number of other arbovirus-related infections possibly transmissible by blood transfusion are endemic or involved in large epidemic outbreaks. Despite being possibly present in the blood at asymptomatic phases of the disease, documented cases of transfusion-transmitted infections involving these arboviruses have been very rare (Zika), without a discernible clinical impact (Dengue), or absent (Chikungunya). Route of infection (i.e., intravenous vs mosquito bite), pathogen dose, ability to

survive in the BC, storage temperature and duration, recipient immune status, and ongoing treatments may all impact the ability of a pathogen in the donor to induce a disease in the recipient. Estimated frequencies of transfusion-relevant infections in donors and of transfusion-transmitted infections are reported in Table 113-6. Such frequencies depend heavily on variables such as local epidemiology, donor deferral rules, risk reduction measures, and data reporting, and may vary considerably.

ALTERNATIVES AND PERSPECTIVES

In addition to promoting appropriate transfusion indications, patient blood management programs have highlighted a number of transfusion-sparing strategies, such as the treatment of anemia and/or iron deficiency before surgery, minimization of blood loss, and optimization of patient red cell mass. Erythropoietin stimulates erythrocyte production in patients with anemia from chronic renal failure and other conditions, thus avoiding or reducing the need for transfusion. Thrombopoietin receptor agonists has been shown to reduce platelet transfusion needs resulting from chemotherapy-induced thrombopenia. Gene therapy approaches in patients with sickle cell or major thalassemia offer the potential of dramatically reducing their transfusion needs. Stem cell-derived blood cells such as RBCs or platelets may in the future become a suitable alternative to rare blood donors.

Importantly, issues surrounding transfusion safety have evolved significantly and now fully encompass transfusion efficacy. New means of assessing transfusion efficacy are needed. Large-scale biological and population-based databases pertaining to blood donors and transfused patients will also be instrumental in assessing and understanding the basis of transfusion efficacy. Optimal transfusion care may soon require consideration of new criteria in relation to donor, blood product, and/or recipient characteristics.

A
The authors are indebted to Jeffery S. Dzieczkowski and Kenneth C. Anderson, who co-authored the chapter in the previous edition and expertly paved the way for this chapter.

FURTHER READING

- C JL et al: Indications for and adverse effects of red-cell transfusion. *N Engl J Med* 377:1261, 2017.
- D M et al: Transfusion reactions: Prevention, diagnosis, and treatment. *Lancet* 388:2825, 2016.
- P SR et al: Hemolytic transfusion reactions. *N Engl J Med* 381:150, 2019.

can survive freezing and thawing with little, if any, damage, making it possible to remove and store a portion of the patient's own bone marrow for later reinfusion following treatment of the patient with high-dose myelotoxic therapy.

CATEGORIES OF HEMATOPOIETIC CELL TRANSPLANTATION

Hematopoietic cell transplantation can be described according to the relationship between the patient and the donor and by the anatomic source of stem cells. In ~1% of cases, patients have identical twins who can serve as donors. With the use of syngeneic donors, there is no risk of graft-versus-host disease (GVHD), and unlike the use of autologous marrow, there is no risk that the stem cells are contaminated with tumor cells.

Allogeneic transplantation involves a donor and a recipient who are not genetically identical. Following allogeneic transplantation, immune cells transplanted with the stem cells or developing from them can react against the patient, causing GVHD. Alternatively, if the immunosuppressive preparative regimen used to treat the patient before transplant is inadequate, immunocompetent cells of the patient can cause graft rejection. The risks of these complications are greatly influenced by the degree of matching between donor and recipient for human leukocyte antigen (HLA) molecules encoded by genes of the major histocompatibility complex.

HLA molecules are responsible for binding antigenic proteins and presenting them to T cells. The antigens presented by HLA molecules may derive from exogenous sources (e.g., during active infections) or may be endogenous proteins. If individuals are not HLA-matched, T cells from one individual will react strongly to the mismatched HLA, or "major antigens," of the second. Even if the individuals are HLA-matched, the T cells of the donor may react to differing endogenous or "minor antigens" presented by the HLA of the recipient. Reactions to minor antigens tend to be less vigorous. The genes of major relevance to transplantation include HLA-A, -B, -C, and -D; they are closely linked and therefore tend to be inherited as haplotypes, with only rare crossovers between them. Thus, the odds that any one full sibling will match a patient are one in four, and the probability that the patient has an HLA-identical sibling is $1 - (0.75)n$, where n equals the number of siblings.

With conventional techniques, the risk of graft rejection is 1–3%, and the risk of severe, life-threatening acute GVHD is ~15% following transplantation between HLA-identical siblings. The incidence of graft rejection and GVHD increases progressively with the use of family member donors mismatched for one, two, or three antigens. Although survival following a one-antigen mismatched transplant is not markedly altered, survival following two- or three-antigen mismatched transplants is reduced. Newer approaches to GVHD prophylaxis, including the use of posttransplant high-dose cyclophosphamide, make transplantation between donor/recipient pairs who share only one HLA haplotype possible. Since the formation of the National Marrow Donor Program and other registries, HLA-matched unrelated donors can be identified for many patients. The genes encoding HLA antigens are highly polymorphic, and thus the odds of any two unrelated individuals being HLA identical are extremely low, somewhat less than 1 in 10,000. However, by recruiting >30 million volunteer donors, HLA-matched donors can be found for ~60% of patients for whom a search is initiated, with higher rates among whites and lower rates among minorities and patients of mixed race. It takes, on average, 3–4 months to complete a search and schedule and initiate an unrelated donor transplant. With improvements in HLA typing and supportive care measures, survival following matched unrelated donor transplantation is essentially the same as that seen with HLA-matched siblings.

Allogeneic hematopoietic cell transplantation can be carried out across ABO blood barriers by removing isoagglutinins and/or incompatible red blood cells from the donor graft. However, depending on the direction of the mismatch, hemolysis of donor cells by persistent isoagglutinins in the host, or hemolysis of recipient red cells by isoagglutinins in the graft or developing from it may occur despite appropriate manipulation of the donor cell product.

Autologous transplantation involves the removal and storage of the patient's own stem cells with subsequent reinfusion after the patient

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Hematopoietic Cell Transplantation

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Bone marrow transplantation was the original term used to describe the collection and transplantation of hematopoietic stem cells, but with the demonstration that peripheral blood and umbilical cord blood are also useful sources of stem cells, *hematopoietic cell transplantation* has become the preferred generic term for this process. Hematopoietic cell transplantation is used to treat patients with an abnormal but nonmalignant lymphohematopoietic system by replacing it with one from a normal donor. Hematopoietic cell transplantation is also used to treat malignancy by allowing the administration of higher doses of myelosuppressive therapy than would otherwise be possible and, in the setting of allogeneic Hematopoietic cell transplantation, by conferring an immunologic graft-versus-tumor effect. The use of hematopoietic cell transplantation is increasing, as it becomes safer and applicable to more diseases and as donor availability expands.

The Center for International Blood and Marrow Transplant Research (<http://www.cibmtr.org>) estimates that worldwide about 100,000 transplants were performed in 2020. The frequency of transplantation varied widely from country to country, with a close association of transplant rates with gross national income (GNI) per capita. However, even among countries with similar GNIs per capita, there are substantial differences between countries and regions regarding the frequency of transplantation, disease indications, and choice of donor type.

THE HEMATOPOIETIC STEM CELL

Several features of the hematopoietic stem cell make transplantation clinically feasible, including its remarkable regenerative capacity, its ability to home to the marrow space following intravenous injection, and the ability of the stem cell to be cryopreserved (Chap. 96). Transplantation of a single stem cell can replace the entire lymphohematopoietic system of an adult mouse. In humans, transplantation of a small percentage of a donor's bone marrow volume regularly results in complete and sustained replacement of the recipient's entire lymphohematopoietic system, including all red cells, granulocytes, B and T lymphocytes, and platelets, as well as cells comprising the fixed macrophage population, including Kupffer cells of the liver, pulmonary alveolar macrophages, osteoclasts, and Langerhans cells of the skin. The ability of the hematopoietic stem cell to home to the marrow following intravenous injection is mediated, in part, by an interaction between CXCL12, also known as stromal cell-derived factor 1, produced by marrow stromal cells and the alpha-chemokine receptor CXCR4 found on stem cells. Homing is also influenced by the interaction of cell-surface molecules, termed *selectins*, including E- and L-selectin, on bone marrow endothelial cells with ligands, termed *integrins*, such as VLA-4, on early hematopoietic cells. Human hematopoietic stem cells

receives high-dose myeloablative therapy. Unlike allogeneic transplantation, there is no risk of GVHD or graft rejection with autologous transplantation. On the other hand, autologous transplantation lacks a graft-versus-tumor (GVT) effect, and the autologous stem cell product can be contaminated with tumor cells, which could lead to relapse. A variety of techniques have been developed to “purge” autologous products of tumor cells, but no prospective randomized trials have shown that any approach decreases relapse rates or improves disease-free or overall survival.

Bone marrow aspirated from the posterior and anterior iliac crests initially was the source of hematopoietic stem cells for transplantation. Typically, anywhere from 1.5 to 5×10^8 nucleated marrow cells per kilogram are collected for allogeneic transplantation. Several studies have found improved survival following both matched sibling and unrelated transplantation by transplanting higher numbers of bone marrow cells.

Hematopoietic stem cells circulate in the peripheral blood but in very low concentrations. Following the administration of a myeloid growth factor such as granulocyte colony-stimulating factor (G-CSF) and during recovery from intensive chemotherapy, the concentration of hematopoietic progenitor cells in blood, as measured either by colony-forming units or expression of the CD34 antigen, increases markedly. This makes it possible to harvest adequate numbers of stem cells from the peripheral blood for transplantation. Donors are typically treated with 4 or 5 days of hematopoietic growth factor, following which stem cells are collected in one or two 4-h pheresis sessions. In the autologous setting, transplantation of $>2.5 \times 10^6$ CD34 cells per kilogram, a number that can be collected in most circumstances, leads to rapid and sustained engraftment in virtually all cases. In the 5–10% of patients who fail to mobilize enough CD34+ cells with growth factor alone, the addition of plerixafor, an antagonist of CXCR4, may be useful. Blocking CXCR4 allows more stem cells to escape the marrow. When compared to the use of autologous marrow, use of peripheral blood stem cells results in more rapid hematopoietic recovery. Although this more rapid recovery diminishes the morbidity rate of transplantation, no studies show improved survival.

In the setting of allogeneic transplantation, the use of growth factor-mobilized peripheral blood stem cells also results in faster engraftment than seen with marrow but at the cost of more chronic GVHD because of donor T-cell contamination. With matched sibling donors, the increased chronic GVHD is more than balanced by reductions in relapse rates and nonrelapse mortality rates, resulting in improved overall survival. However, in the setting of matched unrelated donor transplantation, use of peripheral blood results in more chronic GVHD without a compensatory survival advantage, favoring the use of bone marrow.

Umbilical cord blood contains a high concentration of hematopoietic progenitor cells, allowing for its use as a source of stem cells for transplantation. Cord blood transplantation from family members has been used when the immediate need for transplantation precludes waiting the 9 or so months generally required for the baby to mature to the point of donating marrow. Use of cord blood results in slower peripheral count recovery than seen with marrow but a lower incidence of GVHD, perhaps reflecting the low number of T cells in cord blood. Multiple cord blood banks have been developed to harvest and store cord blood for possible transplantation to unrelated patients from material that would otherwise be discarded. Currently $>800,000$ units are cryopreserved and available for use. The advantages of unrelated cord blood are rapid availability and decreased immune reactivity allowing for the use of partially matched units, which is of particular importance for those without matched unrelated donors. The risks of graft failure and transplant-related mortality are related to the dose of cord blood cells per kilogram, which previously limited the application of single cord blood transplantation to pediatric and smaller adult patients. Subsequent trials have found that for patients without suitable single cord units, the use of double cord transplants diminishes the risk of graft failure and early mortality even though only one of the donors ultimately engrafts. Given the similar survival rates seen with cord blood, matched unrelated, and haploidentical family member donors, a source of allogeneic stem cells can now be found for almost every patient in need ([Table 114-1](#)).

TABLE 114-1 Probability of Identifying a Donor Based on Stem Cell Source and Patient Ethnicity

	UNRELATED ADULT %	UNRELATED CORD %	HAPLOIDENTICAL
Ethnicity	8/8 ^a	7/8 ^b	≥4/6 ^c
Caucasian	75	90	>95
Hispanic	35	75	95
Black	18	70	90

^aMatching for HLA-A, -B, -C, and DRB1. ^bMatching for HLA-A, -B, and DRB1.

THE TRANSPLANT PREPARATIVE REGIMEN

The treatment regimen administered to patients immediately preceding transplantation is designed to eradicate the patient's underlying disease and, in the setting of allogeneic transplantation, immunosuppress the patient adequately to prevent rejection of the transplanted stem cells. The appropriate regimen therefore depends on the disease setting and graft source. For example, when transplantation is performed to treat severe combined immunodeficiency and the donor is a histocompatible sibling, no treatment is needed because no host cells require eradication and the patient is already too immune-incompetent to reject the transplanted graft. For aplastic anemia, there is no large population of cells to eradicate, and high-dose cyclophosphamide plus antithymocyte globulin are sufficient to immunosuppress the patient adequately to accept the marrow graft. In the setting of thalassemia and sickle cell anemia, high-dose busulfan is frequently added to cyclophosphamide to eradicate hyperplastic host hematopoiesis. A variety of different regimens have been developed to treat malignant diseases. Most regimens include agents with high activity against the tumor in question at conventional doses and with myelosuppression as their predominant dose-limiting toxicity. Therefore, these regimens commonly include busulfan, cyclophosphamide, melphalan, thioguanine, carmustine, etoposide, and total-body irradiation in various combinations.

Although high-dose treatment regimens were the initial approach to transplantation for malignancies, the realization that much of the antitumor effect of transplantation derives from an immunologically mediated GVT response led investigators to ask if reduced-intensity conditioning regimens might be effective and more tolerable. Evidence for a GVT effect comes from studies showing that posttransplant relapse rates are lowest in patients who develop acute and chronic GVHD, higher in those without GVHD, and higher still in recipients of T cell-depleted allogeneic or syngeneic marrow. The demonstration that complete remissions can be obtained in many patients who have relapsed after transplant by simply administering viable lymphocytes from the original donor further strengthens the argument for a potent GVT effect. Accordingly, a variety of alternative regimens have been studied, ranging from nonmyeloablative, which are the very minimum required to achieve engraftment (e.g., fludarabine plus 200 cGy total-body irradiation) and would cause only transient myelosuppression if no transplant were performed, to so-called reduced-intensity regimens, which would cause significant but not necessarily fatal myelosuppression in the absence of transplantation (e.g., fludarabine plus melphalan). Studies to date document that engraftment can be readily achieved with less toxicity than seen with conventional transplantation. Complete sustained responses have been documented in many patients, particularly those with more indolent hematologic malignancies. In general, relapse rates are higher following reduced-intensity conditioning, but transplant-related mortality is lower, favoring the use of reduced-intensity conditioning in patients with significant comorbidities. High-dose regimens are favored in those felt able to tolerate the treatment, particularly if patients have any evidence of measurable disease at the time of transplantation.

THE TRANSPLANT PROCEDURE

Marrow is usually collected from the donor's posterior and sometimes anterior iliac crests, with the donor under general or spinal anesthesia. Typically, 10–15 mL/kg of marrow is aspirated, placed in heparinized media, and filtered through 0.3- and 0.2-mm screens to remove fat and bony spicules. The collected marrow may undergo further processing

depending on the clinical situation, such as the removal of red cells to prevent hemolysis in ABO-incompatible transplants, the removal of donor T cells to prevent GVHD, or attempts to remove possible contaminating tumor cells in autologous transplantation. Marrow donation is safe, with only very rare complications reported.

Peripheral blood stem cells are collected by leukapheresis after the donor has been treated with hematopoietic growth factors or, in the setting of autologous transplantation, sometimes after treatment with a combination of chemotherapy and growth factors. Stem cells for transplantation are infused through a large-bore central venous catheter. Such infusions are usually well tolerated, although occasionally patients develop fever, cough, or shortness of breath. These symptoms typically resolve with slowing of the infusion. When the stem cell product has been cryopreserved using dimethyl sulfoxide, patients sometimes experience short-lived nausea or vomiting due to the taste (and smell) of the cryoprotectant.

■ ENGRAFTMENT AND IMMUNE RECONSTITUTION

Peripheral blood counts reach their nadir several days to a week after transplant as a consequence of the preparative regimen; then cells produced by the transplanted stem cells begin to appear in the peripheral blood. The rate of recovery depends on the source of stem cells and use of posttransplant growth factors. If marrow is the source, recovery to 100 granulocytes/ μ L occurs on average by day 16 and to 500/ μ L by day 22. Use of G-CSF-mobilized peripheral blood stem cells speeds the rate of recovery by ~1 week compared to marrow, whereas engraftment following cord blood transplantation is typically delayed by ~1 week. Use of a myeloid growth factor after transplant accelerates recovery by 3–5 days. Platelet counts usually recover shortly after granulocytes.

While granulocytes and other components of innate immunity recover rapidly after hematopoietic cell transplantation, adaptive immunity, which consists of cellular (T cell) and humoral (B cell) immunity, may take 1–2 years to fully recover. Survival and peripheral expansion of infused donor T cells is the dominant mechanism for T cell recovery in the first months after hematopoietic cell transplantation and results in mostly CD8+ T cells with a limited repertoire. After several months, de novo generation of donor derived CD4+ and CD8+ T cells becomes dominant providing a more diverse T-cell repertoire. B-cell counts recover by 6 months after autologous hematopoietic cell transplantation and 9 months after allogeneic hematopoietic cell transplantation. In general, immune recovery occurs more rapidly after autologous than allogeneic hematopoietic cell transplantation and after receipt of unmodified grafts compared to the setting of in vivo or ex vivo T-cell depletion.

Following allogeneic transplantation, engraftment can be documented using fluorescence in situ hybridization of sex chromosomes if donor and recipient are sex-mismatched or by analysis of short tandem repeat polymorphisms after DNA amplification.

■ COMPLICATIONS FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION

Early Direct Chemoradiotoxicities The transplant preparative regimen may cause a spectrum of acute toxicities that vary according to intensity of the regimen and the specific agents used but frequently include nausea, vomiting, and mild skin erythema (Fig. 114-1). High-dose cyclophosphamide can result in hemorrhagic cystitis, which can usually be prevented by bladder irrigation or with the sulfhydryl compound mercaptoethanesulfonate (MESNA). Most high-dose preparative regimens will result in oral mucositis, which typically develops 5–7 days after transplant and often requires narcotic analgesia. Use of a patient-controlled analgesic pump provides the greatest patient satisfaction and results in a lower cumulative dose of narcotic. Keratinocyte growth factor (palifermin) can shorten the duration of mucositis by several days following autologous transplantation. Patients begin losing their hair 5–6 days after transplant and by 1 week are usually profoundly pancytopenic.

Depending on the intensity of the conditioning regimen, 3–10% of patients will develop sinusoidal obstruction syndrome (SOS) of the liver (formerly called venoocclusive disease), a syndrome that results from direct cytotoxic injury to hepatic-venular and sinusoidal endothelium, with subsequent deposition of fibrin and the development of

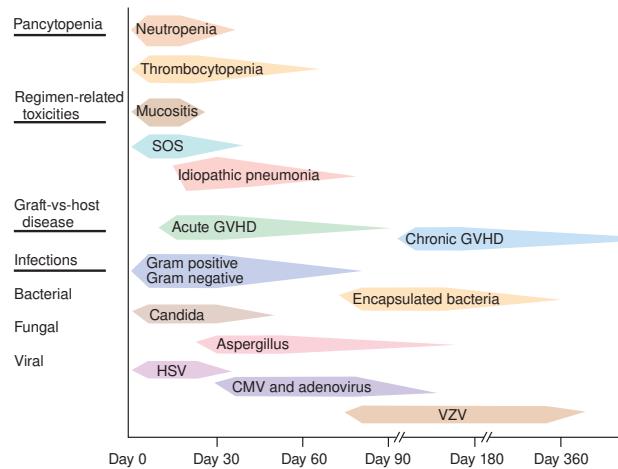


FIGURE 114-1 Major syndromes complicating marrow transplantation. CMV, cytomegalovirus; GVHD, graft-versus-host disease; HSV, herpes simplex virus; SOS, sinusoidal obstructive syndrome (formerly venoocclusive disease); VZV, varicella-zoster virus. The size of the shaded area roughly reflects the period of risk of the complication.

a local hypercoagulable state. This chain of events leads to the clinical symptoms of tender hepatomegaly, ascites, jaundice, and fluid retention. These symptoms can develop any time during the first month after transplant, with the peak incidence at day 16. Predisposing factors include prior exposure to intensive chemotherapy, pretransplant hepatitis of any cause, and use of more intense conditioning regimens. The mortality rate of sinusoidal obstruction syndrome is ~30%, with progressive hepatic failure culminating in a terminal hepatorenal syndrome. Treatment of severe SOS with defibrotide, a polydeoxyribonucleotide, reduces mortality.

Although most pneumonias developing early after transplant are caused by infectious agents, in a small percentage of patients, a diffuse interstitial pneumonia will develop that is a result of direct toxicity of high-dose preparative regimens. Bronchoalveolar lavage usually shows alveolar hemorrhage, and biopsies are typically characterized by diffuse alveolar damage, although some cases may have a more clearly interstitial pattern. High-dose glucocorticoids or antitumor necrosis factor therapies are sometimes used as treatment, although randomized trials proving their utility have not been reported.

Transplant-associated thrombotic microangiopathy is seen in 5–10% of patients, appearing on average about 1 month after transplant. The syndrome is characterized by presence of schistocytes on peripheral smear, elevated lactate dehydrogenase, thrombocytopenia, and acute kidney injury and is the result of endothelial injury and complement activation. Since calcineurin inhibitors are thought to contribute to the pathogenesis of the syndrome, changing immunosuppressive regimens is sometimes effective. Patients sometimes respond to eculizumab.

Late Direct Chemoradiotoxicities Two categories of chronic pulmonary disease occur in patients >3 months after hematopoietic cell transplantation. Cryptogenic organizing pneumonia is a restrictive lung disease characterized by dry cough, shortness of breath, and chest imaging showing a diffuse, fluffy infiltrate. Biopsy shows granulation tissue within alveolar spaces and small airways and no infectious agents. The disease responds well to corticosteroids and is entirely reversible. Bronchiolitis obliterans is an obstructive disease presenting with cough, progressive dyspnea, and radiologic evidence of air trapping. Pathology shows collagen and granulation tissue in and around bronchial structures and eventually obliteration of small airways. The disease is usually associated with chronic GVHD, and although it may respond to increasing immunosuppression, complete reversal is uncommon.

Other late complications of the preparative regimen include decreased growth velocity in children and delayed development of secondary sex characteristics. These complications can be partly ameliorated with the use of appropriate growth and sex hormone

replacement. Most men become azoospermic, and most postpubertal women will develop ovarian failure, which should be treated. However, pregnancy is possible after transplantation, and patients should be counseled accordingly. Thyroid dysfunction, usually well compensated, is sometimes seen. Cataracts develop in 10–20% of patients and are most common in patients treated with total-body irradiation and those who receive glucocorticoid therapy after transplant for treatment of GVHD. Aseptic necrosis of the femoral head is seen in 10% of patients and is particularly frequent following chronic glucocorticoid therapy. Both acute and late chemoradiotoxicities (except those due to glucocorticoids and other agents used to treat GVHD) are less frequent in recipients of reduced-intensity compared to high-dose preparative regimens.

Graft Failure Although complete and sustained engraftment is usually seen after transplant, occasionally marrow function either does not return or, after a brief period of engraftment, is lost. Graft failure after autologous transplantation can be the result of inadequate numbers of stem cells being transplanted, damage during ex vivo treatment or storage, or exposure of the patient to myelotoxic agents after transplant. Infections with cytomegalovirus (CMV) or human herpesvirus type 6 have also been associated with loss of marrow function. Graft failure after allogeneic transplantation can also be due to immunologic rejection of the graft by immunocompetent host cells. Such rejection is generally thought to be mostly T-cell mediated, but the presence pre-hematopoietic cell transplantation of donor-specific HLA antibodies in the patient is associated with poor engraftment, leading to the recommendation for screening for donor-directed anti-HLA antibodies in recipients prior to transplant. Immunologically based graft rejection is more common following use of less immunosuppressive preparative regimens, in recipients of T cell-depleted stem cell products, and in patients receiving grafts from HLA-mismatched donors or cord blood.

Treatment of graft failure involves removing all potentially myelotoxic agents from the patient's regimen and attempting a short trial of a myeloid growth factor. Persistence of lymphocytes of host origin in allogeneic transplant recipients with graft failure indicates immunologic rejection. Reinfusion of donor stem cells in such patients is usually unsuccessful unless preceded by a second immunosuppressive preparative regimen. Standard high-dose preparative regimens are tolerated poorly if administered within 100 days of a first transplant because of cumulative toxicities. However, reduced-intensity conditioning regimens have been effective in some cases.

Graft-Versus-Host Disease Acute GVHD occurs within the first 3 months after allogeneic transplant with a peak onset around 4 weeks and is characterized by an erythematous maculopapular rash; by persistent anorexia or diarrhea, or both; and by liver disease with increased serum levels of bilirubin, alanine and aspartate aminotransferase, and alkaline phosphatase. Because many conditions can mimic acute GVHD, the diagnosis usually requires skin, liver, or endoscopic biopsy for confirmation. In all these organs, endothelial damage and lymphocytic infiltrates are seen. In skin, the epidermis and hair follicles are damaged; in liver, the small bile ducts show segmental disruption; and in intestines, destruction of the crypts and mucosal ulceration may be noted. A commonly used rating system for acute GVHD is shown in Table 114-2. Grade I acute GVHD is of little clinical significance, does

not affect the likelihood of survival, and does not require treatment. In contrast, grades II to IV GVHD are associated with significant symptoms and a poorer probability of survival and require aggressive therapy. The incidence of acute GVHD is higher in recipients of stem cells from mismatched or unrelated donors, in older patients, and in patients unable to receive full doses of drugs used to prevent the disease.

Currently, the standard approach to GVHD prevention is the administration of a calcineurin inhibitor (cyclosporine or tacrolimus) combined with an antimetabolite (methotrexate or mycophenolate mofetil) following transplantation. The addition of anti-T-cell immune globulin (ATG) may further reduce the incidence of GVHD but has not been shown to improve survival. Other approaches being tested in phase 3 studies include the addition of sirolimus to the standard two-drug regimen, the removal of subsets or all T cells from the stem cell inoculum, and the use of cyclophosphamide administered several days after transplant in an effort to deplete activated alloreactive T cells.

Despite prophylaxis, significant acute GVHD will develop in ~30% of recipients of stem cells from matched siblings. Factors associated with a greater risk of acute GVHD include HLA-mismatching between recipient and donor, patient and donor age, use of more intense preparative regimens, and use of multiparous women as donors. Presumably, multiparous women have more alloreactivity based on carriage of genetically disparate fetuses. Disruption of the intestinal microbiota leading to loss of diversity and overgrowth by a single taxon is associated with a higher risk of GVHD and transplant-associated mortality. Biomarkers, including ST2, REG32, and TNF R1, have been identified that predict the severity of acute GVHD. The disease is usually treated with prednisone at a daily dose of 1–2 mg/kg. Patients in whom the acute GVHD fails to respond to prednisone sometimes respond to the oral JAK2 inhibitor ruxolitinib.

Chronic GVHD occurs most commonly between 3 months and 2 years after allogeneic transplant, developing in 20–50% of recipients. The disease is more common in older patients, with the use of peripheral blood rather than marrow as the stem cell source, in recipients of mismatched or unrelated stem cells, and in those with a preceding episode of acute GVHD. The disease resembles an autoimmune disorder with malar rash, sicca syndrome, arthritis, obliterative bronchiolitis, and bile duct degeneration with cholestasis. Mild chronic GVHD can sometimes be managed using local therapies (topical glucocorticoids to skin and cyclosporine eye drops). More severe disease requires systemic therapy usually with prednisone alone or in combination with cyclosporine. Ibrutinib is sometimes effective in patients whose disease does not respond to initial therapy. Mortality rates from chronic GVHD average around 15%, but range from 5 to 50% depending on severity. In most patients, chronic GVHD resolves, but it may require 1–3 years of immunosuppressive treatment before these agents can be withdrawn without the disease recurring. Because patients with chronic GVHD are susceptible to significant infection, they should receive prophylactic trimethoprim-sulfamethoxazole, and all suspected infections should be investigated and treated aggressively.

Although onset before or after 3 months after transplant is often used to discriminate between acute and chronic GVHD, occasional patients will develop signs and symptoms of acute GVHD after 3 months (late-onset acute GVHD), whereas others will exhibit signs

TABLE 114-2 Clinical Staging and Grading of Acute Graft-versus-Host Disease

CLINICAL STAGE	SKIN	LIVER—BILIRUBIN, μ mol/L (mg/dL)	GUT
1	Rash <25% body surface	34–51 (2–3)	Diarrhea 500–1000 mL/d
2	Rash 25–50% body surface	51–103 (3–6)	Diarrhea 1000–1500 mL/d
3	Generalized erythroderma	103–257 (6–15)	Diarrhea >1500 mL/d
4	Desquamation and bullae	>257 (>15)	Ileus
OVERALL CLINICAL GRADE	SKIN STAGE	LIVER STAGE	GUT STAGE
I	1–2	0	0
II	1–3	1	1
III	1–3	2–3	2–3
IV	2–4	2–4	2–4

and symptoms of both acute and chronic GVHD (overlap syndrome). There are as yet no data to suggest that these patients should be treated differently than those with classic acute or chronic GVHD.

From 3 to 5% of patients will develop an autoimmune disorder following allogeneic hematopoietic cell transplantation, most commonly autoimmune hemolytic anemia or idiopathic thrombocytopenic purpura. Unrelated donor source and chronic GVHD are risk factors, but autoimmune disorders have been reported in patients with no obvious GVHD. Treatment is with prednisone, cyclosporine, or rituximab.

Infection Posttransplant patients, particularly recipients of allogeneic transplantation, require unique approaches to the problem of infection. Early after transplantation, patients are profoundly neutropenic, and because the risk of bacterial infection is so great, most centers place patients on broad-spectrum antibiotics once the granulocyte count falls to <500/ μ L. Prophylaxis against fungal infections reduces rates of infection and improves overall survival. Fluconazole is often used for patients with standard risk, while prophylaxis with mold active agents (voriconazole or posaconazole) should be considered for patients at higher risk, such as those with a prior fungal infection. Patients seropositive for herpes simplex should receive acyclovir prophylaxis. One approach to infection prophylaxis is shown in Table 114-3. Despite these prophylactic measures, most patients will develop fever and signs of infection after transplant. The management of patients who become febrile despite bacterial and fungal prophylaxis is a difficult challenge and is guided by individual aspects of the patient and by the institution's experience.

The general problem of infection in the immunocompromised host is discussed in Chap. 143.

Once patients engraft, the incidence of bacterial infection diminishes; however, patients, particularly allogeneic transplant recipients, remain at significant risk of infection. During the period from engraftment until about 3 months after transplant, the most common causes of infection are gram-positive bacteria, fungi (particularly *Aspergillus*), and viruses including CMV. CMV disease, which in the past was frequently seen and often fatal, can be prevented in seronegative patients transplanted from seronegative donors by the use of either seronegative blood products or products from which the white blood cells have been removed. In seropositive patients or patients transplanted from seropositive donors, either prophylaxis or preemptive therapy is used. Letermovir administered over the first 3 months after transplant is effective as prophylaxis. An alternative approach is to monitor blood of patients after transplant using polymerase chain reaction assays for viral DNA and to treat reactivation preemptively with ganciclovir before clinical disease develops. Foscarnet is effective for some patients who develop CMV antigenemia or infection despite the use of ganciclovir or who cannot tolerate the drug, but it can be associated with severe electrolyte wasting.

Pneumocystis jirovecii pneumonia, once seen in 5–10% of patients, can be prevented by treating patients with oral trimethoprim-sulfamethoxazole for 1 week before transplant and resuming the treatment once patients engraft.

TABLE 114-3 Approach to Infection Prophylaxis in Allogeneic Transplant Recipients

ORGANISM	AGENT	APPROACH
Bacterial	Levofloxacin	750 mg PO or IV daily
Fungal	Fluconazole	400 mg PO qd to day 75 posttransplant
<i>Pneumocystis jirovecii</i>	Trimethoprim-sulfamethoxazole	1 double-strength tablet PO bid 2 days/week until day 180 or off immunosuppression
Viral		
Herpes simplex	Acyclovir	800 mg PO bid to day 30
Varicella-zoster	Acyclovir	800 mg PO bid to day 365
Cytomegalovirus	Ganciclovir	5 mg/kg IV bid for 7 days, then 5 (mg/kg)/d 5 days/week to day 100

Respiratory viruses that cause community-acquired infections, including respiratory syncytial virus (RSV), parainfluenza virus, influenza virus, and metapneumovirus, can be life threatening or fatal in the post-transplant patient. Protection of patients from infected visitors and staff by avoiding such contacts is critical. Neuraminidase inhibitors are effective for influenza infections. Inhaled ribavirin is sometimes used for RSV.

The risk of infection diminishes considerably beyond 3 months after transplant unless chronic GVHD requiring continuous immunosuppression develops. Most transplant centers recommend continuing trimethoprim-sulfamethoxazole prophylaxis while patients are receiving any immunosuppressive drugs and also recommend careful monitoring for late CMV reactivation. In addition, many centers recommend prophylaxis against varicella-zoster, using acyclovir for 1 year after transplant. Patients should be revaccinated against tetanus, diphtheria, *Haemophilus influenzae*, polio, and pneumococcal pneumonia starting at 12 months after transplant and against measles, mumps, and rubella (MMR), varicella-zoster virus, and possibly pertussis at 24 months.

TREATMENT

Nonmalignant Diseases

Evidence-based indications for hematopoietic cell transplantation have been published by several organizations and are guided not only by disease-related factors but also by patient comorbidities, socioeconomic issues, caregiver and donor availability, and patient preference.

IMMUNODEFICIENCY DISORDERS

By replacing abnormal stem cells with cells from a normal donor, hematopoietic cell transplantation can cure patients of a variety of immunodeficiency disorders including severe combined immunodeficiency, Wiskott-Aldrich syndrome, and Chédiak-Higashi syndrome. The widest experience is with severe combined immunodeficiency disease, where cure rates of 90% can be expected with HLA-identical donors and success rates of 50–70% have been reported using haplotype-mismatched parents as donors (Table 114-4).

APLASTIC ANEMIA

Transplantation from matched siblings after a preparative regimen of high-dose cyclophosphamide and antithymocyte globulin cures up to 90% of patients age <40 years with severe aplastic anemia. Results in older patients and in recipients of mismatched family member or unrelated marrow are less favorable; therefore, a trial of immunosuppressive therapy is generally recommended for such patients before considering transplantation. Transplantation is effective in all forms of aplastic anemia including, for example, the syndromes associated with paroxysmal nocturnal hemoglobinuria and Fanconi's anemia. Patients with Fanconi's anemia are abnormally sensitive to the toxic effects of alkylating agents, and so less intensive preparative regimens are used in their treatment (Chap. 102).

HEMOGLOBINOPATHIES

Marrow transplantation from an HLA-identical sibling following a preparative regimen of busulfan and cyclophosphamide can cure 80–90% of patients with thalassemia major. The best outcomes can be expected if patients are transplanted before they develop hepatomegaly or portal fibrosis and if they have been given adequate iron chelation therapy. Among such patients, the probabilities of 5-year survival and disease-free survival are 95 and 90%, respectively. Although prolonged survival can be achieved with aggressive chelation therapy, transplantation is the only curative treatment for thalassemia. Transplantation is potentially curative for patients with sickle cell anemia. Two-year survival and disease-free survival rates of 95 and 85%, respectively, have been reported following matched sibling or cord blood transplantation. Decisions about patient selection and the timing of transplantation remain difficult, but transplantation is a reasonable option for children and young

TABLE 114-4 Estimated 5-Year Survival Rates Following Transplantation^a

DISEASE	ALLOGENEIC, %	AUTOLOGOUS, %
Severe combined immunodeficiency	90	N/A
Aplastic anemia	90	N/A
Thalassemia	90	N/A
Acute myeloid leukemia		
First remission	55–60	50
Second remission	40	30
Acute lymphocytic leukemia		
First remission	50	40
Second remission	40	30
Chronic myeloid leukemia		
Chronic phase	70	ID
Accelerated phase	40	ID
Blast crisis	15	ID
Chronic lymphocytic leukemia	50	ID
Myelodysplasia	45	ID
Multiple myeloma—initial therapy	N/A	60
Non-Hodgkin's lymphoma		
First relapse/second remission	40	40
Hodgkin's disease		
First relapse/second remission	40	50

^aThese estimates are generally based on data reported by the International Bone Marrow Transplant Registry. The analysis has not been reviewed by their Advisory Committee.

Abbreviations: ID, insufficient data; N/A, not applicable.

adults who have suffered complications of sickle cell anemia including stroke, recurrent vasoocclusive pain, sickle cell lung disease, or sickle nephropathy (*Chap. 98*).

OTHER NONMALIGNANT DISEASES

Theoretically, hematopoietic cell transplantation should be able to cure any disease that results from an inborn error of the lymphohematopoietic system. Transplantation has been used successfully to treat congenital disorders of white blood cells such as Kostmann's syndrome, chronic granulomatous disease, and leukocyte adhesion deficiency. Congenital anemias such as Blackfan-Diamond anemia can also be cured with transplantation. Since the penetrance of some congenital marrow failure states is variable, potential family member donors should be carefully screened before use to assure they are not affected. Infantile malignant osteopetrosis is due to an inability of the osteoclast to resorb bone, and because osteoclasts derive from the marrow, transplantation can cure this rare inherited disorder.

Hematopoietic cell transplantation has been used as treatment for a number of storage diseases caused by enzymatic deficiencies, such as Gaucher's disease, Hurler's syndrome, Hunter's syndrome, and infantile metachromatic leukodystrophy. Transplantation for these diseases has not been uniformly successful, but treatment early in the course of these diseases, before irreversible damage to extramedullary organs has occurred, increases the chance for success.

Transplantation is being explored as a treatment for severe acquired autoimmune disorders. These trials are based on studies demonstrating that transplantation can reverse autoimmune disorders in animal models and on the observation that occasional patients with coexisting autoimmune disorders and hematologic malignancies have been cured of both with transplantation. A prospective randomized trial found that patients with severe scleroderma have improved event-free and overall survival if treated with hematopoietic cell transplantation.

ACUTE LEUKEMIA

Allogeneic hematopoietic cell transplantation cures 15–20% of patients who do not achieve complete response after induction chemotherapy for acute myeloid leukemia (AML) and is the only form of therapy that can cure such patients. Thus, all patients with AML who are possible transplant candidates should have their HLA type determined soon after diagnosis to enable hematopoietic cell transplantation for those who fail to enter remission. Cure rates of 30–35% are seen when patients are transplanted in second remission or in first relapse. The best results with allogeneic transplantation are achieved when applied during first remission, with disease-free survival rates averaging 55–60%. Meta-analyses of studies comparing matched related donor transplantation to chemotherapy for adult AML patients age <60 years show a survival advantage with transplantation. This advantage is greatest for those with unfavorable-risk AML and is lost in those with favorable-risk disease. While hematopoietic cell transplantation can be performed in patients up to age 75 and possibly beyond, prospective trials comparing hematopoietic cell transplantation with chemotherapy are lacking for older patients. The role of autologous transplantation in the treatment of AML is less well defined. The rates of disease recurrence with autologous transplantation are higher than those seen after allogeneic transplantation, and cure rates are somewhat less.

Similar to patients with AML, adults with acute lymphocytic leukemia who do not achieve a complete response to induction chemotherapy can be cured in 15–20% of cases with immediate transplantation. Cure rates improve to 30–50% in second remission, and therefore, transplantation can be recommended for adults who have persistent disease after induction chemotherapy or who subsequently relapse. Transplantation in first remission results in cure rates of about 55%. Transplantation appears to offer a survival advantage over chemotherapy for patients with high-risk disease as defined by molecular profiling. Debate continues about whether adults with standard-risk disease should be transplanted in first remission or whether transplantation should be reserved until relapse. Autologous transplantation is associated with a higher relapse rate but a somewhat lower risk of nonrelapse mortality when compared to allogeneic transplantation. There is no obvious role of autologous transplantation for acute lymphocytic leukemia in first remission, and for second-remission patients, most experts recommend use of allogeneic stem cells if an appropriate donor is available.

CHRONIC LEUKEMIA

Allogeneic hematopoietic cell transplantation is indicated for patients with chronic myeloid leukemia (CML) who are in chronic phase but have failed therapy with two or more tyrosine kinase inhibitors. In such patients, cure rates of 70% can be expected. Hematopoietic cell transplantation is also recommended for patients with CML who present or progress to accelerated phase or blast crisis, although lower cure rates are seen in such patients (*Chap. 105*).

Although allogeneic transplantation can cure patients with chronic lymphocytic leukemia (CLL), it has not been extensively studied because of the chronic nature of the disease, the age profile of patients, and more recently, the availability of multiple effective therapies. In those cases where it was studied, complete remissions were achieved in the majority of patients, with disease-free survival rates of ~50% at 3 years, despite the advanced stage of the disease at the time of transplant.

MYELODYSPLASIA AND MYELOPROLIFERATIVE DISORDERS

Between 20 and 65% of patients with myelodysplasia appear to be cured with allogeneic transplantation. Results are better among younger patients and those with less advanced disease. However, patients with early-stage myelodysplasia can live for extended periods without intervention, and so transplantation is generally reserved for patients with an International Prognostic Scoring System (IPSS) score of Int-2 or higher, or for selected patients with an IPSS score of Int-1 who have other poor prognostic features

(Chap. 102). Allogeneic hematopoietic cell transplantation can cure patients with primary myelofibrosis or myelofibrosis secondary to polycythemia vera or essential thrombocythemia, with 5-year progression-free survival rates in excess of 65% being reported. It may require many months for the fibrosis to resolve.

LYMPHOMA

Patients with disseminated intermediate- or high-grade non-Hodgkin's lymphoma who have not been cured by first-line chemotherapy and are transplanted in first relapse or second remission can still be cured in 40–50% of cases. This represents a clear advantage over results obtained with conventional-dose salvage chemotherapy. It is unsettled whether patients with high-risk disease benefit from transplantation in first remission. Most experts favor the use of autologous rather than allogeneic transplantation for patients with intermediate- or high-grade non-Hodgkin's lymphoma, because fewer complications occur with this approach and survival appears equivalent. Although autologous transplantation results in high response rates in patients with recurrent disseminated indolent non-Hodgkin's lymphoma, the availability of newer agents for this category of patient leaves the role of transplantation unsettled. Reduced-intensity conditioning regimens followed by allogeneic transplantation result in high rates of complete and enduring complete responses in patients with recurrent indolent lymphomas.

The role of transplantation in Hodgkin's disease is similar to that in intermediate- and high-grade non-Hodgkin's lymphoma. With transplantation, 5-year disease-free survival is 20–30% in patients who never achieve a first remission with standard chemotherapy and up to 70% for those transplanted in second remission. Transplantation has no defined role in first remission in Hodgkin's disease.

MYELOMA

Patients with myeloma whose disease progresses after first-line therapy can sometimes benefit from allogeneic or autologous transplantation. Prospective randomized studies demonstrate that the inclusion of autologous transplantation as part of initial therapy results in improved disease-free survival and overall survival. Further benefit is seen with the use of lenalidomide maintenance therapy following transplantation. The use of autologous transplantation followed by nonmyeloablative allogeneic transplantation has yielded mixed results.

SOLID TUMORS

Patients with testicular cancer in whom first-line platinum-containing chemotherapy has failed can still be cured in ~50% of cases if treated with high-dose chemotherapy with autologous stem cell support, an outcome better than that seen with low-dose salvage chemotherapy. The use of high-dose chemotherapy with autologous stem cell support is being studied for several other solid tumors, including neuroblastoma and pediatric sarcomas. As in most other settings, the best results were obtained in patients with limited amounts of disease and in whom the remaining tumor remains sensitive to conventional-dose chemotherapy. Few randomized trials of transplantation in these diseases have been completed.

POSTTRANSPLANT RELAPSE

Patients who relapse following autologous transplantation sometimes respond to further chemotherapy and may be candidates for possible allogeneic transplantation, particularly if the remission following the initial autologous transplant was long. Several options are available for patients who relapse following allogeneic transplantation. Treatment with infusions of unirradiated donor lymphocytes results in complete responses in as many as 75% of patients with chronic myeloid leukemia, 40% with myelodysplasia, 25% with AML, and 15% with myeloma. Major complications of donor lymphocyte infusions include transient myelosuppression and the development of GVHD. These complications depend on the number of donor lymphocytes given and the schedule of infusions, with less GVHD seen with lower dose, fractionated schedules.

FURTHER READING

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Section 3 Disorders of Hemostasis

115 Disorders of Platelets and Vessel Wall

Barbara A. Konkle



Hemostasis is a dynamic process in which the platelet and the blood vessel wall play key roles. Platelets are activated upon adhesion to von Willebrand factor (VWF) and collagen in the exposed subendothelium after injury. Platelet activation is also mediated through shear forces imposed by blood flow itself, particularly in areas where the vessel wall is diseased, and is also affected by the inflammatory state of the endothelium. The activated platelet surface provides the major physiologic site for coagulation factor activation, which results in further platelet activation and fibrin formation. Genetic and acquired influences on the platelet and vessel wall, as well as on the coagulation and fibrinolytic systems, determine whether normal hemostasis or bleeding or clotting symptoms will result.

THE PLATELET

Platelets are released from the megakaryocyte, likely under the influence of flow in the capillary sinuses. The normal blood platelet count is 150,000–450,000/ μ L. The major regulator of platelet production is the hormone thrombopoietin (TPO), which is synthesized in the liver and other organs. Synthesis is increased with inflammation and specifically by interleukin 6. TPO binds to its receptor on platelets and megakaryocytes, by which it is removed from the circulation. Thus, a reduction in platelet and megakaryocyte mass increases the level of TPO, which then stimulates platelet production. Platelets circulate with an average life span of 7–10 days. Approximately one-third of the platelets reside in the spleen, and this number increases in proportion to splenic size, although the platelet count rarely decreases to <40,000/ μ L as the spleen

enlarges. Platelets are physiologically very active, but are anucleate, and thus have limited capacity to synthesize new proteins.

Normal vascular endothelium contributes to preventing thrombosis by inhibiting platelet function (Chap. 65). When vascular endothelium is injured, these inhibitory effects are overcome, and platelets adhere to the exposed intimal surface primarily through VWF, a large multimeric protein present in both plasma and in the extracellular matrix of the subendothelial vessel wall. Platelet adhesion results in the generation of intracellular signals that lead to activation of the platelet glycoprotein (Gp) IIb/IIIa ($\alpha_{IIb}\beta_3$) receptor and resultant platelet aggregation.

Activated platelets undergo release of their granule contents, which include nucleotides, adhesive proteins, growth factors, and procoagulants that serve to promote platelet aggregation and blood clot formation and influence the environment of the forming clot. During platelet aggregation, additional platelets are recruited to the site of injury, leading to the formation of an occlusive platelet thrombus. The platelet plug is stabilized by the fibrin mesh that develops simultaneously as the product of the coagulation cascade.

THE VESSEL WALL

Endothelial cells line the surface of the entire circulatory tree, totaling $1\text{--}6 \times 10^{13}$ cells, enough to cover a surface area equivalent to about six tennis courts. The endothelium is physiologically active, controlling vascular permeability, flow of biologically active molecules and nutrients, blood cell interactions with the vessel wall, the inflammatory response, and angiogenesis.

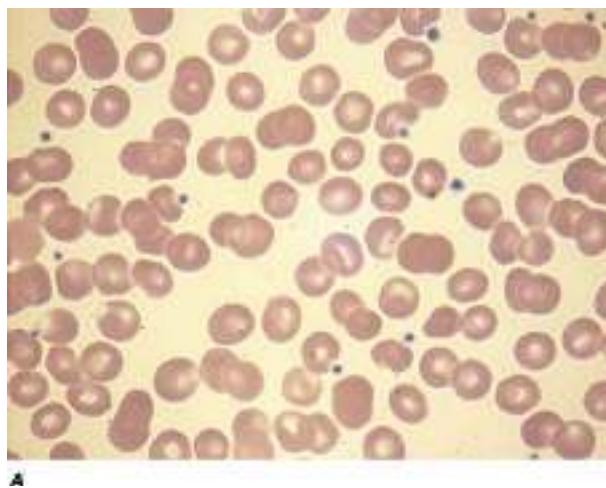
The endothelium normally presents an antithrombotic surface (Chap. 65) but rapidly becomes prothrombotic when stimulated,

which promotes coagulation, inhibits fibrinolysis, and activates platelets. In many cases, endothelium-derived vasodilators are also platelet inhibitors (e.g., nitric oxide), and conversely, endothelium-derived vasoconstrictors (e.g., endothelin) can also be platelet activators. The net effect of vasodilation and inhibition of platelet function is to promote blood fluidity, whereas the net effect of vasoconstriction and platelet activation is to promote thrombosis. Thus, blood fluidity and hemostasis are regulated by the balance of antithrombotic/prothrombotic and vasodilatory/vasoconstrictor properties of endothelial cells.

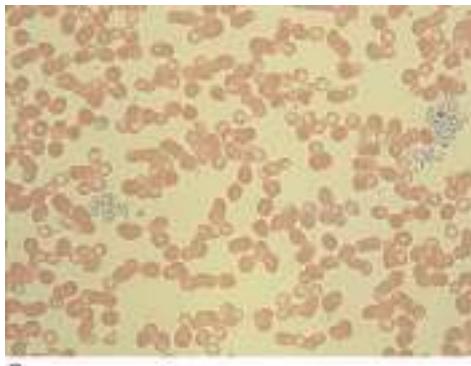
DISORDERS OF PLATELETS

THROMBOCYTOPENIA

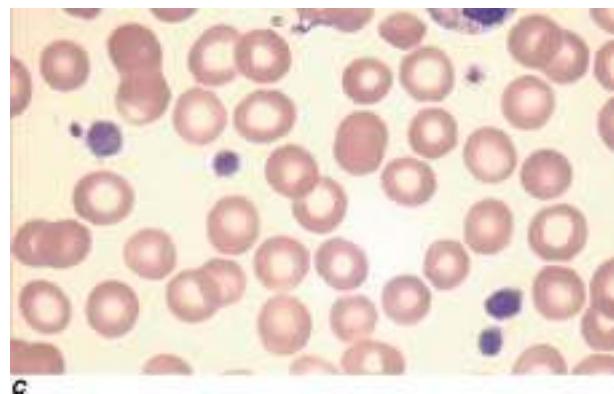
Thrombocytopenia results from one or more of three processes: (1) decreased bone marrow production; (2) sequestration, usually in an enlarged spleen; and/or (3) increased platelet destruction. Disorders of production may be either inherited or acquired. In evaluating a patient with thrombocytopenia, a key step is to review the peripheral blood smear and to first rule out "pseudothrombocytopenia," particularly in a patient without an apparent cause for the thrombocytopenia. Pseudothrombocytopenia (Fig. 115-1B) is an *in vitro* artifact resulting from platelet agglutination via antibodies (usually IgG, but also IgM and IgA) when the calcium content is decreased by blood collection in ethylenediamine tetraacetic (EDTA) (the anticoagulant present in tubes [purple top] used to collect blood for complete blood counts [CBCs]). If a low platelet count is obtained in EDTA-anticoagulated blood, a blood smear should be evaluated and a platelet count determined in blood collected into sodium citrate (blue top tube) or heparin (green



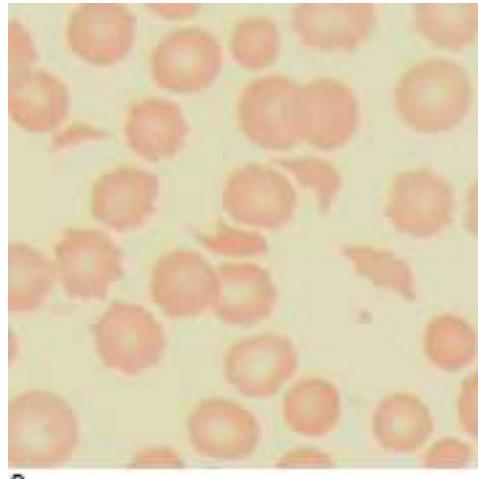
A



B



C



D

FIGURE 115-1 Photomicrographs of peripheral blood smears. A. Normal peripheral blood. B. Platelet clumping in pseudothrombocytopenia. C. Abnormal large platelet in autosomal dominant macrothrombocytopenia. D. Schistocytes and decreased platelets in microangiopathic hemolytic anemia.

top tube), or a smear of freshly obtained unanticoagulated blood, such as from a finger stick, can be examined.

APPROACH TO THE PATIENT

Thrombocytopenia

The history and physical examination, results of the CBC, and review of the peripheral blood smear are all critical components in the initial evaluation of thrombocytopenic patients (Fig. 115-2). The overall health of the patient and whether he or she is receiving drug treatment will influence the differential diagnosis. A healthy young adult with thrombocytopenia will have a much more limited differential diagnosis than an ill hospitalized patient who is receiving multiple medications. Except in unusual inherited disorders, decreased platelet production usually results from bone marrow disorders that also affect red blood cell (RBC) and/or white blood cell (WBC) production. Because myelodysplasia can present with isolated thrombocytopenia, the bone marrow should be examined in patients presenting with isolated thrombocytopenia who are older than 60 years of age or who do not respond to initial therapy. While inherited thrombocytopenia is rare, any prior platelet counts should be retrieved and a family history regarding thrombocytopenia obtained. A careful history of drug ingestion should be obtained, including nonprescription and herbal remedies, because drugs are the most common cause of thrombocytopenia.

The physical examination can document an enlarged spleen, evidence of chronic liver disease, and other underlying disorders. Mild to moderate splenomegaly may be difficult to appreciate in many individuals due to body habitus and/or obesity but can be easily assessed by abdominal ultrasound. A platelet count of approximately 5000–10,000 is required to maintain vascular integrity in the microcirculation. When the count is markedly decreased, petechiae first appear in areas of increased venous pressure, the ankles and feet in an ambulatory patient. Petechiae are pinpoint, nonblanching hemorrhages and are usually a sign of a decreased platelet number

and not platelet dysfunction. Wet purpura, blood blisters that form on the oral mucosa, are thought to denote an increased risk of life-threatening hemorrhage in the thrombocytopenic patient. Excessive bruising is seen in disorders of both platelet number and function.

Infection-Induced Thrombocytopenia Many viral and bacterial infections result in thrombocytopenia and are the most common noniatrogenic cause of thrombocytopenia. This may or may not be associated with laboratory evidence of disseminated intravascular coagulation (DIC), which is most commonly seen in patients with systemic infections with gram-negative bacteria and is seen in patients ill with COVID-19. Infections can affect both platelet production and platelet survival. In addition, immune mechanisms can be at work, as in infectious mononucleosis and early HIV infection. Late in HIV infection, pancytopenia and decreased and dysplastic platelet production are more common. Immune-mediated thrombocytopenia in children usually follows a viral infection and almost always resolves spontaneously. This association of infection with immune thrombocytopenic purpura is less clear in adults.

Drug-Induced Thrombocytopenia Many drugs have been associated with thrombocytopenia. A predictable decrease in platelet count occurs after treatment with many chemotherapeutic drugs due to bone marrow suppression (Chap. 73). Drugs that cause isolated thrombocytopenia and have been confirmed with positive laboratory testing are listed in Table 115-1, but all drugs should be suspect in a patient with thrombocytopenia without an apparent cause and should be stopped, or substituted, if possible. Although not as well studied, herbal and over-the-counter preparations may also result in thrombocytopenia and should be discontinued in patients who are thrombocytopenic.

Classic drug-dependent antibodies are antibodies that react with specific platelet surface antigens and result in thrombocytopenia only when the drug is present. Many drugs are capable of inducing these antibodies, but for some reason, they are more common with quinine and sulfonamides. Drug-dependent antibody binding can be demonstrated by laboratory assays, showing antibody binding in the presence of, but not without, the drug present in the assay. The thrombocytopenia typically occurs after a period of initial exposure (median length 21 days), or upon reexposure, and usually resolves in 7–10 days after drug withdrawal. The thrombocytopenia caused by the platelet Gp IIb/IIIa inhibitory drugs, such as abciximab, differs in that it may occur within 24 h of initial exposure. This appears to be due to the presence

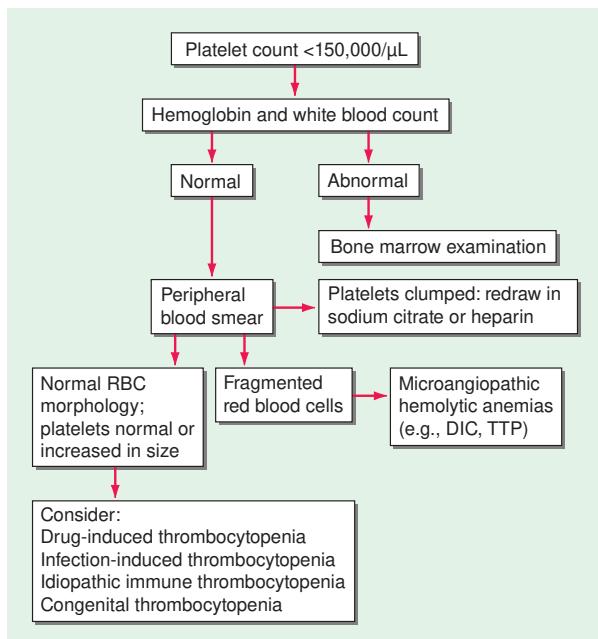


FIGURE 115-2 Algorithm for evaluating the thrombocytopenic patient. DIC, disseminated intravascular coagulation; RBC, red blood cell; TTP, thrombotic thrombocytopenic purpura.

TABLE 115-1 Drugs Reported as Definitely or Probably Causing Isolated Thrombocytopenia^a

Abciximab	Mirtazapine
Acetaminophen	Naproxen
Amiodarone	Oxaliplatin
Amlodipine	Penicillin
Ampicillin	Phenytoin
Carbamazepine	Piperacillin
Ceftriaxone	Quinidine
Cephalexin	Quinine
Ciprofloxacin	Ranitidine
Diazepam	Rosiglitazone
Eptifibatide	Roxifiban
Furosemide	Sulfisoxazole
Gold	Suramin
Haloperidol	Tirofiban
Heparin	Tranilast
Ibuprofen	Trimethoprim/sulfamethoxazole
Lorazepam	Vancomycin

^aBased on scoring requiring a compatible clinical picture and positive laboratory testing.

Source: Adapted from DM Arnold et al: J Thromb Hemost 11:169, 2013.

of naturally occurring antibodies that cross-react with the drug bound to the platelet.

Heparin-Induced Thrombocytopenia Drug-induced thrombocytopenia due to heparin differs from that seen with other drugs in two major ways. (1) The thrombocytopenia is not usually severe, with nadir counts rarely <20,000/ μ L. (2) Heparin-induced thrombocytopenia (HIT) is not associated with bleeding and, in fact, markedly increases the risk of thrombosis. The pathogenesis of HIT is complex. It results from antibody formation to a complex of the platelet-specific protein platelet factor 4 (PF4) and heparin or other glycosaminoglycans. The anti-heparin/PF4 antibody can activate platelets through the Fc γ RIIa receptor and also activate monocytes, endothelial cells, and coagulation proteins. Many patients exposed to heparin develop antibodies to heparin/PF4 but do not appear to have adverse consequences. A fraction of those who develop antibodies will develop HIT, and a portion of those (up to 50%) will develop thrombosis (HITT).

HIT can occur after exposure to low-molecular-weight heparin (LMWH) as well as unfractionated heparin (UFH), although it is more common with the latter. Most patients develop HIT after exposure to heparin for 5–14 days (Fig. 115-3). It occurs before 5 days in those who were exposed to heparin in the prior few weeks or months (<~100 days) and have circulating anti-heparin/PF4 antibodies. Rarely, thrombocytopenia and thrombosis begin several days after all heparin has been stopped (termed *delayed-onset HIT*), and more rarely, spontaneous HIT, or autoimmune HIT syndrome, occurs where there is no history of heparin exposure and termed *vaccine-induced immune thrombocytopenia and thrombosis* (VITT). A syndrome similar to spontaneous HIT has been described rarely post-COVID-19 vaccination mainly with the ChAdOx1-S/nCoV-19 vaccine. The “4T’s” have been recommended to be used in a diagnostic algorithm for HIT: thrombocytopenia, timing of platelet count drop, thrombosis and other sequelae such as localized skin reactions, and other causes of thrombocytopenia not evident. Application of the 4T scoring system is very useful in excluding a diagnosis of HIT but will result in overdiagnosis of HIT in situations where thrombocytopenia and thrombosis due to other etiologies are common, such as in the intensive care unit. Alternative scoring systems have been recommended, including for patients after cardiopulmonary bypass.

LABORATORY TESTING FOR HIT Because of the prevalence of antiheparin antibodies without clinical disease, testing should be done in individuals who are at intermediate or high risk based on clinical pretest assessment. HIT (anti-heparin/PF4) antibodies can be detected using two types of assays. The most widely available is an enzyme-linked immunoassay (ELISA) with PF4/polyanion complex as the antigen. Because many patients develop antibodies but do not develop clinical HIT, the test has a low specificity for the diagnosis of HIT. This is especially true in patients who have undergone surgery requiring cardiopulmonary bypass, where approximately 50% of patients develop these antibodies postoperatively. IgG-specific ELISAs increase specificity but may decrease sensitivity. The other assay is a platelet activation assay, most commonly the serotonin release assay, which measures the ability of the patient's serum to activate platelets in the presence of heparin in a concentration-dependent manner. This test has lower sensitivity

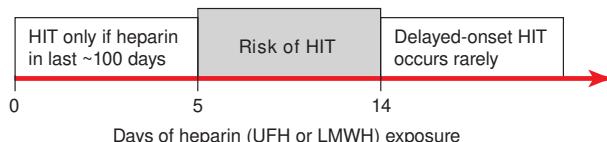


FIGURE 115-3 Time course of heparin-induced thrombocytopenia (HIT) development after heparin exposure. The timing of development after heparin exposure is a critical factor in determining the likelihood of HIT in a patient. HIT occurs early after heparin exposure in the presence of preexisting heparin/platelet factor 4 (PF4) antibodies, which disappear from circulation by ~100 days following a prior exposure. Rarely, HIT may occur later after heparin exposure (termed *delayed-onset HIT*). In this setting, heparin/PF4 antibody testing is usually markedly positive. HIT can occur after exposure to either unfractionated (UFH) or low-molecular-weight heparin (LMWH).

but higher specificity than the ELISA. However, HIT remains a clinical diagnosis.

TREATMENT

Heparin-Induced Thrombocytopenia

Early recognition is key in treatment of HIT, with prompt discontinuation of heparin and use of alternative anticoagulants if bleeding risk does not outweigh thrombotic risk. Thrombosis is a common complication of HIT, even after heparin discontinuation, and can occur in both the venous and arterial systems. In patients diagnosed with HIT, imaging studies to evaluate the patient for thrombosis (at least lower extremity duplex Doppler imaging) are recommended. Patients requiring anticoagulation should be switched from heparin to an alternative anticoagulant. The direct thrombin inhibitor (DTI) argatroban is effective in HITT. The DTI bivalirudin and the antithrombin-binding pentasaccharide fondaparinux are also effective but not approved by the U.S. Food and Drug Administration (FDA) for this indication. Direct oral anticoagulants (DOACs) are being used for treatment, although they are not FDA approved for this indication. Studies in small numbers of patients suggest their use in this setting may be a viable option. HIT antibodies cross-react with LMWH, and these drugs should not be used in the treatment of HIT.

Because of the high rate of thrombosis in patients with HIT, anticoagulation should be considered, even in the absence of thrombosis. In patients with thrombosis, anticoagulation is continued for 3–6 months, but in patients without thrombosis, the duration of anticoagulation is less well defined. An increased risk of thrombosis is present for at least 1 month after diagnosis; however, most thromboses occur early, and whether thrombosis occurs later if the patient is initially anticoagulated is unknown. Options include continuing anticoagulation until a few days after platelet recovery or for 1 month. Introduction of warfarin alone in the setting of HIT or HITT may precipitate thrombosis, particularly venous gangrene, presumably due to clotting activation and severely reduced levels of proteins C and S. Warfarin therapy, if started, should be overlapped with a DTI or fondaparinux and started after resolution of the thrombocytopenia and lessening of the prothrombotic state. Evidence for use of an oral direct Xa inhibitor in this setting is growing.

The rare VITT syndrome is characterized by high D-dimer levels and thrombosis in unusual sites like the cerebral venous sinuses. Fatal in about 20%, treatment is usually IgIV to block platelet activation through Fc receptors, the pathogenic effect of the anti-PF4-polyanion antibody.

Immune Thrombocytopenic Purpura Immune thrombocytopenic purpura (ITP; also termed *idiopathic thrombocytopenic purpura*) is an acquired disorder in which there is immune-mediated destruction of platelets and possibly inhibition of platelet release from the megakaryocyte. In children, it is usually an acute disease, most commonly following an infection, and with a self-limited course. In adults, it is a more chronic disease, although in some adults, spontaneous remission occurs, usually within months of diagnosis. ITP is termed *secondary* if it is associated with an underlying disorder; autoimmune disorders, particularly systemic lupus erythematosus (SLE), and infections, such as HIV and hepatitis C, are common causes. The association of ITP with *Helicobacter pylori* infection is unclear but appears to have a geographic distribution.

ITP is characterized by mucocutaneous bleeding and a low, often very low, platelet count, with an otherwise normal peripheral blood cells and smear. Patients usually present either with ecchymoses and petechiae or with thrombocytopenia incidentally found on a routine CBC. Mucocutaneous bleeding, such as oral mucosa, gastrointestinal, or heavy menstrual bleeding, may be present. Rarely, life-threatening, including central nervous system, bleeding can occur. Wet purpura (blood blisters in the mouth) and retinal hemorrhages may herald life-threatening bleeding.

LABORATORY TESTING IN ITP Laboratory testing for antibodies (serologic testing) is usually not helpful due to the low sensitivity and specificity of the current tests. Bone marrow examination can be reserved for those who have other signs or laboratory abnormalities not explained by ITP or in patients who do not respond to initial therapy. The peripheral blood smear may show large platelets, with otherwise normal morphology. Depending on the bleeding history, iron-deficiency anemia may be present.

Laboratory testing is performed to evaluate for secondary causes of ITP and should include testing for HIV infection and hepatitis C (and other infections if indicated). Serologic testing for SLE, serum protein electrophoresis, immunoglobulin levels to potentially detect hypogammaglobulinemia, selective testing for IgA deficiency or monoclonal gammopathies, and testing for *H. pylori* infection should be considered, depending on the clinical circumstance. If anemia is present, direct antiglobulin testing (Coombs' test) should be performed to rule out combined autoimmune hemolytic anemia with ITP (Evans' syndrome).

TREATMENT

Immune Thrombocytopenic Purpura

The treatment of ITP uses drugs that decrease reticuloendothelial uptake of the antibody-bound platelet, decrease antibody production, and/or increase platelet production. The diagnosis of ITP does not necessarily mean that treatment must be instituted. Patients with platelet counts >30,000/ μ L appear not to have increased mortality related to the thrombocytopenia.

Initial treatment in patients without significant bleeding symptoms, severe thrombocytopenia (<5000/ μ L), or signs of impending bleeding (e.g., retinal hemorrhage or large oral mucosal hemorrhages) can be instituted as an outpatient using single agents. Traditionally, this has been prednisone at 1 mg/kg or a 4-day course of dexamethasone, 40 mg/d, although Rh₀(D) immune globulin therapy (WinRho SDF), at 50–75 μ g/kg, is also being used in this setting. Rh₀(D) immune globulin must be used only in Rh-positive patients because the mechanism of action is production of limited hemolysis, with antibody-coated cells “saturating” the Fc receptors, inhibiting Fc receptor function. Monitoring patients for 8 h after infusion is now advised by the FDA because of the rare complication of severe intravascular hemolysis. Intravenous gamma globulin (IVIgG), which is pooled, primarily IgG antibodies, also blocks the Fc receptor system, but appears to work primarily through different mechanism(s). IVIgG has more efficacy than anti-Rh₀(D) in postsplenectomy patients. IVIgG is dosed at 1–2 g/kg total, given over 1–5 days. Side effects are usually related to the volume of infusion and infrequently include aseptic meningitis and renal failure. All immunoglobulin preparations are derived from human plasma and undergo treatment for viral inactivation.

For patients with severe ITP and/or symptoms of bleeding, hospital admission is required, and combined-modality therapy is given using high-dose glucocorticoids with IVIgG or anti-Rh₀(D) therapy and, as needed, additional immunosuppressive agents. Rituximab, an anti-CD20 (B cell) antibody, has shown efficacy in the treatment of refractory ITP, although long-lasting remission only occurs in approximately 30% of patients.

TPO receptor agonists, one administered subcutaneously (romiprilostim) and another orally (eltrombopag), are effective in raising platelet counts in patients with ITP and are recommended for patients who relapse or who are unresponsive to at least one other therapy.

Other immunosuppressive drugs have also been tested. The combination of glucocorticoids with mycophenolate mofetil (500 mg PO bid, increasing to 1000 mg PO bid as tolerated) appears to be more effective than glucocorticoids alone.

Splenectomy has been used for treatment of patients who relapse after glucocorticoids are tapered and remains a treatment option. However, with the recognition that ITP will resolve spontaneously in some adult patients, observation, if the platelet count is high enough, or intermittent treatment with anti-Rh₀(D) or IVIgG, or

initiation of treatment with a TPO receptor agonist may be a reasonable approach to see if the ITP will resolve, prior to splenectomy or other therapies. Vaccination against encapsulated organisms (especially pneumococcus, but also meningococcus and *Haemophilus influenzae*, depending on patient age and potential exposure) is recommended before splenectomy. Accessory spleens are a very rare cause of relapse.

Inherited Thrombocytopenia Thrombocytopenia is rarely inherited, either as an isolated finding or as part of a syndrome, and may be inherited in an autosomal dominant, autosomal recessive, or X-linked pattern. Many forms of autosomal dominant macrothrombocytopenia are now known to be associated with variants in the non-muscle myosin heavy chain *MYH9* gene. Interestingly, these include the May-Hegglin anomaly, and Sebastian, Epstein's, and Fechtner syndromes, all of which have distinct distinguishing features. A common feature of these disorders is large platelets (Fig. 115-1C). Autosomal recessive disorders include congenital amegakaryocytic thrombocytopenia, thrombocytopenia with absent radii, and Bernard-Soulier syndrome. The latter is primarily a functional platelet disorder due to absence of Gp Ib-IX-V, the VWF adhesion receptor. X-linked disorders include Wiskott-Aldrich syndrome and a dyshematopoietic syndrome resulting from a mutation in *GATA-1*, an important transcriptional regulator of hematopoiesis.

■ THROMBOTIC THROMBOCYTOPENIC PURPURA AND HEMOLYTIC UREMIC SYNDROME

Thrombotic thrombocytopenic microangiopathies are a group of disorders characterized by microangiopathic hemolytic anemia (MAHA) defined by thrombocytopenia and fragmented RBCs (Fig. 115-1D) on peripheral blood smear, laboratory evidence of hemolysis (elevated lactate dehydrogenase [LDH] and unconjugated bilirubin and decreased haptoglobin), and microvascular thrombosis. They include thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS), as well as syndromes complicating bone marrow transplantation, certain medications and infections, pregnancy, and vasculitis. In DIC, although thrombocytopenia and microangiopathy are seen, a coagulopathy predominates, with consumption of clotting factors and fibrinogen resulting in an elevated prothrombin time (PT) and often activated partial thromboplastin time (aPTT). The PT and aPTT are characteristically normal in TTP or HUS.

Thrombotic Thrombocytopenic Purpura TTP was first described in 1924 by Eli Moschcowitz and characterized by a pentad of findings that include microangiopathic hemolytic anemia, thrombocytopenia, renal failure, neurologic findings, and fever. The full-blown syndrome is less commonly seen now, probably due to earlier diagnosis. The introduction of treatment with plasma exchange markedly improved the prognosis in patients, with a decrease in mortality from 85–100% to 10–30%.

The pathogenesis of inherited (Upshaw-Schulman syndrome) and idiopathic TTP (ITTP) is related to a deficiency of, or antibodies to, the metalloprotease ADAMTS13, which cleaves VWF. VWF is normally secreted as ultra-large multimers, which are then cleaved by ADAMTS13. The persistence of ultra-large VWF molecules is thought to contribute to pathogenic platelet adhesion and aggregation (Fig. 115-4). This defect alone, however, is not sufficient to result in TTP because individuals with a congenital absence of ADAMTS13 develop TTP only episodically, including during first pregnancy. The level of ADAMTS13 activity, as well as antibodies to ADAMTS13, can be detected by laboratory assays, which play a critical role in the differential diagnosis of MAHA. ADAMTS13 activity levels of <10% are diagnostic of TTP.

Idiopathic TTP appears to be more common in women than in men. No geographic or racial distribution has been defined. TTP is more common in patients with HIV infection and in pregnant women. Medication-related MAHA may be secondary to antibody formation (ticlopidine and possibly clopidogrel) or direct endothelial toxicity (cyclosporine, mitomycin C, tacrolimus, quinine), although this is not always so clear, and fear of withholding treatment, as well as lack of

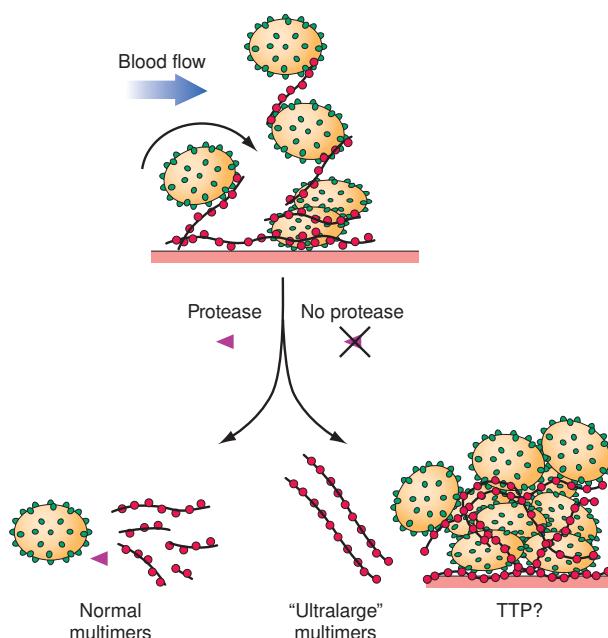


FIGURE 115-4 Pathogenesis of thrombotic thrombocytopenic purpura (TTP). Normally the ultra-high-molecular-weight multimers of von Willebrand factor (VWF) produced by the endothelial cells are processed into smaller multimers by a plasma metalloproteinase called ADAMTS13. In TTP, the activity of the protease is inhibited, and the ultra-high-molecular-weight multimers of VWF initiate platelet aggregation and thrombosis.

other treatment alternatives, may result in initial application of plasma exchange. However, withdrawal, or reduction in dose, of endothelial toxic agents usually decreases the microangiopathy.

TREATMENT

Thrombotic Thrombocytopenic Purpura

TTP is a devastating disease if not diagnosed and treated promptly. In patients presenting with new thrombocytopenia, with or without evidence of renal insufficiency and other elements of classic TTP, laboratory data (PT, aPTT, CBC with platelet count and peripheral smear, ADAMTS13 activity, LDH, bilirubin, haptoglobin, direct antiglobulin assay) should be obtained to rule out DIC and to evaluate for evidence of MAHA.

Therapeutic plasma exchange (TPE) remains the mainstay of treatment of TTP. TPE is continued until the platelet count is normal and signs of hemolysis are resolved for at least 2 days. Although never evaluated in clinical trials, the use of glucocorticoids seems a reasonable approach but should only be used as an adjunct to plasma exchange. The addition of rituximab to initial therapy decreases duration of TPE and relapses. Caplacizumab, an anti-VWF nanobody, decreases mortality and burden of care when used in patients with ADAMTS13 <10% or with high clinical probability of disease. Guidelines from the International Society of Thrombosis and Hemostasis recommend starting caplacizumab and rituximab only in individuals with diagnostic ADAMTS13 levels (usually <10%) and, additionally for rituximab, in patients with evidence of an inhibitor, given potential side effects and costs.

Patients with persistently low ADAMTS13 have a greater risk of ongoing sequelae including stroke. There is a significant relapse rate; in patients treated with TPE, 25–45% of patients relapse within 30 days of initial ‘remission,’ and 12–40% of patients have late relapses. Relapses are more frequent in patients with severe ADAMTS13 deficiency at presentation. Treatment of patients with

TTP relapses should be initiated before confirmatory laboratory assays are available.

Hemolytic-Uremic Syndrome HUS is a syndrome characterized by acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia. It is seen preceded by an episode of diarrhea, often hemorrhagic in nature, predominantly in children. *Escherichia coli* O157:H7 is the most frequent, although not only, etiologic serotype. HUS not associated with diarrhea is more heterogeneous in presentation and course. Atypical HUS (aHUS) is usually due to genetic defects in complement genes or antibodies directed against complementary regulatory proteins that result in chronic complement activation. Laboratory testing for DNA variants in complement regulatory genes is available, although assigning pathogenicity to variants remains challenging. Currently, a commercially available functional assay is not available that is diagnostic of the disease.

TREATMENT

Hemolytic-Uremic Syndrome

Treatment of HUS is primarily supportive. In HUS associated with diarrhea, many (~40%) children require at least some period of support with dialysis; however, the overall mortality is <5%. In HUS not associated with diarrhea, the mortality is higher, approximately 26%. Plasma infusion or plasma exchange has not been shown to alter the overall course in HUS or aHUS, except in patients with antibodies to factor H. ADAMTS13 levels are generally reported to be normal in HUS, although occasionally they have been reported to be decreased. In patients with aHUS, eculizumab, a humanized monoclonal antibody against C5 that blocks terminal complement, has efficacy in resolution of aHUS and improving or preserving renal function. Patients with aHUS may initially be treated with plasma exchange, until the ADAMTS13 level is returned and the diagnosis is more clear, since aHUS remains a diagnosis of exclusion. However, plasma exchange has not been shown to affect clinical outcomes in aHUS.

THROMBOCYTOSIS

Thrombocytosis is almost always due to (1) iron deficiency; (2) inflammation, cancer, or infection (reactive thrombocytosis); or (3) an underlying myeloproliferative process (essential thrombocythemia or polycythemia vera) (Chap. 103) or, rarely, the 5q- myelodysplastic process (Chap. 102). Patients presenting with an elevated platelet count should be evaluated for underlying inflammation and malignancy, and iron deficiency should be ruled out. Thrombocytosis in response to acute or chronic inflammation has not been clearly associated with an increased thrombotic risk. In fact, patients with markedly elevated platelet counts (>1.5 million), usually seen in the setting of a myeloproliferative disorder, have an increased risk of bleeding. This appears to be due, at least in part, to acquired von Willebrand disease (VWD) due to platelet-VWF binding and removal from the circulation.

QUALITATIVE DISORDERS OF PLATELET FUNCTION

Inherited Disorders of Platelet Function Inherited platelet function disorders are thought to be relatively rare, although the prevalence of mild disorders of platelet function is unclear, in part because our testing for such disorders is suboptimal. Rare qualitative disorders include the autosomal recessive disorders Glanzmann's thrombasthenia (absence of the platelet Gp IIb/IIIa receptor) and Bernard-Soulier syndrome (absence of the platelet Gp Ib-IX-V receptor). Both are inherited in an autosomal recessive fashion and present with bleeding symptoms in childhood.

Platelet storage pool disorder (SPD) is the classic autosomal dominant qualitative platelet disorder. This results from abnormalities of platelet granule formation. It is also seen as a part of inherited disorders of granule formation, such as Hermansky-Pudlak syndrome. Bleeding

symptoms in SPD are variable but often are mild. The most common inherited disorders of platelet function prevent normal secretion of granule content and are termed *secretion defects*. An increasing number of genetic variants are being found in patients with these disorders, although assigning pathogenicity remains challenging.

TREATMENT

Inherited Disorders of Platelet Dysfunction

Bleeding symptoms or prevention of bleeding in patients with severe platelet dysfunction frequently requires platelet transfusion. Care must be taken to limit the risk of alloimmunization by limiting exposure and using HLA-matched single donor platelets for transfusion when needed. rFVIIa is FDA approved in Glanzmann's thrombasthenia and Bernard Soulier syndrome where use can avoid platelet alloimmunization and anti-receptor antibody formation. Platelet disorders associated with milder bleeding symptoms frequently respond to desmopressin (1-deamino-8- α -arginine vasopressin [DDAVP]). DDAVP increases plasma VWF and factor VIII levels; it may also have a direct effect on platelet function. Particularly for mucosal bleeding symptoms, antifibrinolytic therapy (tranexamic acid or ϵ -aminocaproic acid) is used alone or in conjunction with DDAVP or platelet therapy.

Acquired Disorders of Platelet Function Acquired platelet dysfunction is common, usually due to medications, either intentionally as with antiplatelet therapy or unintentionally as with high-dose penicillins. Acquired platelet dysfunction occurs in uremia. This is likely multifactorial, but the resultant effect is defective adhesion and activation. The platelet defect is improved most by dialysis but may also be improved by increasing the hematocrit to 27–32%, giving DDAVP (0.3 $\mu\text{g}/\text{kg}$), or use of conjugated estrogens. Platelet dysfunction also occurs with cardiopulmonary bypass due to the effect of the artificial circuit on platelets, and bleeding symptoms respond to platelet transfusion. Platelet dysfunction seen with underlying hematologic disorders can result from nonspecific interference by circulating paraproteins or intrinsic platelet defects in myeloproliferative and myelodysplastic syndromes.

VON WILLEBRAND DISEASE

VWD is the most common inherited bleeding disorder, with prevalence of symptomatic disease of 1 in 1000 to 1 in 10,000 individuals. VWF serves two roles: (1) as the major adhesion molecule that tethers the platelet to the exposed subendothelium; and (2) as the binding protein for factor VIII (FVIII), resulting in significant prolongation of the FVIII half-life in circulation. The platelet-adhesive function of VWF is critically dependent on the presence of large VWF multimers, whereas FVIII binding is not. Most of the symptoms of VWD are "platelet-like" except in more severe VWD when the FVIII is low enough to produce symptoms similar to those found in FVIII deficiency (hemophilia A).

VWD has been classified into three major types, with four subtypes of type 2 (Table 115-2). By far, the most common type of VWD is type 1 disease, with a parallel decrease in VWF protein, VWF function, and FVIII levels, accounting for at least 80% of cases. In type 1 VWD, patients have predominantly mucosal bleeding symptoms, although postoperative bleeding can also be seen. Bleeding symptoms are uncommon in infancy and usually manifest later in childhood with excessive bruising and epistaxis. Because these symptoms occur commonly in childhood, the clinician should particularly note bruising at sites unlikely to be traumatized and/or prolonged epistaxis requiring medical attention. Heavy menstrual bleeding is a common manifestation of VWD. Menstrual bleeding resulting in anemia should warrant an evaluation for VWD and, if negative, functional platelet disorders. Type 1 VWD may first manifest with dental extractions, particularly wisdom tooth extraction, or tonsillectomy.

Not all patients with low VWF levels have bleeding symptoms. Whether patients bleed or not will depend on the overall hemostatic balance they have inherited, along with environmental influences and the type of hemostatic challenges they experience. Although the

TABLE 115-2 Laboratory Diagnosis of von Willebrand Disease (VWD)					
TYPE	aPTT	VWF ANTIGEN	VWF ACTIVITY	FVIII ACTIVITY	MULTIMER
1	NI or ↑	↓	↓	↓	Normal distribution, decreased in quantity
2A	NI or ↑	↓	↓↓	↓	Loss of high- and intermediate-MW multimers
2B ^a	NI or ↑	↓	↓↓	↓	Loss of high-MW multimers
2M	NI or ↑	↓	↓↓	↓	Normal distribution, decreased in quantity
2N	↑↑	NI or ↓ ^b	NI or ↓ ^b	↓↓	Normal distribution
3	↑↑	↓↓	↓↓	↓↓	Absent

^aUsually also decreased platelet count. ^bFor type 2N, in the homozygous state, factor VIII is very low; in the heterozygous state, it is only seen in conjunction with type 1 VWD.

Abbreviations: aPTT, activated partial thromboplastin time; F, factor; MW, molecular weight; NI, normal; VWF, von Willebrand factor.

inheritance of VWD is autosomal, many factors modulate both VWF levels and bleeding symptoms. These have not all been defined, but include blood type, thyroid hormone status, race, stress, exercise, hormonal (both endogenous and exogenous) influences, and modulators of VWF clearance. Patients with type O blood have VWF protein levels of approximately one-half those of patients with AB blood type, and in fact, the normal range for patients with type O blood overlaps that which has been considered diagnostic for VWD. Patients with mildly decreased VWF levels should be diagnosed with VWD only in the setting of bleeding symptoms and/or a family history of VWD.

Patients with type 2 VWD have functional defects; thus, the VWF antigen measurement is significantly higher than the test of function. For types 2A, 2B, and 2M VWD, platelet-binding and/or collagen-binding VWF activity is decreased. In type 2A VWD, the impaired function is due either to increased susceptibility to cleavage by ADAMTS13, resulting in loss of intermediate- and high-molecular-weight multimers, or to decreased production of these multimers by the cell. Type 2B VWD results from gain-of-function DNA variants that result in increased ADAMTS13 cleavage and binding of VWF to platelets in circulation, with subsequent clearance of this complex by the reticuloendothelial system. The resulting VWF in the patients' plasma lacks the highest molecular-weight multimers, and the platelet count is usually modestly reduced. Type 2M occurs as a consequence of a group of DNA variants that cause dysfunction but do not affect multimer structure.

Type 2N VWD is due to variants in the VWF gene that affect binding of FVIII. As FVIII is stabilized by binding to VWF, the FVIII in patients with type 2N VWD has a very short half-life, and the FVIII level is markedly decreased. This is sometimes termed *autosomal hemophilia*. Type 3 VWD, or severe VWD, describes patients with virtually no VWF protein and usually FVIII levels <10%. Patients experience mucosal and joint bleeding, surgery-related bleeding, and other bleeding symptoms. Some patients with type 3 VWD, particularly those with large VWF gene deletions, are at risk of developing antibodies to infused VWF.

Acquired VWD or von Willebrand syndrome is most commonly seen in patients with underlying lymphoproliferative disorders, including monoclonal gammopathies of undetermined significance (MGUS), multiple myeloma, and Waldenström's macroglobulinemia. It is seen most commonly in the setting of MGUS and should be suspected in patients, particularly elderly patients, with a new onset of severe mucosal bleeding symptoms. Laboratory evidence of acquired VWD is found in some patients with cardiac valvular disease. Heyde's syndrome (aortic stenosis with gastrointestinal bleeding) is attributed to the presence of angiogenesis of the gastrointestinal tract in patients with aortic stenosis. The shear stress on blood passing through the stenotic aortic valve appears to unfold VWF, making it susceptible to proteolysis. Consequently, large multimer forms are lost, leading to an acquired type 2 VWD, but return when the stenotic valve is replaced.

TREATMENT

Von Willebrand Disease

The mainstay of treatment for type 1 VWD is DDAVP (desmopressin), which results in release of VWF and FVIII from endothelial stores. DDAVP can be given intravenously, by high-concentration intranasal spray (1.5 mg/mL), or when a concentrated form is available, by subcutaneous injection. The peak activity when given intravenously is approximately 30 min, whereas it is 2 h when given intranasally. The usual dose is 0.3 µg/kg intravenously or two squirts (one in each nostril) for patients >50 kg (one squirt for those <50 kg). It is recommended that patients with VWD be tested with DDAVP to assess their response before using it. In patients who respond well (increase in laboratory values of two- to fourfold), it can be used for procedures with minor to moderate risk of bleeding. Depending on the procedure, additional doses may be needed; it is usually given every 12–24 h. Less frequent dosing may result in less tachyphylaxis, which occurs when synthesis cannot compensate for the released stores. The major side effect of DDAVP is hyponatremia due to decreased free water clearance. This occurs most commonly in the very young and the very old, but fluid restriction should be advised for all patients for the 24 h following each dose.

Some patients with types 2A VWD respond to DDAVP such that it can be used for minor procedures. For the other subtypes, for type 3 disease, and for major procedures requiring longer periods of normal hemostasis, VWF replacement can be given. Virally inactivated VWF-plasma-derived and recombinant factor concentrates are safer than cryoprecipitate as the replacement product.

Antifibrinolytic therapy using either tranexamic acid (TXA) or ϵ -aminocaproic acid is an important therapy, either alone or in an adjunctive capacity, particularly for the prevention or treatment of mucosal bleeding. These agents are particularly useful in treatment of heavy menstrual bleeding (TXA 1300 mg every 8 h) and postpartum hemorrhage, as prophylaxis for dental procedures, and with DDAVP or factor concentrate for dental extractions, tonsillectomies, and prostate procedures. Antifibrinolytic agents are contraindicated in the setting of upper urinary tract bleeding due to the risk of ureteral obstruction.

DISORDERS OF THE VESSEL WALL

The vessel wall is an integral part of hemostasis, and separation of a fluid phase is artificial, particularly in disorders such as TTP or HIT that clearly involve the endothelium as well. Inflammation localized to the vessel wall, such as vasculitis, and inherited connective tissue disorders are abnormalities inherent to the vessel wall.

Metabolic and Inflammatory Disorders Acute febrile illnesses may result in vascular damage. This can result from immune complexes containing viral antigens or the viruses themselves. Certain pathogens, such as the rickettsiae causing Rocky Mountain spotted fever, replicate in endothelial cells and damage them. SARS-CoV-2 also infects endothelial cells, resulting in activation and damage contributing to COVID-19 pathogenicity. Vascular purpura may occur in patients with polyclonal gammopathies but more commonly occurs in those with monoclonal gammopathies, including Waldenström's macroglobulinemia, multiple myeloma, and cryoglobulinemia. Patients with mixed cryoglobulinemia develop a more extensive maculopapular rash due to immune complex-mediated damage to the vessel wall.

Patients with scurvy (vitamin C deficiency) develop painful episodes of perifollicular skin bleeding as well as more systemic bleeding symptoms. Vitamin C is needed to synthesize hydroxyproline, an essential constituent of collagen. Patients with Cushing's syndrome or on chronic glucocorticoid therapy develop skin bleeding and easy bruising due to atrophy of supporting connective tissue. A similar phenomenon is seen with aging, where following minor trauma, blood spreads superficially under the epidermis. This has been termed *senile purpura*. It is most common on skin that has been previously damaged by sun exposure.

Henoch-Schönlein, or anaphylactoid, purpura is a distinct, self-limited type of vasculitis that occurs in children and young adults. Patients have an acute inflammatory reaction with IgA and complement components in capillaries, mesangial tissues, and small arterioles leading to increased vascular permeability and localized hemorrhage. The syndrome is often preceded by an upper respiratory infection, commonly with streptococcal pharyngitis, or is triggered by drug or food allergies. Patients develop a purpuric rash on the extensor surfaces of the arms and legs, usually accompanied by polyarthralgias or arthritis, abdominal pain, and hematuria from focal glomerulonephritis. All coagulation tests are normal, but renal impairment may occur. Glucocorticoids can provide symptomatic relief but do not alter the course of the illness.

Inherited Disorders of the Vessel Wall Patients with inherited disorders of the connective tissue matrix, such as Marfan's syndrome, Ehlers-Danlos syndrome, and pseudoxanthoma elasticum, frequently report easy bruising. Inherited vascular abnormalities can result in increased bleeding. This is notably seen in hereditary hemorrhagic telangiectasia (HHT, or Osler-Weber-Rendu disease), a disorder where abnormal telangiectatic capillaries result in frequent bleeding episodes, primarily from the nose and gastrointestinal tract. Arteriovenous malformation (AVM) in the lung, brain, and liver may also occur in HHT. The telangiectasia can often be visualized on the oral and nasal mucosa. Signs and symptoms develop over time. Epistaxis begins, on average, at the age of 12 and occurs in >95% of affected individuals by middle age. Approximately 25% have gastrointestinal bleeding usually beginning after the age of 50. HHT is caused by pathogenic DNA variants in number of genes involved in the TGF β /BMP signaling cascade.

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Coagulation Disorders

Jean M. Connors



Deficiencies of coagulation factors have been recognized for centuries. Patients with genetic deficiencies of plasma coagulation factors exhibit lifelong recurrent bleeding episodes into joints, muscles, and closed spaces, either spontaneously or following an injury. The most common inherited factor deficiencies are the hemophilias, X-linked diseases caused by deficiency of factor (F) VIII (hemophilia A) or FIX

TABLE 116-1 Genetic and Laboratory Characteristics of Inherited Coagulation Disorders

CLOTTING FACTOR DEFICIENCY	INHERITANCE	PREVALENCE IN GENERAL POPULATION	LABORATORY ABNORMALITY ^a			MINIMUM HEMOSTATIC LEVELS	TREATMENT	PLASMA HALF-LIFE
			aPTT	PT	TT			
Fibrinogen	AR	1 in 1,000,000	+	+	+	100 mg/dL	Cryoprecipitate	2–4 d
Prothrombin	AR	1 in 2,000,000	+	+	-	20%–30%	FFP/PCC	3–4 d
Factor V	AR	1 in 1,000,000	+/-	+/-	-	15%–20%	FFP ^c	36 h
Factor VII	AR	1 in 500,000	-	+	-	15%–20%	FFP/PCC	4–6 h
Factor VIII	X-linked	1 in 5000	+	-	-	30%	FVIII concentrates	8–12 h
Factor IX	X-linked	1 in 30,000	+	-	-	30%	FIX concentrates	18–24 h
Factor X	AR	1 in 1,000,000	+/-	+/-	-	15%–20%	FFP/PCC	40–60 h
Factor XI	AR	1 in 1,000,000	+	-	-	15%–20%	FFP	40–70 h
Factor XII	AR	ND	+	-	-	b	b	60 h
HK	AR	ND	+	-	-	b	b	150 h
Prekallikrein	AR	ND	+	-	-	b	b	35 h
Factor XIII	AR	1 in 2,000,000	-	-	+/-	2%–5%	Cryoprecipitate/FXIII concentrates	11–14 d

^aValues within normal range (-) or prolonged (+). ^bNo risk for bleeding; treatment is not indicated. ^cSince platelets contain FV, platelet transfusion can be used as therapy.

Abbreviations: aPTT, activated partial thromboplastin time; AR, autosomal recessive; FFP, fresh-frozen plasma; HK, high-molecular-weight kininogen; ND, not determined; PCC, prothrombin complex concentrates; PT, prothrombin time; TT, thrombin time.

(hemophilia B). Rare congenital bleeding disorders due to deficiencies of other factors, including FII (prothrombin), FV, FVII, FX, FXI, FXIII, and fibrinogen, are commonly inherited in an autosomal recessive manner (Table 116-1). Disease phenotype often correlates with the level of factor activity. While patients can have a congenital deficiency of FXII accompanied by a significant prolongation in the activated partial thromboplastin time (aPTT), FXII deficiency is not accompanied by a bleeding phenotype, likely due to redundant paths to activation of the intrinsic pathway of the coagulation cascade, including direct activation of FXI by thrombin generated through the extrinsic pathway (Fig. 116-1). Advances in characterization of the molecular basis of clotting factor deficiencies have contributed to better understanding of the disease phenotypes allowing the development of more targeted therapeutic approaches, including the use of small molecules, recombinant proteins, or cell- and gene-based therapies.

The two most commonly used tests of hemostasis, the prothrombin time (PT) and the aPTT, were designed to perform the first screen for clotting factor deficiency (Fig. 116-1). An isolated prolonged PT suggests FVII deficiency, whereas a prolonged aPTT indicates an intrinsic pathway factor deficiency, most commonly hemophilia A or B (FVIII or FIX, respectively) or FXI deficiency (Fig. 116-1). The prolongation of both PT and aPTT suggests a deficiency of FV, FX, FII, or fibrinogen abnormalities. A mixing study, in which the addition of normal pooled plasma to the patient's plasma, will correct a prolonged aPTT or PT due to a factor deficiency, and is the next step in determining if there is a coagulation factor deficiency. If the clotting time does not correct, it suggests the presence of an inhibitor, an antibody to a specific factor; however, a mixing study will also detect the presence of anticoagulants. Many labs have testing methods for detecting inhibitors that neutralize anticoagulants. If the mixing study corrects with normal plasma, individual factor activity assays are performed to determine which factor is deficient.

Acquired deficiencies of plasma coagulation factors are more frequent than congenital disorders; the most common disorders include hemorrhagic diathesis of liver disease, disseminated intravascular coagulation (DIC), and vitamin K deficiency. In these disorders, blood coagulation

is hampered by the deficiency of more than one clotting factor, and the bleeding episodes are the result of perturbation of both primary (e.g., platelet and vessel wall interactions) and secondary (coagulation) hemostasis.

The development of alloantibodies to coagulation plasma proteins, clinically termed *inhibitors*, is a relatively rare disease that often affects hemophilia A or B and FXI-deficient patients on repetitive exposure to the missing protein to control bleeding episodes. Inhibitory autoantibodies also occur among subjects without genetic deficiency of clotting factors and although rare can be seen in the postpartum setting, as a manifestation of underlying autoimmune or neoplastic disease, or idiopathically. Rare cases of acquired inhibitors to thrombin or FV have been reported in patients receiving topical bovine thrombin preparation as a local hemostatic agent in complex surgeries. The results of a mixing study that does not correct with the addition of normal plasma indicate the presence of an inhibitor, requiring additional tests to identify the specificity of the inhibitor and measure its titer.

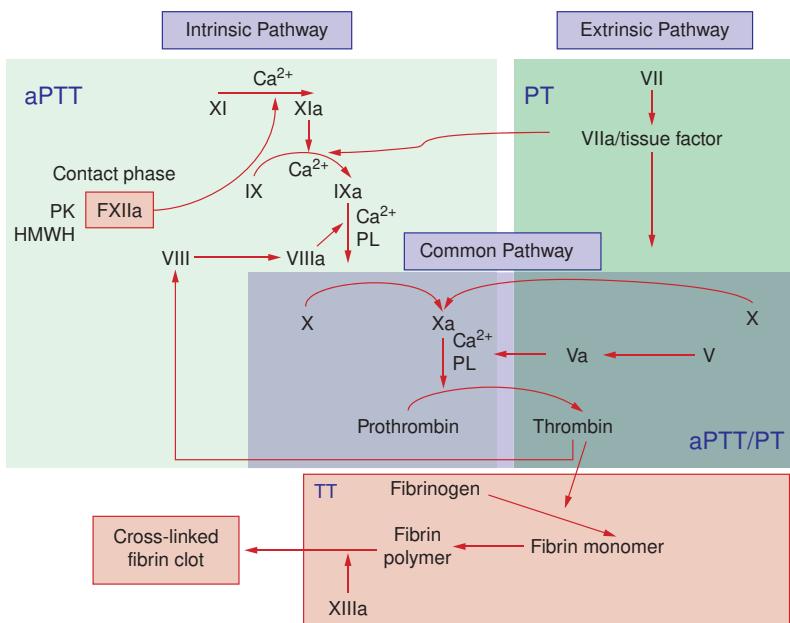


FIGURE 116-1 Coagulation cascade and laboratory assessment of clotting factor deficiency by activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT), and phospholipid (PL).

detection in patients with hemophilia is of particular importance, with yearly screening performed at most hemophilia treatment centers.

The treatment of coagulation factor deficiencies in the setting of bleeding requires replacement of the deficient protein(s) using recombinant or purified plasma-derived products or fresh-frozen plasma (FFP). Prothrombin complex concentrates (PCCs) are intermediate purity plasma-derived factor concentrates initially used as sources of FVIII or FIX for hemophilia patients, but as they contain the vitamin K-dependent factors, are also used for warfarin reversal. Three-factor PCC (3F-PCC) is less frequently used now for warfarin reversal because these preparations contain low levels of FVII, requiring FFP as a source of FVII. Four-factor PCC (4F-PCC), especially the one used in the United States, contains FII, FIX, FX, higher levels of FVII than 3F-PCC, and protein S and protein C.

HEMOPHILIA A AND B

■ PATHOGENESIS AND CLINICAL MANIFESTATIONS

Hemophilia is an X-linked recessive hemorrhagic disease due to mutations in the *F8* gene (hemophilia A or classic hemophilia) or *F9* gene (hemophilia B). The disease affects 1 in 10,000 males worldwide, in all ethnic groups; hemophilia A represents 80% of all cases. The large size of the *F8* gene makes it more susceptible to mutation events than the smaller *F9* gene. Male subjects are clinically affected; women, who carry a single mutated gene, are generally asymptomatic. Family history of the disease is absent in ~30% of cases, and in these cases 80% of the mothers are carriers of the de novo mutated allele. More than 500 different mutations have been identified in the *F8* or *F9*. One of the most common hemophilia A mutations results from an inversion of the intron 22 sequence, and it is present in 40% of cases of severe hemophilia A. Advances in molecular diagnosis now permit precise identification of mutations, allowing accurate diagnosis of women carriers of the hemophilia gene in affected families.

Clinically, hemophilia A and hemophilia B are indistinguishable. The disease phenotype correlates with the activity of FVIII or FIX and can be classified as severe (<1%), moderate (1–5%), or mild (6–30%). In the severe and moderate forms, the disease is characterized by bleeding into the joints (hemarthrosis), soft tissues, and muscles after minor trauma or even spontaneously. Patients with mild disease experience infrequent bleeding that is usually secondary to trauma. Among those with residual FVIII or FIX activity >25% of normal, the disease is discovered only in the event of bleeding after major trauma or during routine preoperative laboratory tests, usually with an isolated prolongation of the APTT that requires further investigation with a mixing study. Factor VIII has a short circulating half-life of 25–30 min that is extended to roughly 12 h when complexed with its carrier protein von Willebrand factor (VWF). In patients without a known history of hemophilia, a diagnosis of von Willebrand disease (VWD) needs to be excluded in patients with a prolonged aPTT and low FVIII activity. Early in life, bleeding may present after circumcision or rarely as intracranial hemorrhages. The disease is more evident when children begin to walk or crawl. In the severe form, the most common bleeding manifestations are recurrent hemarthroses, affecting primarily the knees, elbows, ankles, shoulders, and hips. Acute hemarthroses are painful, and clinical signs are local swelling and erythema. To avoid pain, the patient may adopt a fixed position, which leads eventually to muscle contractures. Very young children unable to communicate verbally show irritability and a lack of movement of the affected joint. Chronic hemarthroses are debilitating with synovial thickening and synovitis in response to the intraarticular blood. After a joint has been damaged, recurrent bleeding episodes result in the clinically recognized “target joint,” which then establishes a vicious cycle of bleeding, resulting in progressive joint deformity that in critical cases requires surgery as the only therapeutic option. Hematomas into the muscle of distal parts of the limbs may lead to external compression of arteries, veins, or nerves that can result in compartment syndrome.

Bleeding into the oropharyngeal spaces, central nervous system (CNS), or retroperitoneum is life-threatening and requires immediate therapy. Retroperitoneal hemorrhages can accumulate large quantities

of blood with formation of masses with calcification and inflammatory tissue reaction (pseudotumor syndrome) and also result in damage to the femoral nerve. Pseudotumors can also form in bones, especially long bones of the lower limbs. Hematuria is frequent among hemophilia patients, even in the absence of genitourinary pathology. It is often self-limited and may not require specific therapy.

TREATMENT

Hemophilia

Without treatment, severe hemophilia may limit life expectancy. Advances in the blood fractionation industry during World War II resulted in the realization that plasma could be used to treat hemophilia, but the volumes required to achieve even modest elevation of circulating factor levels limit the utility of plasma infusion as an approach to disease management. The discovery in the 1960s that the cryoprecipitate fraction of plasma was enriched for FVIII, and the eventual purification of FVIII and FIX from plasma, led to the introduction of home infusion therapy with factor concentrates in the 1970s. The availability of factor concentrates resulted in a dramatic improvement in life expectancy and in quality of life for people with severe hemophilia. However, the contamination of the blood supply with hepatitis viruses, and subsequently HIV, resulted in widespread transmission of these bloodborne infections within the hemophilia population. The introduction of viral inactivation steps in the preparation of plasma-derived products in the mid-1980s greatly reduced the risk of HIV and hepatitis; the risks were further reduced by the production of recombinant FVIII and FIX proteins in the 1990s. It is uncommon for hemophilic patients born after 1985 to have contracted either hepatitis or HIV, and for these individuals, life expectancy is 65 years. In fact, since 1998, new infections with viral hepatitis or HIV have not been reported in hemophilia patients.

Factor replacement for hemophilia has been the mainstay of therapy for half a century; however, advances including uniquely functioning molecules and gene therapy have expanded treatment approaches. Factor replacement has been provided either in response to a bleeding episode or as prophylactic treatment. Primary prophylaxis is defined as maintaining the missing clotting factor at levels ~1% or higher on a regular basis in order to prevent bleeds, especially the onset of hemarthroses. Hemophilic boys receiving regular infusions of FVIII (3 days/week) or FIX (2 days/week) can reach puberty without detectable joint abnormalities. Therefore, prophylactic treatment has become more common. The Centers for Disease Control and Prevention reported that more than 51% of children with severe hemophilia who are aged <6 years receive prophylaxis, increasing considerably from 33% in 1995. Although prophylaxis with factor concentrates is the standard care for children and adults with severe hemophilia, teenagers and young adults do not always maintain treatment due to high cost and lifestyle factors including difficulties accessing peripheral veins for two-to-three times a week infusions, and potential infectious and thrombotic risks of long-term central vein catheters.

Treatment of hemophilia bleeds requires the following: (1) prompt initiation of factor replacement as symptoms often precede objective evidence of bleeding, especially for classic symptoms of bleeding into the joint in a reliable patient, headaches, or major trauma; and (2) avoidance of antiplatelet drugs.

FVIII and FIX are dosed in units. One unit is defined as the amount of FVIII (100 ng/mL) or FIX (5 µg/mL) in 1 mL of normal plasma. One unit of FVIII per kilogram of body weight increases the plasma FVIII level by 2%. One can calculate the dose needed to increase FVIII levels to 100% in a 70-kg severe hemophilia patient (<1%) using the simple formula below. Thus, 3500 units of FVIII will raise the circulating level to 100%.

$$\text{FVIII dose (IU)} = \frac{\text{Target FVIII levels} - \text{FVIII baseline levels}}{\times \text{body weight (kg)} \times 0.5 \text{ unit/kg}}$$

The doses for FIX replacement are different from those for FVIII, because FIX recovery after infusion is usually only 50% of the predicted value. Therefore, the formula for FIX replacement is as follows:

$$\text{FIX dose (IU)} = \text{Target FIX levels} - \text{FIX baseline levels}$$

$$\times \text{body weight (kg)} \times 1 \text{ unit/kg}$$

The FVIII half-life of 8–12 h requires injections twice a day to maintain therapeutic levels, whereas the FIX half-life is longer, ~24 h, so that once-a-day injection is sufficient. In specific situations such as after surgery, continuous infusion of factor may be desirable because of its safety in achieving sustained factor levels at a lower total cost.

Cryoprecipitate is enriched with FVIII protein bound to VWF (each bag contains ~80 IU of FVIII). Because of the risk of blood-borne diseases, this product should be used only in emergencies when factor concentrates are not available, although cryoprecipitate may be the only source of FVIII in developing countries.

Mild bleeds such as uncomplicated hemarthroses or superficial hematomas require achieving an initial factor level of 30–50%. Additional doses to maintain levels of 15–25% for 2 or 3 days are indicated for severe hemarthroses, especially when these episodes affect the “target joint.” Large hematomas, or bleeds into deep muscles, require factor levels of 50% or even higher if the clinical symptoms do not improve, and factor replacement may be required for a period of 1 week or longer. The control of serious bleeds, including those that affect the oropharyngeal spaces, CNS, and the retroperitoneum, requires sustained protein levels of 50–100% for 7–10 days. Prophylactic replacement for surgery is aimed at achieving normal factor levels (100%) for a period of 7–10 days; replacement can then be tapered depending on the extent of the surgical wounds. Oral surgery is associated with extensive tissue damage that usually requires factor replacement for 1–3 days coupled with oral antifibrinolytic drugs.

NONTRANSFUSION THERAPY IN HEMOPHILIA

DDAVP (1-Amino-8-D-Arginine Vasopressin) DDAVP is a synthetic vasopressin analog that causes a transient rise in FVIII and VWF, but not FIX by release from stores in vascular endothelial cells. Patients with moderate or mild hemophilia A should be tested to determine if they respond to DDAVP before use. DDAVP at doses of 0.3 µg/kg body weight, over a 20-min period, is expected to raise FVIII levels by two- to threefold over baseline, peaking between 30 and 60 min after infusion. DDAVP does not improve FVIII levels in severe hemophilia A patients because no stores are available to release. Repeated dosing of DDAVP results in tachyphylaxis as storage pools are depleted. After three consecutive doses, if further therapy is indicated, exogenous FVIII is required.

Antifibrinolytic Drugs Bleeding in the gums, in the gastrointestinal tract, and during oral surgery can be treated with oral antifibrinolytic drugs such as ε-amino caproic acid (EACA) or tranexamic acid to prevent fibrin degradation by plasmin. The duration of the treatment depending on the clinical indication is 1 week or longer. Tranexamic acid is given at doses of 25 mg/kg three to four times a day. EACA treatment requires a loading dose of 200 mg/kg (maximum of 10 g) followed by 100 mg/kg per dose (maximum 30 g/d) every 6 h. These drugs are not indicated to control hematuria because of concern for forming an occlusive clot in the lumen of genitourinary tract structures.

COMPLICATIONS

Inhibitor Formation The formation of alloantibodies to FVIII or FIX is the major complication of hemophilia treatment. The prevalence of inhibitors to FVIII is estimated to be ~30% in severe hemophilia A patients and 10% among patients with nonsevere hemophilia A. Inhibitors to FIX are detected in only 3–5% of all hemophilia B patients. The high-risk group for inhibitor formation includes severe deficiency

(>80% of all cases of inhibitors), familial history of inhibitor, African descent, mutations in the FVIII or FIX gene resulting in deletion of large coding regions, or gross gene rearrangements. Inhibitors usually appear early in life, at a median of 2 years of age, and after 10 cumulative days of exposure. However, intensive replacement therapy such as for major surgery, intracranial bleeding, or trauma increases the risk of inhibitor formation for patients of all ages and degree of clinical severity, such that patients require close laboratory monitoring in the weeks following these events.

The clinical diagnosis of an inhibitor is suspected when patients do not respond to factor replacement at therapeutic doses. Inhibitors increase both morbidity and mortality in hemophilia. Because early detection of an inhibitor is critical to a successful correction of the bleeding or to eradication of the antibody, most hemophilia centers perform annual screening with aPTT and mixing studies. The Bethesda assay uses a similar principle as a mixing study and defines the specificity of the inhibitor and its titer. The results are expressed in Bethesda units (BU), in which 1 BU is the amount of antibody that neutralizes 50% of the FVIII or FIX present in normal plasma after 2 h of incubation at 37°C. Clinically, inhibitor patients are classified as low responders or high responders, with response defined as increase in antibody titer; knowledge of responder type guides therapy. Therapy for inhibitor patients has two goals: the control of acute bleeding episodes and the eradication of the inhibitor. For the control of bleeding episodes, low responders, those with titer <5 BU, respond well to high doses of human FVIII (50–100 U/kg), with minimal or no increase in the inhibitor titers. However, high-responder patients, those with initial inhibitor titer >5 BU or an anamnestic response with increase in the antibody titer to >5 BU, even if low titer initially, do not respond to FVIII. The control of bleeding episodes in high-responder patients can be achieved by using concentrates enriched for prothrombin, FVII, FIX, FX (prothrombin complex concentrates [PCCs] but usually activated PCCs [aPCCs]), and recombinant activated factor VII (FVIIa) known as “bypass agents” as they activate coagulation downstream of the inhibited/absent factor or through a different pathway (Fig. 116-1). For FIX inhibitor patients, high doses of FIX can be used (<5 BU); however, allergic or anaphylactic reactions are common in FIX inhibitor patients; thus bypass products should be used to treat or prevent bleeding as well as for those cases of high titer inhibitors. For eradication of the inhibitory antibody, immunosuppression alone is not effective. The most effective strategy is immune tolerance induction (ITI) based on daily infusion of the missing protein until the inhibitor disappears, typically requiring periods >1 year, with success rates of ~60%. The management of patients with severe hemophilia and inhibitors resistant to ITI is challenging. The use of anti-CD20 monoclonal antibody (rituximab) combined with ITI was thought to be effective but while it reduces the inhibitor titers in some cases, sustained eradication is uncommon.

Other Therapeutic Approaches for Hemophilia A and B Engineered clotting factors, using fusion to polyethylene glycol (FVIII, FIX), IgG1-Fc (FVIII, FIX) or albumin (FIX) with resultant longer half-lives, have been in development, with one currently approved for use. These new-generation products (for FVIII and FIX) aim to facilitate prophylaxis by requiring fewer weekly injections to maintain circulating levels >1%, with infusion frequency decreasing from 3 to 2 days a week in hemophilia A, and notably for hemophilia B, only once-a-week injections of long-acting FIX are required. Other novel approaches to manipulating the coagulation cascade components, such as targeting the natural anticoagulants and inhibitors of activation of coagulation, are in development.

Emicizumab is an asymmetric bispecific antibody with one immunoglobulin variable chain region that binds FIXa and another that binds FX bringing them in close contact resulting in activation of FX by FIXa. FXa subsequently cleaves prothrombin to thrombin—without the need for FVIII (Fig. 116-2). It is effective in patients with severe hemophilia A with or without inhibitors. After initial once-a-week subcutaneous injections (an improvement

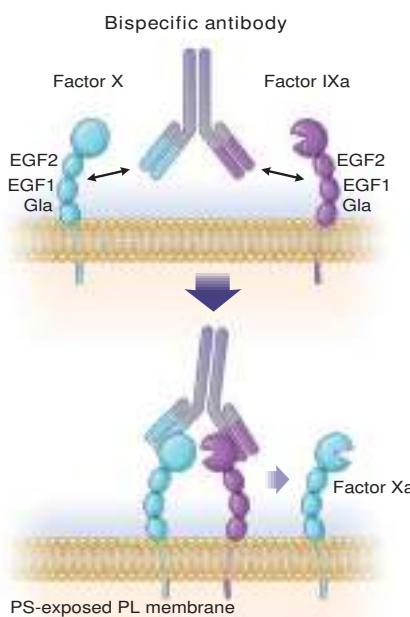


FIGURE 116-2 Mechanism of action of emicizumab. Emicizumab is a bifunctional antibody; the two binding sites recognize different protein sequences, unlike normal antibodies where both variable regions recognize the same antigen. One arm of emicizumab recognizes factor IXa and the other factor X. It functions to bring these two factors in proximity so that factor IXa can activate factor X to factor Xa, which then cleaves prothrombin to thrombin and activates the clotting cascade. (From T Kitazawa, M Shima: Emicizumab, a humanized bispecific antibody to coagulation factors IXa and X with a factor VIIIa-cofactor activity. *Int J Hematol* 111:20, 2020.)

over intravenous administration of factors) for 4 weeks, patients can be maintained with once-a-month dosing to prevent spontaneous bleeds, an overwhelmingly dramatic improvement in quality of life when compared to even the twice-weekly infusion schedule of “long-acting” FVIII compounds. Breakthrough bleeds can occur, however, and need to be carefully managed, as a small number of patients with inhibitors treated with aPCC or recombinant FVIIa developed thrombotic events or fatal thrombotic microangiopathy.

These X-linked disorders are ideally suited for gene therapy as small increases in plasma factor level will result in significant clinical improvement. FIX has been the most studied as the gene is smaller and easier to package in the viral vectors used. In one approach, the sequence of a known spontaneous FIX gain of function mutation that has marked increase in specific activity, FIX Padua, is used so that small increments in plasma level of FIX are also accompanied by even greater increase in functional activity. The larger FVIII gene has also been successfully transferred through an adeno-associated viral vector to a few patients with hemophilia A. The early results appear promising. Complications include transaminitis and loss of gene expression for a variety of reasons; no gene therapy approaches have regulatory approval yet (*Chap. 470*).

INFECTIOUS DISEASES

Hemophilia patients treated with clotting factor concentrates before the development of recombinant factors in the 1990s were almost universally infected with hepatitis C virus (HCV) and HIV. These infections are the major cause of morbidity and the second leading cause of death in these patients. Co-infection of HCV and HIV, present in almost 50% of hemophilia patients, is an aggravating factor for the evolution of liver disease as correction of both genetic and acquired (secondary to liver disease) factor deficiencies may be needed. Improvements in treatment of both HIV and HCV have altered the devastating prognosis for many infected patients.

In some select cases with cirrhosis, liver transplant has been performed, which also is curative for hemophilia.

EMERGING CLINICAL PROBLEMS IN AGING HEMOPHILIA PATIENTS

The number of patients living with hemophilia well into adulthood has increased with the advances in treatments. The life expectancy of patients with severe hemophilia is now only ~10 years shorter than the general male population, and near normal in patients with mild or moderate hemophilia. The older hemophilia population has distinct needs relating to more severe arthropathy, chronic pain, and high rates of HCV and/or HIV infections.

Although mortality from coronary artery disease is lower in hemophilia patients with hypocoagulability decreasing thrombus formation, atherogenesis is not prevented. Typical cardiovascular risk factors such as age, obesity, and smoking, along with physical inactivity, hypertension, and chronic renal disease are seen in hemophilia patients as in the general population.

Management of an acute ischemic event and coronary revascularization should include collaboration among hematologists, cardiologists, and internists. Cancer due to HIV- and HCV-related malignancies is also a concern in this population, with hepatocellular carcinoma (HCC) the most common cause of death in HIV-negative patients. The recommendations for cancer screening for the general population should be the same for age-matched hemophilia patients, including routine screening for HCC. Screening for GU or GI tract neoplasms in patients with hematuria or hematochezia may be delayed. Hemophilia patients benefit from the same preventive and therapeutic approaches to minimize the risk of cardiovascular disease and malignancy as the general population.

MANAGEMENT OF CARRIERS OF HEMOPHILIA

Women carriers of hemophilia with factor levels ~50% of normal may not have an increased risk for bleeding. However, a wide range of factor activity (22–116%) due to random inactivation of the X chromosome (*lyonization*) can occur and lead to unexpected bleeding in women with low levels. The factor level of carriers should be measured to optimize perioperative management. During pregnancy, FVIII levels increase approximately two- to threefold compared to nonpregnant women, whereas the FIX increase is less pronounced. After delivery, a rapid fall in the pregnancy-induced rise of maternal clotting factor levels occurs resulting in an imminent risk of bleeding that can be prevented by infusion of factor concentrate to levels of 50–70% for 3 days for vaginal delivery and up to 5 days for cesarean delivery. In mild cases, the use of DDAVP and/or antifibrinolytic drugs is recommended.

■ FACTOR XI DEFICIENCY

Factor XI deficiency, also known as hemophilia C, is a rare autosomal bleeding disorder that occurs at a frequency of one in a million. However, the disease is highly prevalent among Ashkenazi and Iraqi Jewish populations, reaching a frequency of 6% heterozygotes and 0.1–0.3% homozygotes. More than 65 mutations in the FXI gene have been reported, whereas fewer mutations (two to three) are found among affected Jewish populations.

Normal FXI clotting activity levels range from 70–150 U/dL. Levels vary depending on the presence of heterozygous, homozygous, or double heterozygous mutations with levels <1 U/dL seen in the latter two. Patients with FXI levels <10% of normal have a high risk of bleeding, but the phenotype does not always correlate with FXI clotting activity. The family history is informative, with the bleeding risk based on bleeding in kindreds. Clinically, mucocutaneous hemorrhages such as bruises, gum bleeding, epistaxis, hematuria, and menorrhagia are common, especially following trauma. This hemorrhagic phenotype suggests that tissues rich in fibrinolytic activity are more susceptible to FXI deficiency. Postoperative bleeding is common but not always present, even among patients with very low FXI levels.

FXI replacement is indicated in patients with severe disease for major surgical procedures. A negative history of bleeding complications

following invasive procedures does not exclude the possibility of an increased risk for hemorrhage.

TREATMENT

Factor XI Deficiency

Sources of FXI are limited to FFP in the United States, while a plasma-derived FXI concentrate is available in other countries. FFP at doses of 15–20 mL/kg to maintain trough levels ranging from 10–20% can be given every other day in the setting of bleeding or major surgery as FXI has a half-life of 40–70 h. Antifibrinolytic drugs can be used for minor bleeds and as adjunctive treatment with FXI replacement with the exception of GU tract bleeding. The development of an FXI inhibitor can be seen in 10% of severely FXI-deficient patients. Although inhibitors are not associated with spontaneous bleeding, bleeding with surgery or trauma can be severe; treatment with PCC/aPCC or recombinant activated VII is effective.

RARE BLEEDING DISORDERS

Inherited disorders resulting from deficiencies of clotting factors other than FVIII, FIX, and FXI (Table 116-1) occur infrequently. Bleeding manifestations vary from generally asymptomatic as with dysfibrinogenemia or FVII deficiency to life-threatening as with FX or FXIII deficiency. In contrast to hemophilia, hemarthroses are rare but bleeding in the mucosal tract or after umbilical cord clamping is common. Individuals heterozygous for plasma coagulation deficiencies are often asymptomatic. The laboratory assessment for the specific deficient factor following screening with general coagulation tests (Table 116-1) identifies the diagnosis.

Replacement therapy using FFP or PCCs for deficiencies provides adequate hemostasis for bleeds or prophylactic treatment, although specific concentrates for FX and fibrinogen are available. Cryoprecipitate or FXIII concentrate is needed for FXIII deficiency. VII deficiency, like FXI, has an increased prevalence in the Ashkenazi Jewish population and is best treated with rVIIa rather than FFP or PCCs depending on the severity of bleeding or type of surgery.

FAMILIAL MULTIPLE COAGULATION DEFICIENCIES

Several bleeding disorders are characterized by the inherited deficiency of more than one plasma coagulation factor. To date, the genetic defects in two of these diseases have been characterized, and they provide new insights into the regulation of hemostasis by gene-encoding proteins outside blood coagulation.

Combined Deficiency of FV and FVIII Patients with combined FV and FVIII deficiency exhibit ~5% of residual clotting activity of each factor, yet it is associated with a mild bleeding tendency, often following trauma. A mutation in the lectin mannose binding 1 (*LMAN1*) gene, a mannose-binding protein localized in the Golgi apparatus that functions as a chaperone for both FV and FVIII, is responsible. In other families, mutations in the multiple coagulation factor deficiency 2 (*MCFD2*) gene have been defined; this gene encodes a protein that forms a Ca^{2+} dependent complex with *LMAN1* and provides cofactor activity in the intracellular mobilization of both FV and FVIII. Replacement therapy to control or prevent bleeding consists of FFP to maintain FV levels and DDAVP or FVIII concentrate to achieve FVIII levels of 20–40%. Alternatively, platelets, which contain FV, can also be used.

Multiple Deficiencies of Vitamin K-Dependent Coagulation Factors Two enzymes involved in vitamin K metabolism have been associated with combined deficiency of all vitamin K-dependent proteins, including the procoagulant proteins prothrombin (II), VII, IX, and X and the anticoagulant proteins C and S. Vitamin K is a fat-soluble vitamin that is a cofactor for carboxylation of the gamma carbon of the glutamic acid residues in the vitamin K-dependent factors, a critical step for calcium and phospholipid binding of these proteins

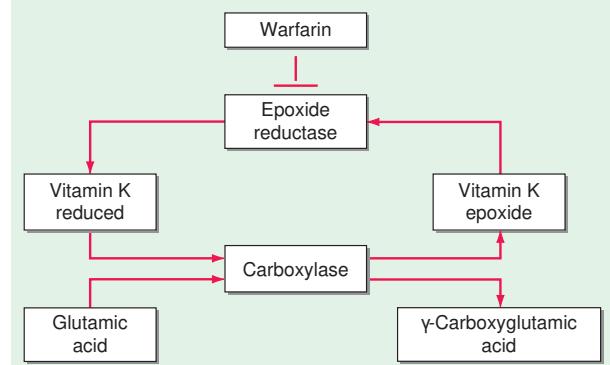


FIGURE 116-3 The vitamin K cycle. Vitamin K is a cofactor for the formation of γ -carboxylglutamic acid residues on coagulation proteins. Vitamin K-dependent γ -glutamylcarboxylase, the enzyme that catalyzes the vitamin K epoxide reductase, regenerates reduced vitamin K. Warfarin blocks the action of the reductase and competitively inhibits the effects of vitamin K.

(Fig. 116-3). The enzymes γ -glutamylcarboxylase and epoxide reductase are critical for the metabolism and regeneration of vitamin K. Mutations in the genes encoding the γ -carboxylase (GGCX) or vitamin K epoxide reductase complex 1 (VKORC1) result in defective enzymes and thus in vitamin K-dependent factors with reduced activity, varying from 1–30% of normal. Patients can have mild to severe bleeding episodes present from birth. Some patients respond to oral vitamin K1 (5–20 mg/d), or parenteral vitamin K1 at doses of 5–20 mg/week. For severe bleeding, replacement therapy with PCC may be necessary.

DISSEMINATED INTRAVASCULAR COAGULATION

In 2001, the International Society on Thrombosis and Haemostasis (ISTH) defined disseminated intravascular coagulation (DIC) as “an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes that can originate from and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction.” Many disparate processes are associated with DIC (Table 116-2).

The most common causes are bacterial sepsis, although viral and fungal sepsis can also cause DIC, trauma, obstetric causes such as abruptio placenta or amniotic fluid embolism, and malignant disorders especially mucin-producing adenocarcinomas and acute promyelocytic leukemia. Activation of inflammatory pathways in response to infectious pathogens results in increased expression of tissue factor, activation of neutrophils and monocytes with release of cytokines and development of neutrophil extracellular traps, and release of polyphosphates that engage in cross talk with the coagulation system to cause thrombin generation; this process is known as *thrombo-inflammation*. Damage to vascular endothelial cells results in the loss of their native antithrombotic properties; such damage especially occurs with sepsis and trauma. Systemic inflammatory response syndrome (SIRS) and cytokine storm are cytokine-mediated exuberant inflammatory responses often in the setting of infection that are associated with increased mortality and DIC. Purpura fulminans is a severe form of DIC resulting in thrombosis of extensive areas of the skin; it affects predominantly young children following viral or bacterial infection, particularly those with inherited or acquired hypercoagulability due to deficiencies of the components of the protein C pathway. Neonates homozygous for protein C deficiency can develop neonatal purpura fulminans with or without thrombosis of large vessels.

The central mechanism of DIC is the uncontrolled generation of thrombin by multiple mechanisms (Fig. 116-4). Simultaneous disruption of the physiologic anticoagulant mechanisms and abnormal fibrinolysis further accelerate the process. These abnormalities contribute to systemic fibrin deposition in small and midsize vessels. The duration and intensity of the fibrin deposition can compromise

TABLE 116-2 Common Clinical Causes of Disseminated Intravascular Coagulation

SEPSIS	IMMUNOLOGIC DISORDERS
<ul style="list-style-type: none"> Bacterial: <ul style="list-style-type: none"> Staphylococci, streptococci, pneumococci, meningococci, gram-negative bacilli Viral Mycotic Parasitic Rickettsial 	<ul style="list-style-type: none"> Acute hemolytic transfusion reaction Organ or tissue transplant rejection Immunotherapy Graft-versus-host disease
TRAUMA AND TISSUE INJURY	DRUGS
<ul style="list-style-type: none"> Brain injury (gunshot) Extensive burns Fat embolism Rhabdomyolysis 	<ul style="list-style-type: none"> Fibrinolytic agents Aprotinin Warfarin (especially in neonates with protein C deficiency) Prothrombin complex concentrates Recreational drugs (amphetamines)
VASCULAR DISORDERS	ENVENOMATION
<ul style="list-style-type: none"> Giant hemangiomas (Kasabach-Merritt syndrome) Large vessel aneurysms (e.g., aorta) 	<ul style="list-style-type: none"> Snake Insects
OBSTETRICAL COMPLICATIONS	LIVER DISEASE
<ul style="list-style-type: none"> Abruption placentae Amniotic fluid embolism Dead fetus syndrome Septic abortion 	<ul style="list-style-type: none"> Fulminant hepatic failure Cirrhosis Fatty liver of pregnancy
CANCER	MISCELLANEOUS
<ul style="list-style-type: none"> Adenocarcinoma (prostate, pancreas, etc.) Hematologic malignancies (acute promyelocytic leukemia) 	<ul style="list-style-type: none"> Shock Respiratory distress syndrome Massive transfusion

the blood supply of many organs, especially the lung, kidney, liver, and brain, with consequent organ failure; for example, pulmonary microvascular thrombosis is a component of adult respiratory distress syndrome (ARDS). The sustained activation of coagulation and formation of fibrin can result in consumption of clotting factors and platelets,

which in turn leads to systemic bleeding that can be aggravated by secondary hyperfibrinolysis that occurs in late stages of DIC.

Clinical manifestations of DIC are related to the magnitude of the imbalance of hemostasis, to the underlying disease, or to both. The most common clinical findings include petechiae, ecchymoses, and bleeding ranging from oozing from venipuncture sites to severe hemorrhage from the gastrointestinal tract, lung, or into the CNS. In chronic DIC, the bleeding symptoms are discrete and restricted to skin or mucosal surfaces. The hypercoagulability of DIC manifests as the occlusion of vessels in the microcirculation and resulting organ failure. Thrombosis of large vessels and cerebral embolism can also occur. Hemodynamic complications and shock are common among patients with acute DIC, due to the underlying disease, with mortality ranging from 30 to >80%.

Making the diagnosis of DIC can be difficult. The ISTH has developed a validated scoring tool to aid in the diagnosis of overt DIC with a separate tool for pregnant women. It incorporates platelet count, -dimer level, prothrombin time (PT), and fibrinogen level, and assigns points for different levels of each with the aggregate score helping to make the diagnosis of DIC (Table 116-3). The peripheral smear should be assessed for schistocytes. The laboratory diagnosis of DIC should prompt a search for the underlying disease if not already apparent. In critically ill patients, these tests should be repeated over a period of 6–8 h as patients can rapidly deteriorate.

Chronic DIC Low-grade, compensated DIC can occur in clinical situations including giant hemangioma, metastatic carcinoma, or the dead fetus syndrome. Plasma levels of FDP or -dimers are elevated. aPTT, PT, and fibrinogen values are within the normal range or high. Mild thrombocytopenia or normal platelet counts are also common findings. Red cell fragmentation is often detected but at a lower degree than in acute DIC.

Differential Diagnosis Distinguishing between DIC and severe liver disease is challenging and requires serial measurements of the laboratory parameters of DIC. Patients with severe liver disease manifest laboratory features including thrombocytopenia due to platelet sequestration, portal hypertension, or hypersplenism; decreased synthesis of coagulation factors and natural anticoagulants; and elevated levels of -dimer. However, in contrast to DIC, these laboratory parameters in liver disease do not change rapidly.

Although microangiopathic disorders such as acquired thrombotic thrombocytopenic purpura present with acute onset accompanied by

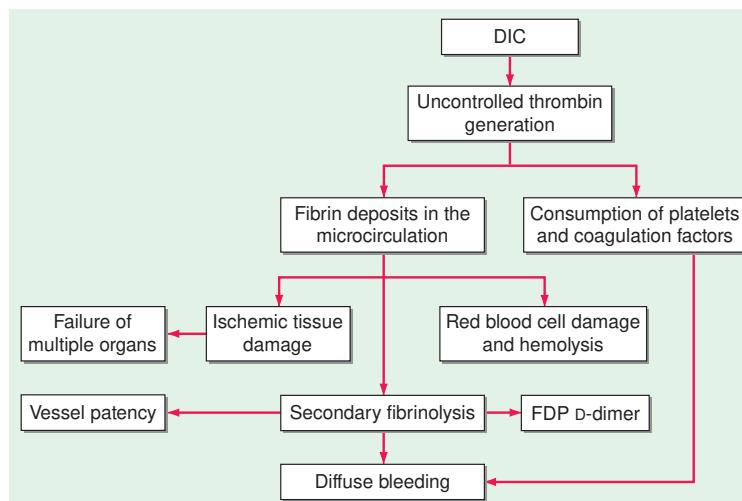


FIGURE 116-4 The pathophysiology of disseminated intravascular coagulation (DIC). Interactions between coagulation and fibrinolytic pathways result in bleeding and thrombosis in the microcirculation in patients with DIC. FDP, fibrin degradation product.

TABLE 116-3 ISTH Criteria for Overt DIC

PARAMETER	VALUE	POINTS
Platelets	>100,000 × 10 ⁹ /L	0
	>50 – <100 × 10 ⁹ /L	1
	<50 × 10 ⁹ /L	2
D-dimer*	Normal	0
	Moderate increase	2
	Severe increase	3
Prothrombin time (PT) prolonged	<3 s	0
	3 – <6 s	1
	>6 s	2
Fibrinogen	>1 g/L	0
	<1 g/L	1
Total Score		<5 Low-grade DIC >5 Overt DIC

* D-dimer assays are not standardized and have different ranges of normal. Check your institution range of normal to assess degree of increase.

Note: A score of <5 suggests non-overt DIC/low-grade DIC and should be repeated every 1–2 days. A score of >5 suggests overt DIC; lab values should be repeated daily to assess critical changes. Not to be used in pregnant patients.

thrombocytopenia, red cell fragmentation, and multiorgan failure, the clinical presentation and laboratory findings such as an inhibitor to ADAMTS13 levels assist in making the microangiopathic disorder diagnosis (*Chap. 115*).

TREATMENT

Disseminated Intravascular Coagulation

The morbidity and mortality associated with DIC are primarily related to the underlying disease. Management of the underlying disease is required to control and eliminate DIC; however, support with platelets and coagulation factors may be needed until the inciting cause is under control. Many patients with overt DIC are critically ill, usually requiring management in the intensive care unit to treat shock physiology and other manifestations of the underlying illness.

MANAGEMENT OF HEMORRHAGIC SYMPTOMS

Patients with active bleeding or at high risk of bleeding during invasive procedures or after chemotherapy require transfusion support; however, transfusion solely to correct mildly to moderately abnormal coagulation parameters is not indicated. Platelet transfusion for platelet counts <10,000–20,000/µL and replacement of fibrinogen and coagulation factors with FFP, with cryoprecipitate or fibrinogen concentrate as a source of fibrinogen, are indicated with amounts determined by the degree of abnormal PT, aPTT, and fibrinogen levels, as well as severity of bleeding or bleeding risk with invasive procedures. For these situations, fibrinogen level should be maintained at >150 mg/dL and PT prolonged no more than 3 s above the upper limit of normal. Vitamin K should be given. Patients should be frequently monitored, and transfusion support adjusted as the patient's condition changes and dictates.

REPLACEMENT OF COAGULATION OR FIBRINOLYSIS INHIBITORS

Anticoagulants such as heparin, antithrombin III (ATIII), and thrombomodulin concentrates, and antifibrinolytic drugs have all been tried in the treatment of DIC. Low doses of continuous-infusion heparin (5–10 U/kg per h) may be effective in patients with low-grade DIC associated with solid tumors, acute promyelocytic leukemia, or in a setting with recognized thrombosis. Heparin is also indicated for the treatment of purpura fulminans, during the surgical resection of giant hemangiomas, and during removal of a dead fetus. In acute hemorrhagic DIC, the use of heparin is likely to aggravate bleeding. The use of heparin in patients with severe DIC, although demonstrating improved coagulation parameters, has not

had a survival benefit; professional society recommendations for use vary widely. Although the use of concentrates of the serine protease inhibitors, antithrombin and thrombomodulin, for sepsis demonstrated little efficacy in all treated patients, post hoc analyses of those with sepsis and confirmed DIC suggest a survival advantage and require further study. Activated protein C treatment for septic shock was withdrawn from the market years ago as findings in clinical practice did not replicate the mortality advantage seen in the clinical trial; impact on DIC was not evaluated.

In patients who have DIC characterized by a primary hyperfibrinolytic state with concomitant severe bleeding, the administration of antifibrinolytics may be considered. However, concern for increasing the risk of thrombosis has led to consideration of concomitant use of heparin. Patients with acute promyelocytic leukemia or those with chronic DIC associated with giant hemangiomas are among the few patients who may benefit from this therapy.

VITAMIN K DEFICIENCY

Vitamin K-dependent proteins are a heterogeneous group, including clotting factor proteins and also proteins found in bone, lung, kidney, and placenta. Vitamin K mediates posttranslational modification of glutamate residues to γ-carboxylglutamate, a critical step for the activity of vitamin K-dependent proteins for calcium binding and proper assembly on phospholipid membranes (Fig. 116-3). Inherited mutations with decreased functional activity of the enzymes GGCX or VKORC1 (see above) result in bleeding disorders. Vitamin K in the diet is often limiting for the carboxylation reaction; thus recycling of the vitamin K by these enzymes is essential to maintain normal levels of vitamin K-dependent proteins. In adults, severe vitamin K deficiency due to low dietary intake in adults is rare but is common in association with the use of broad-spectrum antibiotics, or with disease or surgical interventions that affect the ability of the intestinal tract to absorb vitamin K, through anatomic alterations or by changing the fat content of bile salts and pancreatic enzymes in the proximal small bowel. Chronic liver diseases such as primary biliary cirrhosis also deplete vitamin K stores. Neonatal vitamin K deficiency and the resulting hemorrhagic disease of the newborn have been almost entirely eliminated by routine administration of vitamin K to all neonates. Prolongation of PT values is the most common and earliest finding in vitamin K-deficient patients due to the short half-life of FVII, and occurs before prolongation of the aPTT. Parenteral administration of 10 mg of vitamin K is sufficient to restore normal levels of clotting factor within 8–10 h. More rapid correction of the coagulopathy requires replacement with FFP or PCC, the choice depending on patient intravascular volume status and need for rapidity of correction. The reversal of excessive anticoagulant therapy with vitamin K antagonists, such as warfarin, can be achieved by minimal doses of vitamin K (1 mg orally or by intravenous injection) for asymptomatic patients. This strategy can diminish the risk of bleeding while maintaining therapeutic anticoagulation for an underlying prothrombotic state. For emergent reversal of warfarin in the setting of life-threatening bleeding or need for emergency surgery, use of 4F-PPC is the standard of care.

In patients with underlying vascular disease, vascular trauma, atrial fibrillation, and other comorbidities, re-initiation of anticoagulation needs to be carefully considered to prevent subsequent thromboembolic complications.

COAGULATION DISORDERS ASSOCIATED WITH LIVER FAILURE

The liver is the site of synthesis and clearance of most procoagulant and natural anticoagulant proteins and of essential components of the fibrinolytic system. Liver failure is associated with a high risk of bleeding due to deficient synthesis of procoagulant factors and enhanced fibrinolysis; hepatologists refer to this as accelerated intravascular coagulation and fibrinolysis (AICF). Thrombocytopenia is common in patients with liver disease and may be due to decreased thrombopoietin that is synthesized in the liver, congestive splenomegaly (hypersplenism), or immune-mediated shortened platelet life span

TABLE 116-4 Coagulation Disorders and Hemostasis in Liver Disease

Bleeding	
Portal hypertension	
Esophageal varices	
Thrombocytopenia	
Splenomegaly	
Chronic or acute DIC	
Decreased synthesis of clotting factors	
Hepatocyte failure	
Vitamin K deficiency	
Systemic fibrinolysis	
DIC	
Dysfibrinogenemia	
Thrombosis	
Decreased synthesis of coagulation inhibitors: protein C, protein S, antithrombin	
Hepatocyte failure	
Vitamin K deficiency (protein C, protein S)	
Failure to clear activated coagulation proteins (DIC)	
Dysfibrinogenemia	

Abbreviation: DIC, disseminated intravascular coagulation.

(primary biliary cirrhosis). In addition, several anatomic abnormalities secondary to underlying liver disease further increase the risk of bleeding (Table 116-4). Dysfibrinogenemia is a relatively common finding in patients with liver disease due to impaired fibrin polymerization. The development of DIC in patients with chronic liver disease is not uncommon and may enhance the risk for bleeding. Laboratory evaluation is mandatory for an optimal therapeutic strategy, either to control ongoing bleeding or before invasive procedures. Typically, these patients present with prolonged PT, aPTT, and TT depending on the degree of liver damage, thrombocytopenia, and normal or slight increase in -dimer. Fibrinogen levels are low only in fulminant hepatitis, decompensated cirrhosis, advanced liver disease, or in the presence of DIC. The presence of prolonged TT and normal fibrinogen and -dimer levels suggests dysfibrinogenemia. FVIII levels are often normal or elevated in patients with liver failure, and decreased levels suggest superimposed DIC. FV is only synthesized in the hepatocyte and is not a vitamin K-dependent protein; therefore, reduced levels of FV may be an indicator of liver failure. Normal levels of FV and low levels of FVII suggest vitamin K deficiency. Vitamin K levels may be reduced in patients with liver failure due to compromised storage in hepatocellular disease, changes in bile acids, or cholestasis that can diminish the absorption of vitamin K. Replacement with IV vitamin K may improve hemostasis.

Although treatment of bleeding with FFP was the standard approach to correcting hemostasis in patients with liver failure, the use of 4F-PCC is now favored due to lower volume, less increase in portal pressure, reduced risk of circulatory overload, and other complications associated with FFP transfusion. As in any clinical situation, treatment should not be given simply to correct laboratory abnormalities in a patient who is not bleeding or with no need for invasive procedures. Platelet concentrates are indicated when platelet counts are <10,000–20,000/ μ L to control bleeding

or immediately before an invasive procedure if counts are <50,000/ μ L. Cryoprecipitate is indicated only when fibrinogen levels are <100–150 mg/mL unless the patient is bleeding in which case a higher target is used. The use of antifibrinolytic drugs as adjuncts to control bleeding in patients with liver failure is not thought to result in an increased risk of thrombosis; however, their impact on acute thrombosis propagation is not well studied.

Liver Disease and Thromboembolism Bleeding in patients with stable liver disease is often mild or even asymptomatic. However, as the disease progresses, the hemostatic balance is precarious and easily disturbed; comorbid complications such as infections and renal failure can rapidly upset this balance (Fig. 116-5). Past assumptions based on abnormal coagulation tests have been that patients with liver disease have a decreased risk of thrombosis; however, multiple factors contribute to hypercoagulability, including decreased levels of the natural anticoagulant proteins S and C, as well as endothelial cell changes and hemodynamic changes that result in stasis such that portal vein thrombosis is common. Patients with liver disease can also develop deep-vein thrombosis and pulmonary embolism; those with cirrhosis appear to have a 1.5- to 2-fold increase in the rate of venous thromboembolism (VTE). Patients with compensated cirrhosis do not appear to have increased bleeding with the use of VTE prophylaxis or even therapeutic dose heparin to treat acute portal vein thrombosis when carefully managed. In the outpatient setting, warfarin is avoided but low-molecular-weight heparin and direct oral anticoagulants have been safely used to treat thrombosis.

Acquired Inhibitors of Coagulation Factors An acquired inhibitor is an immune-mediated disease characterized by the presence of an autoantibody against a specific clotting factor. Almost half of patients with an acquired factor inhibitor will have an underlying autoimmune or immunoproliferative disorder, malignancy, or be peripartum. FVIII is the most common target of antibody formation and is sometimes referred to as acquired hemophilia A, but inhibitors to prothrombin (FII), FV, FIX, FX, and FXI are also reported. Acquired inhibitor to FVIII occurs predominantly in older adults (median age of 60 years) but occasionally in pregnant or postpartum women with no previous history of bleeding. Bleeding episodes occur commonly in

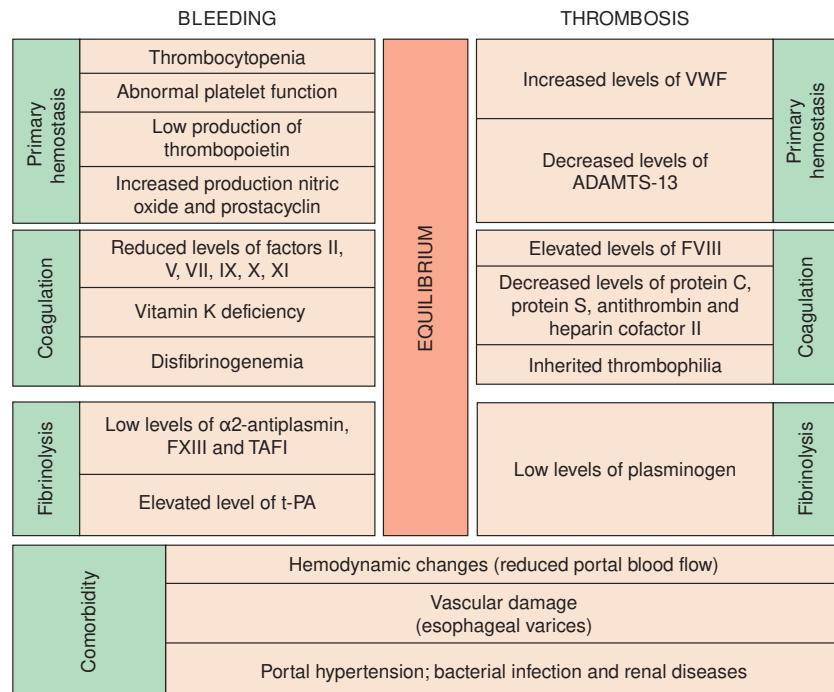


FIGURE 116-5 Balance of hemostasis in liver disease. TAFI, thrombin-activated fibrinolytic inhibitor; t-PA, tissue plasminogen activator; VWF, von Willebrand factor.

soft tissues, the gastrointestinal or urinary tracts, and skin. In contrast to hemophilia, hemarthrosis is rare in these patients. Retroperitoneal hemorrhages and other life-threatening bleeding may appear suddenly. The overall mortality in untreated patients ranges from 8–22%, and most deaths occur within the first few weeks after presentation. The diagnosis is based on the prolonged aPTT with normal PT and TT and a mixing study that does not correct with normal pooled plasma. The Bethesda assay using factor specific-deficient plasma as performed for inhibitor detection in hemophilia will confirm the diagnosis. Treatment of acquired inhibitors of coagulation factors requires control of bleeding and eradication of the inhibitor. Many patients can have life-threatening bleeding. The use of activated “bypass products” such as aPCC or recombinant FVIIa is required. The use of recombinant porcine FVIII can be effective for acquired inhibitors of FVIII. The use of emicizumab to treat acquired FVIII inhibitors has been reported and trials in this population are underway in Europe.

In contrast to hemophilia, inhibitors in nonhemophilic patients are typically responsive to immune suppression, and therapy should be initiated early for most cases. High-dose intravenous γ -globulin and anti-CD20 monoclonal antibody are reported to be effective in patients with autoantibodies to FVIII; however, no firm evidence confirms that these alternatives are superior to the first line of immunosuppressive drugs (glucocorticoids and cyclophosphamide), effective in 70% of patients. Relapse of an inhibitor to FVIII is relatively common (up to 20%) within the first 6 months following withdrawal of immunosuppression; patients should be followed up regularly for relapse.

Topical plasma-derived bovine and human thrombin are commonly used during major cardiovascular, thoracic, neurologic, and pelvic surgeries as well as in trauma patients with extensive burns. Antibody formation to the xenoantigen or its contaminant (bovine clotting protein) has the potential to cross-react with human clotting factors, particularly FV and thrombin and can result in bleeding that can be life-threatening. The development of antibodies to FV with the use of topical preparations of recombinant human thrombin has also been reported. The clinical diagnosis of these acquired coagulopathies is rare but is often complicated by the fact that the bleeding episodes may be detectable during or immediately following major surgery and could be assumed to be due to the procedure itself.

The risk of developing a cross-reacting antibody is increased by repeated exposure to topical thrombin preparations. Thus, a careful medical history of previous surgical interventions that may have occurred even decades earlier is critical to assessing risk.

The laboratory abnormalities include a combined prolongation of the aPTT and PT that often fails to improve by transfusion of FFP and vitamin K, and a mixing study that does not correct with normal pooled plasma. The specificity of the antibody is determined by the measurement of the residual activity of human FV or other suspected human clotting factor. No assays specific for bovine thrombin coagulopathy are commercially available.

No treatment guidelines have been established. Platelet transfusions have been used as a source of FV replacement for patients with FV inhibitors. FFP and vitamin K supplementation may function as co-adjuvants rather than as effective treatments for the coagulopathy itself. Experience with recombinant FVIIa as a bypass agent is limited, and outcomes have been generally poor. Specific treatments to eradicate the antibodies based on immunosuppression with glucocorticoids, intravenous immunoglobulin, or serial plasmapheresis have been sporadically reported. Patients should be advised to avoid any topical thrombin sealant in the future.

The presence of lupus anticoagulant can be associated with venous or arterial thrombotic disease. However, bleeding has also been reported rarely with lupus anticoagulants due to antibodies to prothrombin, resulting in hypoprothrombinemia. Both disorders show a prolonged aPTT that does not correct on mixing. To distinguish acquired inhibitors from lupus anticoagulant, note that the dilute Russell viper venom time (dRVVT) and the hexagonal-phase phospholipids test will be negative in patients with an acquired inhibitor and positive in patients with lupus anticoagulants. Moreover, lupus anticoagulant interferes with the clotting activity of many factors (FVIII, FIX, FXI, FXII), which can be

assessed in the clinical laboratory; acquired inhibitors are specific to a single factor.

A

Valder Arruda and Katherine High wrote this chapter in prior editions and some material from their chapter is included here.

■ FURTHER READING

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Arterial and Venous Thrombosis

Jane E. Freedman, Joseph Loscalzo



OVERVIEW OF THROMBOSIS

■ GENERAL OVERVIEW

Thrombosis, the obstruction of blood flow due to the formation of clot, may result in tissue anoxia and damage, and it is a major cause of morbidity and mortality in a wide range of arterial and venous diseases and patient populations. As reported in 2020, 655,000 Americans die from heart disease each year, accounting for about 1 in 4 deaths. In 2017, coronary disease killed 365,914 people in the United States, and approximately 805,000 people experienced a heart attack and 795,000 had a stroke.

It is estimated that as many as 600,000 people each year have a pulmonary embolism or deep-venous thrombotic event, and 60,000–80,000 Americans die from these conditions annually. In the nondiseased state, physiologic hemostasis reflects a delicate interplay between factors that promote and inhibit blood clotting, favoring the former. This response is crucial as it prevents uncontrolled hemorrhage and exsanguination following injury. In specific settings, the same processes that regulate normal hemostasis can cause pathologic thrombosis, leading to arterial or venous occlusion. Importantly, many commonly used therapeutic interventions may also alter the thrombotic–hemostatic balance adversely.

Hemostasis and thrombosis primarily involve the interplay among three factors: the vessel wall, coagulation and fibrinolytic proteins, and platelets. Many prevalent acute vascular diseases are due to thrombus formation within a vessel, including myocardial infarction, thrombotic cerebrovascular events, and venous thrombosis. Although the end result is vessel occlusion and tissue ischemia, the pathophysiologic processes governing these pathologies have similarities as well as distinct differences. While many of the pathways regulating thrombus formation are similar to those that regulate hemostasis, the processes triggering or perpetuating thrombosis may be distinct and can vary in different clinical and genetic settings. In venous thrombosis, primary hypercoagulable states reflecting defects in the proteins governing coagulation and/or fibrinolysis or secondary hypercoagulable states involving abnormalities of blood vessels and blood flow or stasis lead to

thrombosis. By contrast, arterial thrombosis is highly dependent on the state of the vessel wall, the platelet, and factors related to blood flow.

ARTERIAL THROMBOSIS

OVERVIEW OF ARTERIAL THROMBOSIS

In arterial thrombosis, platelets and abnormalities of the vessel wall typically play a key role in vessel occlusion. Arterial thrombus forms via a series of sequential steps in which platelets adhere to the vessel wall, additional platelets are recruited, and thrombin is activated (Fig. 117-1). The regulation of platelet adhesion, activation, aggregation, and recruitment will be described in detail below. In addition, while the primary function of platelets is regulation of hemostasis, our understanding of their role in other processes, such as immunity, metastasis, wound healing, and inflammation, continues to evolve.

ARTERIAL THROMBOSIS AND VASCULAR DISEASE

Arterial thrombosis is a major cause of morbidity and mortality both in the United States and, increasingly, worldwide. Although the rates have declined in the United States, the overall burden remains high. Overall, in 2020, heart disease was estimated to cause about 1 of every 4 deaths in the United States. In addition to the 605,000 Americans who will have a new coronary event annually, an additional 200,000 myocardial infarctions occur in those with previous heart attacks. Although the rate of strokes has fallen, each year about 795,000 people experience a new or recurrent ischemic stroke. In 2018, about 1 in 6 deaths from cardiovascular disease were due to stroke in the United States.

THE PLATELET

Many processes in platelets have parallels with other cell types, such as the presence of specific receptors and signaling pathways; however, unlike most cells, platelets lack a nucleus and are unable to adapt to changing biologic settings by altered gene transcription. Platelets sustain limited protein synthetic capacity from megakaryocyte-derived and intracellularly transported messenger RNA (mRNA) and microRNA (miRNA). Most of the molecules needed to respond to various stimuli, however, are maintained in storage granules and membrane compartments.

Platelets are disc-shaped, very small, anucleate cells ($1\text{--}5 \mu\text{m}$ in diameter) that circulate in the blood at concentrations of $200\text{--}400,000/\mu\text{L}$, with an average life span of 7–10 days. Platelets are derived from megakaryocytes, polyploid hematopoietic cells found in the bone marrow. The primary regulator of platelet formation is thrombopoietin (TPO). The precise mechanism by which megakaryocytes produce and release fully formed platelets is unclear, but the process likely involves formation of proplatelets, pseudopod-like structures generated by the evagination of the cytoplasm from which platelets bud. After release into the circulation, (young, large) platelets may continue to divide. Platelet granules are synthesized in megakaryocytes before thrombopoiesis and contain an array of prothrombotic, proinflammatory, and antimicrobial mediators. The two major types of platelet granules, alpha and dense, are distinguished by their size, abundance, and content. Alpha-granules contain soluble coagulation proteins, adhesion molecules, growth factors, integrins, cytokines, and inflammatory modulators. Platelet dense-granules are smaller than alpha-granules

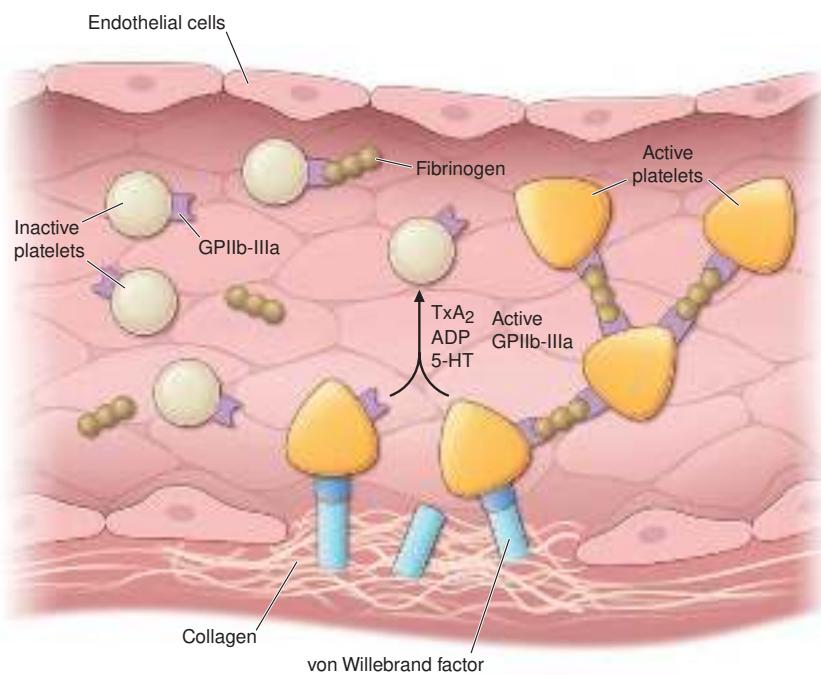


FIGURE 117-1 Platelet activation and thrombosis. Platelets circulate in an inactive form in the vasculature. Damage to the endothelium and/or external stimuli activates platelets that adhere to the exposed subendothelial von Willebrand factor and collagen. This adhesion leads to activation of the platelet, shape change, and the synthesis and release of thromboxane (TxA_2), serotonin (5-HT), and adenosine diphosphate (ADP). Platelet stimuli cause conformational change in the platelet integrin glycoprotein (GP) IIb/IIIa receptor, leading to the high-affinity binding of fibrinogen and the formation of a stable platelet thrombus.

and less abundant. Whereas alpha-granules contain proteins that may be more important in the inflammatory response, dense-granules contain high concentrations of small molecules, including adenosine diphosphate (ADP) and serotonin, that influence platelet aggregation and other related vascular processes, such as vasomotor tone.

Platelet Adhesion (See Fig. 117-1) The formation of a thrombus is initiated by the adherence of platelets to the damaged vessel wall. Damage exposes subendothelial components responsible for triggering platelet reactivity, including collagen, von Willebrand factor, fibronectin, and other adhesive proteins, such as vitronectin and thrombospondin. The hemostatic response may vary, depending on the extent of damage, the specific proteins exposed, and flow conditions. Certain proteins are expressed on the platelet surface that subsequently regulate collagen-induced platelet adhesion, particularly under flow conditions, and include glycoprotein (GP) IV, GPVI, and the integrin $\alpha_2\beta_1$. The platelet GPIb-IX-V complex adhesive receptor is central both to platelet adhesion and to the initiation of platelet activation. Damage to the blood vessel wall exposes subendothelial von Willebrand factor and collagen to the circulating blood. The GPIb-IX-V complex binds to the exposed von Willebrand factor, causing platelets to adhere (Fig. 117-1). In addition, the engagement of the GPIb-IX-V complex with ligand induces signaling pathways that lead to platelet activation. von Willebrand factor-bound GPIb-IX-V promotes a calcium-dependent conformational change in the GPIIb/IIIa receptor, transforming it from an inactive low-affinity state to an active high-affinity receptor for fibrinogen.

Platelet Activation The activation of platelets is controlled by a variety of surface receptors that regulate various functions in the activation process. Platelet receptors control many distinct processes and are stimulated by a wide variety of agonists and adhesive proteins that result in variable degrees of activation. In general terms, the stimulation of platelet receptors triggers two specific processes: (1) activation of internal signaling pathways that lead to further platelet activation and granule release, and (2) the capacity of the platelet to bind to other

adhesive proteins/platelets. Both of these processes contribute to the formation of a thrombus. Stimulation of nonthrombotic receptors results in platelet adhesion or interaction with other vascular cells, including endothelial cells, neutrophils, and mononuclear cells.

Many families and subfamilies of receptors are found on platelets that regulate a variety of platelet functions. These include the seven transmembrane receptor family, which is the main agonist-stimulated receptor family. Several seven transmembrane receptors are found on platelets, including the ADP receptors, prostaglandin receptors, lipid receptors, and chemokine receptors. Receptors for thrombin comprise the major seven transmembrane receptors found on platelets. Among this last group, the first identified was the protease activation receptor 1 (PAR1). The PAR class of receptors has a distinct mechanism of activation that involves specific cleavage of the N-terminus by thrombin, which, in turn, acts as a ligand for the receptor. Other PAR receptors are present on platelets, including PAR2 (not activated by thrombin) and PAR4. Adenosine receptors are responsible for transduction of ADP-induced signaling events, which are initiated by the binding of ADP to purinergic receptors on the platelet surface. There are several distinct ADP receptors, classified as P2X₁, P2Y₁, and P2Y₁₂. The activation of both the P2Y₁₂ and P2Y₁ receptors is essential for ADP-induced platelet aggregation. The thienopyridine derivatives, clopidogrel and prasugrel, are clinically used inhibitors of ADP-induced platelet aggregation.

Platelet Aggregation Activation of platelets results in a rapid series of signal transduction events, including tyrosine kinase, serine/threonine kinase, and lipid kinase activation. In unstimulated platelets, the major platelet integrin GPIIb/IIIa is maintained in an inactive conformation and functions as a low-affinity adhesion receptor for fibrinogen. This integrin is unique as it is only expressed on platelets. After stimulation, the interaction between fibrinogen and GPIIb/IIIa forms intercellular connections between platelets, leading to the formation of a platelet aggregate (Fig. 117-1). A calcium-sensitive conformational change in the extracellular domain of GPIIb/IIIa enables the high-affinity binding of soluble plasma fibrinogen as a result of a complex network of inside-out signaling events. The GPIIb/IIIa receptor serves as a bidirectional conduit with GPIIb/IIIa-mediated signaling (outside-in) occurring immediately after the binding of fibrinogen. This leads to additional intracellular signaling that further stabilizes the platelet aggregate and transforms platelet aggregation from a reversible to an irreversible process (inside-out).

THE ROLE OF PLATELETS AND THROMBOSIS IN INFLAMMATION

Inflammation plays an important role during the acute thrombotic phase of acute coronary and other vascular occlusive syndromes. In the setting of acute upper respiratory infections, people are at higher risk of myocardial infarction and thrombotic stroke. Patients with acute coronary syndromes have not only increased interactions between platelets (homotypic aggregates), but also increased interactions between platelets and leukocytes (heterotypic aggregates) detectable in circulating blood. These latter aggregates form when platelets are activated, often directly by pathogens, and adhere to circulating leukocytes as part of their contribution to the immune process. Platelets bind via P-selectin (CD62P) expressed on the surface of activated platelets to the leukocyte receptor, P-selectin glycoprotein ligand 1 (PSGL-1). This association leads to increased expression of CD11b/CD18 (Mac-1) on leukocytes, which amplifies immunity but may also support further interactions with platelets partially via bivalent fibrinogen linking this integrin with its platelet surface counterpart, GPIIb/IIIa. Platelet surface P-selectin also induces the expression of tissue factor on monocytes, which promotes fibrin formation.

In addition to platelet-monocyte aggregates, the immunomodulator, soluble CD40 ligand (CD40L or CD154), also reflects a link between thrombosis and inflammation. The CD40 ligand is a trimeric transmembrane protein of the tumor necrosis factor family and, with its receptor CD40, is an important contributor to the inflammatory process leading both to thrombosis and atherosclerosis. While many

immunologic and vascular cells have been found to express CD40 and/or CD40 ligand, in platelets, CD40 ligand is rapidly translocated to the surface after stimulation and is upregulated in the newly formed thrombus. The surface-expressed CD40 ligand is cleaved from the platelet to generate a soluble fragment (soluble CD40 ligand).

Links have also been established among platelets, infection, immunity, and inflammation. Bacterial and viral infections are associated with a transient increase in the risk of acute thrombotic events, such as acute myocardial infarction and stroke. In addition, platelets contribute significantly to the pathophysiology and high mortality rates of sepsis. The expression, functionality, and signaling pathways of Toll-like receptors (TLRs) have been established in platelets. Stimulation of platelet TLR2, TLR3, and TLR4 directly and indirectly activates the platelet's thrombotic and inflammatory responses, and live bacteria induce a proinflammatory response in platelets in a TLR2-dependent manner, suggesting a mechanism by which specific bacteria and bacterial components can directly activate platelet-dependent thrombosis. Additionally, viruses, such as SARS-CoV-2, HIV, hepatitis C virus, and Dengue, are also known to cause elevated levels of thrombosis, and recently, platelets have been shown to regulate immune responses to viruses via receptors TLR7 and TLR8.

Risk Factors for Arterial Thrombosis In addition to immune burden, various factors increase the risk of developing arterial thrombosis. Classically, the cardiovascular-dependent risk factors implicated in thrombosis have been hypertension, high levels of low-density lipoprotein cholesterol, and smoking. However, diabetes, pregnancy, age, and chemotherapeutic agents may also contribute to arterial thrombosis. Stillbirth and loss of multiple pregnancies may increase the risk of ischemic stroke and myocardial infarction as does hormonal replacement therapy. Systemic lupus erythematosus and rheumatoid arthritis are now well-recognized risks for thrombosis, and the former, in particular, may contribute in the pediatric population. The anti-phospholipid syndrome is also another widely recognized autoimmune prothrombotic risk for arterial (and venous) thrombosis.

GENETICS OF ARTERIAL THROMBOSIS

Some studies have associated arterial thrombosis with genetic variants (Table 117-1A); however, the associations have been weak and not confirmed in larger series. Platelet count and mean platelet volume have been studied by genome-wide association studies (GWAS), and this approach identified signals located to noncoding regions. Of 15 quantitative trait loci associated with mean platelet volume and platelet count, one located at 12q24 is also a risk locus for coronary artery disease.

In the area of genetic variability and platelet function, studies have primarily dealt with pharmacogenetics, the field of pharmacology dealing with the interindividual variability in drug response based on genetic determinants (Table 117-2). This focus has been driven by the wide variability among individuals in terms of response to antithrombotic drugs and the lack of a common explanation for this variance. The best described is the issue of "aspirin resistance," although heterogeneity for other antithrombotics (e.g., clopidogrel) has also been extensively examined. Primarily, platelet-dependent genetic determinants have been defined at the level of (1) drug effect, (2) drug compliance, and (3) drug metabolism. Many candidate platelet genes have been studied for their interaction with antiplatelet and antithrombotic agents.

Many patients have an inadequate response to the inhibitory effects of aspirin. Heritable factors contribute to the variability; however, ex vivo tests of residual platelet responsiveness after aspirin administration have not provided firm evidence for a pharmacogenetic interaction between aspirin and COX1 or other relevant platelet receptors. As such, currently, there is no clinical indication for genotyping to optimize aspirin's antiplatelet efficiency. For the platelet P2Y12 receptor inhibitor clopidogrel, additional data suggest that genetics may affect the drug's responsiveness and utility. The responsible genetic variant appears not to be the expected P2Y12 receptor but an enzyme responsible for drug metabolism. Clopidogrel is a prodrug, and liver metabolism by specific

TABLE 117-1 Heritable Causes of Arterial and Venous Thrombosis

A. Arterial Thrombosis**Platelet Receptors**

$\beta 3$ and $\alpha 2$ integrins
 $P_1 A2$ polymorphism
 $Fc(\gamma)$ RIA
 $GPIV T13254C$ polymorphism
 $GPIb$

Thrombin receptor PAR1-5061 → D

Redox Enzymes

Plasma glutathione peroxidase, GPx3, promoter haplotype H2
H2 promoter haplotype
Endothelial nitric oxide synthase
-786T/C, -922A/G, -1468T/A
Paraoxonase
-107T allele, 192R allele

Homocysteine

Cystathione β -synthase 833T → C
5,10-Methylene tetrahydrofolate reductase (MTHFR) 677C → T

B. Venous Thrombosis**Procoagulant Proteins**

Fibrinogen
-455G/A, -854G/A
Prothrombin (20210G → A)

Protein C Anticoagulant Pathway

Factor V Leiden: 1691G → A (Arg506Gln)
Thrombomodulin 1481C → T (Ala455Val)

Fibrinolytic Proteins with Known Polymorphisms

Tissue plasminogen activator (tPA)
7351C/T, 20 099T/C in exon 6, 27 445T/A in intron 10
Plasminogen activator inhibitor (PAI-1)
4G/5G insertion/deletion polymorphism at position -675
Homocysteine
Cystathione β -synthase 833T → C
5,10-MTHFR 677C → T

cytochrome P450 enzymes is required for activation. The genes encoding the CYP-dependent oxidative steps are polymorphic, and carriers of specific alleles of the CYP2C19 and CYP3A4 loci have increased platelet aggregability. Increased platelet activity has also been specifically associated with the CYP2C19 2 allele, which causes loss of platelet function in select patients. Because these are common genetic variants, this observation has been shown to be clinically relevant in large studies. In summary, although the loss-of-function polymorphism in CYP2C19 is the strongest individual variable affecting pharmacokinetics and antiplatelet response to clopidogrel, it only accounts for 5–12% of the variability in ADP-induced platelet aggregation on clopidogrel. In addition, genetic variables do not appear to contribute significantly to the clinical outcomes of patients treated with the P2Y12 receptor antagonists prasugrel or ticagrelor.

TABLE 117-2 Genetic Variation and Pharmacogenetic Responses to Platelet Inhibitors

POTENTIAL GENE ALTERED	TARGET THERAPEUTIC CLASS	SPECIFIC DRUG
<i>P2Y1</i> and <i>P2Y12</i> , <i>CYP2C19</i> , <i>CYP3A4</i> , <i>CYP3A5</i>	ADP receptor inhibitors	Clopidogrel, prasugrel
<i>COX1</i> , <i>COX2</i>	Cyclooxygenase inhibitors	Aspirin
<i>PIA1/A2</i>	Receptor inhibitors	Abciximab, eptifibatide, tirofiban
<i>INTB3</i> , <i>GPIbA</i>	Glycoprotein IIb-IIIa receptor inhibitors	

VENOUS THROMBOSIS**OVERVIEW OF VENOUS THROMBOSIS**

Coagulation is the process by which thrombin is activated and soluble plasma fibrinogen is converted into insoluble fibrin. These steps account for both normal hemostasis and the pathophysiologic processes influencing the development of venous thrombosis. The primary forms of venous thrombosis are deep-vein thrombosis (DVT) in the extremities and the subsequent embolization to the lungs (pulmonary embolism [PE]), referred to together as venous thromboembolic disease (VTE). Although the majority of venous thromboembolic events occur as PE or DVT of the lower extremities, up to 10% of events may occur in other vascular locations. Venous thrombosis occurs due to heritable causes (Table 117-1B) and acquired causes (Table 117-3).

DEEP VENOUS THROMBOSIS AND PULMONARY EMBOLISM

It is estimated that DVT or PE occurs in ~1–2 individuals per 1000 each year, resulting in 300,000–600,000 new cases of VTE each year in the United States. Approximately, 60,000–80,000 deaths are attributed to DVT or PE annually. Of new cases, up to 30% of patients die within 30 days and one-fifth suffer sudden death due to PE; 30% go on to develop recurrent VTE within 10 years. Data from the Atherosclerosis Risk in Communities (ARIC) study reported a 9% 28-day fatality rate from DVT and a 15% fatality rate from PE. PE in the setting of cancer has a 25% fatality rate. The mean incidence of first DVT in the general population is 5 per 10,000 person-years; the incidence is similar in males and females when adjusting for factors related to reproduction and birth control and increases dramatically with age from 2–3 per 10,000 person-years at 30–49 years of age to 20 per 10,000 person-years at 70–79 years of age.

OVERVIEW OF THE COAGULATION CASCADE AND ITS ROLE IN VENOUS THROMBOSIS

Coagulation is defined as the formation of fibrin by a series of linked enzymatic reactions in which each reaction product converts the subsequent inactive zymogen into an active serine protease (Fig. 117-2). This coordinated sequence is called the coagulation cascade and is a key mechanism for regulating hemostasis. Central to the function of the coagulation cascade is the principle of amplification: due to a series of linked enzymatic reactions, a small stimulus can lead to much greater quantities of fibrin, the end product that prevents hemorrhage at the site of vascular injury. In addition to the known risk factors relevant to hypercoagulopathy, stasis, and vascular dysfunction, newer areas of research have identified contributions from procoagulant microparticles, inflammatory cells, microvesicles, and fibrin structure.

The coagulation cascade is primarily initiated by vascular injury exposing tissue factor to blood components (Fig. 117-2). Tissue factor may also be found in bloodborne cell-derived microparticles and, under pathophysiologic conditions, in leukocytes or platelets. Plasma

TABLE 117-3 Acquired Causes of Venous Thrombosis

Surgery
Neurosurgery
Major abdominal surgery
Malignancy
Antiphospholipid syndrome
Other
Trauma
Pregnancy
Long-distance travel
Obesity
Oral contraceptives/hormone replacement
Myeloproliferative disorders
Polycythemia vera

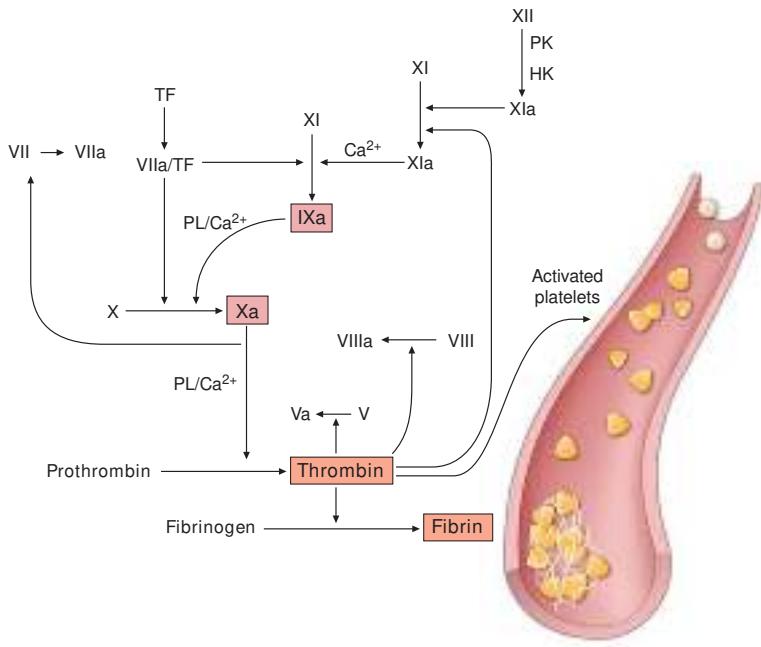


FIGURE 117-2 Summary of the coagulation pathways. Specific coagulation factors (“a” indicates activated form) are responsible for the conversion of soluble plasma fibrinogen into insoluble fibrin. This process occurs via a series of linked reactions in which the enzymatically active product subsequently converts the downstream inactive protein into an active serine protease. In addition, the activation of thrombin leads to stimulation of platelets. HK, high-molecular-weight kininogen; PK, prekallikrein; TF, tissue factor.

factor VII (FVII) is the ligand for and is activated (FVIIa) by binding to tissue factor exposed at the site of vessel damage. The binding of FVII/FVIIa to tissue factor activates the downstream conversion of factor X (FX) to active FX (FXa). In an alternative reaction, the FVII/FVIIa-tissue factor complex initially converts FIX to FIXa, which then activates FX in conjunction with its cofactor factor VIII (FVIIIa). Factor Xa with its cofactor FVa converts prothrombin to thrombin, which then converts soluble plasma fibrinogen to insoluble fibrin, leading to clot or thrombus formation. Thrombin also activates FXIII to FXIIIa, a transglutaminase that covalently cross-links and stabilizes the fibrin clot. Formation of thrombi is affected by mechanisms governing fibrin structure and stability, including specific fibrinogen variants and how they alter fibrin formation, strength, and structure.

Several antithrombotic factors also regulate coagulation; these include antithrombin, tissue factor pathway inhibitor (TFPI), heparin cofactor II, and protein C/protein S. Under normal conditions, these factors limit the production of thrombin to prevent the perpetuation of coagulation and thrombus formation. Typically, after the clot has caused occlusion at the damaged site and begins to expand toward adjacent uninjured vessel segments, the anticoagulant reactions governed by the normal endothelium become pivotal in limiting the extent of this hemostatically protective clot.

RISK FACTORS FOR VENOUS THROMBOSIS

An array of different factors contributes to the risk of VTE, and it is notable that women and men of all ages, races, and ethnicities are at risk for VTE. The risk factors for venous thrombosis are primarily related to hypercoagulability, which can be genetic (Table 117-1) or acquired, or due to immobilization and venous stasis. Independent predictors for recurrence include increasing age, obesity, malignant neoplasm, and acute extremity paresis. It is estimated that 5–8% of the U.S. population has a genetic risk factor known to predispose to venous thrombosis. Often, multiple risk factors are present in a single individual. Significant risk is incurred by major orthopedic, abdominal, or neurologic surgeries. Cancer patients have an approximately fourfold increased risk of VTE as compared with the general population, and cancer patients with VTE have reduced survival.

Hospitalized patients have a greatly increased risk of venous thrombosis with risk factors (increased age, male, ethnicity) and comorbid conditions, including infection, renal disease, and weight loss. Community- or hospital-acquired infection is also associated with increased risk of VTE. Supportive of this, nearly 20% of hospitalized COVID-19 patients are noted to have coagulation abnormalities as well as increased PE, DVT, and peripheral thrombotic risk. Moderate risk is promoted by prolonged bedrest, certain types of cancer, pregnancy, hormone replacement therapy or oral contraceptive use, and other sedentary conditions such as long-distance plane travel. It has been reported that the risk of developing a venous thromboembolic event doubles after air travel lasting 4 h, although the absolute risk remains low (1 in 6000). The relative risk of VTE among pregnant or postpartum women is 4.3, and the overall incidence (absolute risk) is 199.7 per 100,000 woman-years.

GENETICS OF VENOUS THROMBOSIS

(See Table 117-2) Less common causes of venous thrombosis are those due to genetic variants. These abnormalities include loss-of-function mutations of endogenous anticoagulants as well as gain-of-function mutations of procoagulant proteins. Heterozygous antithrombin deficiency and homozygosity of the factor V Leiden mutation significantly increase the risk of venous thrombosis. While homozygous protein C or protein S deficiencies are rare and may

lead to fatal purpura fulminans, heterozygous deficiencies are associated with a moderate risk of thrombosis. Activated protein C impairs coagulation by proteolytic degradation of FVa. Patients resistant to the activity of activated protein C may have a point mutation in the FV gene located on chromosome 1, a mutant denoted factor V Leiden. Mildly increased risk has been attributed to elevated levels of procoagulant factors, as well as low levels of tissue factor pathway inhibitor. Polymorphisms of methylene tetrahydrofolate reductase as well as hyperhomocysteinemia have been shown to be independent risk factors for venous thrombosis, as well as arterial vascular disease; however, many of the initial descriptions of genetic variants and their associations with thromboembolism are being questioned in larger, more contemporary studies.

FIBRINOLYSIS AND THROMBOSIS

Specific abnormalities in the fibrinolytic system have been associated with enhanced thrombosis. Factors such as elevated levels of tissue plasminogen activator (tPA) and plasminogen activator inhibitor type 1 (PAI-1) have been associated with decreased fibrinolytic activity and an increased risk of arterial thrombotic disease. Specific genetic variants have been associated with decreased fibrinolytic activity, including the 4G/5G insertion/deletion polymorphism in the *PAI-1* gene. Additionally, the 311-bp Alu insertion/deletion in tPA's intron 8 has been associated with enhanced thrombosis; however, genetic abnormalities have not been associated consistently with altered function or tPA levels, raising questions about the relevant pathophysiologic mechanism. Thrombin-activatable fibrinolysis inhibitor (TAFI) is a carboxypeptidase that regulates fibrinolysis; elevated plasma TAFI levels have been associated with an increased risk of both DVT and cardiovascular disease.

The metabolic syndrome also is accompanied by altered fibrinolytic activity. This syndrome, which comprises abdominal fat (central obesity), altered glucose and insulin metabolism, dyslipidemia, and hypertension, has been associated with atherosclerosis. The mechanism for enhanced thrombosis appears to be due both to altered platelet function and to a procoagulant and hypofibrinolytic state. One of the most frequently documented prothrombotic abnormalities reported in this syndrome is an increase in plasma levels of PAI-1.

In addition to contributing to platelet function, inflammation plays a role in both coagulation-dependent thrombus formation and thrombus resolution. Both polymorphonuclear neutrophils and monocytes/macrophages contribute to multiple overlapping thrombotic functions, including fibrinolysis, chemokine and cytokine production, and phagocytosis.

THE DISTINCTION BETWEEN ARTERIAL AND VENOUS THROMBOSIS

Although there is overlap, venous thrombosis and arterial thrombosis are initiated differently, and clot formation progresses by somewhat distinct pathways. In the setting of stasis or states of hypercoagulability, venous thrombosis is activated with the initiation of the coagulation cascade primarily due to exposure of tissue factor; this leads to the formation of thrombin and the subsequent conversion of fibrinogen to fibrin. In the artery, thrombin formation also occurs, but thrombosis is primarily promoted by the adhesion of platelets to an injured vessel and stimulated by exposed extracellular matrix (Figs. 117-1 and 117-2). There is wide variation in individual responses to vascular injury, an important determinant of which is the predisposition an individual has to arterial or venous thrombosis. This concept has been supported indirectly in prothrombotic animal models in which there is poor correlation between the propensity to develop venous versus arterial thrombosis.

Despite considerable progress in understanding the role of hypercoagulable states in VTE, the contribution of hypercoagulability to arterial vascular disease is much less well understood. Although specific thrombophilic conditions, such as factor V Leiden and the prothrombin G20210A mutation, are risk factors for DVT, pulmonary embolism, and other venous thromboembolic events, their contribution to arterial thrombosis is less well defined. In fact, to the contrary, many of these thrombophilic factors have not been found to be clinically important risk factors for arterial thrombotic events, such as acute coronary syndromes.

Clinically, although the pathophysiology is distinct, arterial and venous thrombosis do share common risk factors, including age, obesity, cigarette smoking, diabetes mellitus, arterial hypertension, hyperlipidemia, and metabolic syndrome. Select genetic variants, including those of the glutathione peroxidase-3 (GPx3) gene, have also been associated with arterial and venous thrombo-occlusive disease. Importantly, arterial and venous thrombosis may both be triggered by pathophysiologic stimuli responsible for activating inflammatory and oxidative pathways.

The diagnosis and treatment of ischemic heart disease are discussed in Chap. 273. Stroke diagnosis and management are discussed in Chap. 307. The diagnosis and management of DVT and PE are discussed in Chap. 279.

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Antiplatelet, Anticoagulant, and Fibrinolytic Drugs

Jeffrey I. Weitz



Thromboembolic disorders are major causes of morbidity and mortality. Thrombosis can occur in arteries or veins. Arterial thrombosis is the most common cause of acute myocardial infarction (MI), ischemic stroke, and limb gangrene. Venous thromboembolism encompasses deep vein thrombosis (DVT), which can lead to postthrombotic syndrome, and pulmonary embolism (PE), which can be fatal or can result in chronic thromboembolic pulmonary hypertension.

Most arterial thrombi are superimposed on disrupted atherosclerotic plaque because plaque rupture exposes thrombogenic material in the core to the blood. This material then triggers platelet aggregation and fibrin formation, which results in the generation of a platelet-rich thrombus that can temporarily or permanently occlude blood flow. In contrast, venous thrombi rarely form at sites of obvious vascular disruption. Although they can develop after surgical trauma to veins or secondary to indwelling venous catheters, venous thrombi usually originate in the valve cusps of the deep veins of the calf or in the muscular sinuses. Sluggish blood flow reduces the oxygen supply to the avascular valve cusps. Endothelial cells lining these valve cusps become activated and express adhesion molecules on their surface. Tissue factor-bearing leukocytes and microvesicles adhere to these activated cells and induce coagulation. DNA extruded from neutrophils forms neutrophil extracellular traps (NETs) that provide a scaffold that binds platelets and promotes their activation and aggregation and activate factor XII. Local thrombus formation is exacerbated by reduced clearance of activated clotting factors because of impaired blood flow. If the thrombi extend from the calf veins into the popliteal and more proximal veins of the leg, thrombus fragments can dislodge, travel to the lungs, and produce a PE.

Arterial and venous thrombi are composed of platelets, fibrin, and trapped red blood cells, but the proportions differ. Arterial thrombi are rich in platelets because of the high shear in the injured arteries. In contrast, venous thrombi, which form under low shear conditions, contain relatively few platelets and are predominantly composed of fibrin and trapped red cells. Because of the predominance of platelets, arterial thrombi appear white, whereas venous thrombi are red in color, reflecting the trapped red cells.

Antithrombotic drugs are used for prevention and treatment of thrombosis. Targeting the components of thrombi, these agents include (1) antiplatelet drugs, (2) anticoagulants, and (3) fibrinolytic agents (Fig. 118-1). With the predominance of platelets in arterial thrombi, strategies to attenuate arterial thrombosis focus mainly on antiplatelet agents, although, in the acute setting, they may include anticoagulants and fibrinolytic agents. The addition of low-dose rivaroxaban, an oral factor Xa inhibitor, to dual-antiplatelet therapy reduces recurrent ischemic events and stent thrombosis in patients with acute coronary syndrome, whereas its addition to aspirin reduces the risk of major adverse coronary and limb events in patients with stable coronary or peripheral artery disease. These findings highlight the utility of combining low

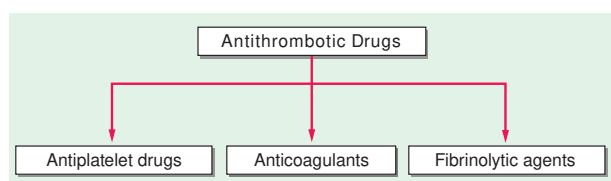


FIGURE 118-1 Classification of antithrombotic drugs.

dose anticoagulants with antiplatelet agents for secondary prevention in patients at risk for recurrent atherothrombotic events.

Anticoagulants are the mainstay of prevention and treatment of venous thromboembolism because fibrin is the predominant component of venous thrombi. Antiplatelet drugs are less effective than anticoagulants in this setting because of the limited platelet content of venous thrombi. Fibrinolytic therapy is used in selected patients with venous thromboembolism. For example, patients with massive PE can benefit from systemic or catheter-directed fibrinolytic therapy. Pharmacomechanical therapy also is used to restore blood flow in patients with extensive DVT involving the iliac and/or femoral veins.

ANTIPLATELET DRUGS

■ ROLE OF PLATELETS IN ARTERIAL THROMBOSIS

In healthy vasculature, circulating platelets are maintained in an inactive state by nitric oxide (NO) and prostacyclin released by endothelial cells lining the blood vessels. In addition, endothelial cells also express CD39 on their surface, a membrane-associated ecto-adenosine diphosphatase (ADPase) that degrades ADP released from activated platelets. When the vessel wall is damaged, release of these substances is impaired and subendothelial matrix is exposed. Platelets adhere to exposed collagen via $\alpha_2\beta_1$ and glycoprotein (Gp) V_IV and to von Willebrand factor (VWF) via Gp Ib α and Gp IIb/IIIa ($\alpha_{IIb}\beta_3$)—receptors that are constitutively expressed on the platelet surface. Adherent platelets undergo a change in shape, secrete ADP from their dense granules, and synthesize and release thromboxane A₂. Released ADP and thromboxane A₂, which are platelet agonists, activate ambient platelets and recruit them to the site of vascular injury (Fig. 118-2).

Disruption of the vessel wall also exposes tissue factor-expressing cells to the blood. Tissue factor binds factor VIIa and initiates coagulation. Activated platelets potentiate coagulation by providing a surface that binds clotting factors and supports the assembly of activation complexes that enhance thrombin generation. In addition to converting fibrinogen to fibrin, thrombin serves as a potent platelet agonist and recruits more platelets to the site of vascular injury. Thrombin also amplifies its own generation by feedback activation of factors V, VIII,

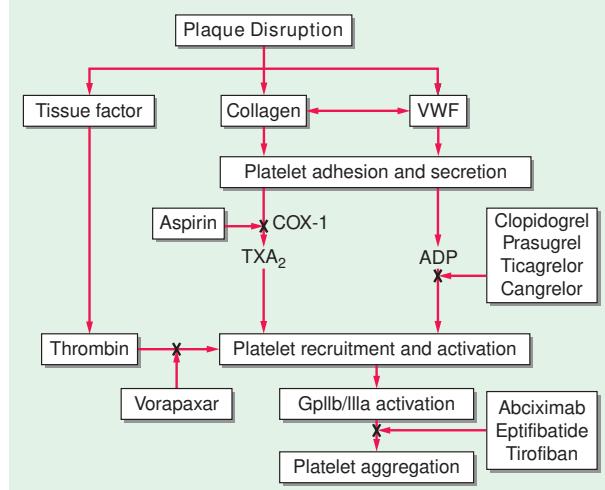


FIGURE 118-3 Site of action of antiplatelet drugs. Aspirin inhibits thromboxane A₂ (TXA₂) synthesis by irreversibly acetylating cyclooxygenase-1 (COX-1). Reduced TXA₂ release attenuates platelet activation and recruitment to the site of vascular injury. Clopidogrel and prasugrel irreversibly block P2Y₁₂, a key ADP receptor on the platelet surface; cangrelor and ticagrelor are reversible inhibitors of P2Y₁₂. Abciximab, eptifibatide, and tirofiban inhibit the final common pathway of platelet aggregation by blocking fibrinogen and von Willebrand factor binding to activated glycoprotein (Gp) IIb/IIIa. Vorapaxar inhibits thrombin-mediated platelet activation by targeting protease-activated receptor-1 (PAR-1), the major thrombin receptor on human platelets.

and XI and solidifies the fibrin network by activating factor XIII, which then cross-links the fibrin strands.

When platelets are activated, Gp IIb/IIIa, the most abundant receptor on the platelet surface, undergoes a conformational change that enables it to bind fibrinogen and, under high shear conditions, VWF. Divalent fibrinogen or multivalent VWF molecules bridge adjacent platelets together to form platelet aggregates. Fibrin strands, generated through the action of thrombin, then weave these aggregates together to form a platelet/fibrin mesh.

Antiplatelet drugs target various steps in this process. The commonly used drugs include aspirin, ADP receptor inhibitors, which include the thienopyridines (clopidogrel and prasugrel) as well as ticagrelor and cangrelor, dipyridamole, Gp IIb/IIIa antagonists, and vorapaxar.

■ ASPIRIN

The most widely used antiplatelet agent worldwide is aspirin. As a cheap and effective antiplatelet drug, aspirin serves as the foundation of most antiplatelet strategies.

Mechanism of Action Aspirin produces its antithrombotic effect by irreversibly acetylating and inhibiting platelet cyclooxygenase (COX)-1 (Fig. 118-3), a critical enzyme in the biosynthesis of thromboxane A₂. At high doses (~1 g/d), aspirin also inhibits COX-2, an inducible COX isoform found in endothelial cells and inflammatory cells. In endothelial cells, COX-2 initiates the synthesis of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation.

Indications Aspirin is widely used for secondary prevention of cardiovascular events in patients with established coronary artery, cerebral artery, or peripheral artery disease. Compared with placebo in this setting, aspirin produces a 25% reduction in the risk of cardiovascular death, MI, or stroke. Use of aspirin for primary prevention is controversial. Recent studies have questioned whether the benefits of daily aspirin for primary cardiac protection outweigh its associated risks for gastrointestinal and intracerebral hemorrhage. Consequently, aspirin is no longer recommended for primary cardiac prevention unless the baseline cardiovascular risk is at least 1% per year and 10% at 10 years and patients are at low risk for bleeding.

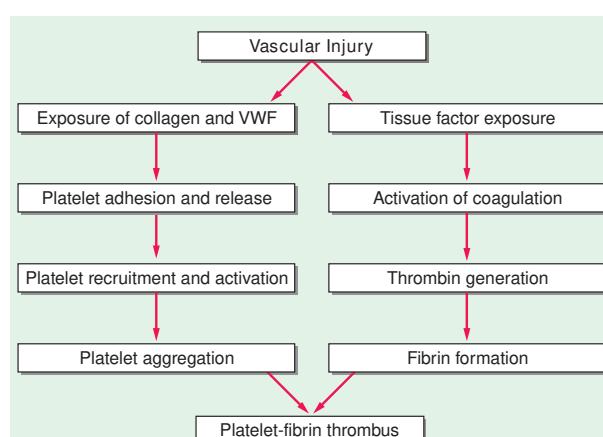


FIGURE 118-2 Coordinated role of platelets and the coagulation system in thrombogenesis. Vascular injury simultaneously triggers platelet activation and aggregation and activation of the coagulation system. Platelet activation is initiated by exposure of subendothelial collagen and von Willebrand factor (VWF), onto which platelets adhere. Adherent platelets become activated and release ADP and thromboxane A₂, platelet agonists that activate ambient platelets and recruit them to the site of injury. When platelets are activated, glycoprotein IIb/IIIa on their surface undergoes a conformational change that enables it to ligate fibrinogen and/or VWF and mediate platelet aggregation. Coagulation is triggered by tissue factor exposed at the site of injury. Tissue factor triggers thrombin generation. As a potent platelet agonist, thrombin amplifies platelet recruitment to the site of injury. Thrombin also converts fibrinogen to fibrin, and the fibrin strands then weave the platelet aggregates together to form a platelet/fibrin thrombus.

Dosages Aspirin is usually administered at doses of 75–325 mg once daily. Higher doses of aspirin are not more effective than lower aspirin doses, and some analyses suggest reduced efficacy with higher doses. Because the side effects of aspirin are dose-related, daily aspirin doses of 75–100 mg are recommended for most indications. When rapid platelet inhibition is required, an initial aspirin dose of at least 160 mg should be given.

Side Effects The most common side effects are gastrointestinal and range from dyspepsia to erosive gastritis or peptic ulcers with bleeding and perforation. These side effects are dose-related. Use of enteric-coated or buffered aspirin in place of plain aspirin does not eliminate gastrointestinal side effects. The overall risk of major bleeding with aspirin is 1–3% per year. The risk of bleeding is increased two- to threefold when aspirin is given in conjunction with other antiplatelet drugs, such as clopidogrel, or with anticoagulants, such as warfarin. When dual or triple therapy is prescribed, low-dose aspirin should be given (75–100 mg daily). Eradication of *Helicobacter pylori* infection and administration of proton pump inhibitors may reduce the risk of aspirin-induced upper gastrointestinal bleeding in patients with peptic ulcer disease.

Aspirin should not be administered to patients with a history of aspirin allergy characterized by bronchospasm. This problem occurs in ~0.3% of the general population but is more common in those with chronic urticaria or asthma, particularly in individuals with nasal polyps or chronic rhinitis. Hepatic and renal toxicity are observed with aspirin overdose.

Aspirin Resistance Clinical aspirin resistance is defined as the failure of aspirin to protect patients from ischemic vascular events. This is not a helpful definition because it is made after the event occurs. Furthermore, it is not realistic to expect aspirin, which only blocks thromboxane A₂-induced platelet activation, to prevent all vascular events.

Aspirin resistance has also been described biochemically as failure of the drug to produce its expected inhibitory effects on tests of platelet function, such as thromboxane A₂ synthesis or arachidonic acid-induced platelet aggregation. Potential causes of aspirin resistance include poor compliance, reduced absorption, drug-drug interaction with ibuprofen, and overexpression of COX-2. Unfortunately, the tests for aspirin resistance have not been well standardized, and there is little evidence that they identify patients at increased risk of recurrent vascular events, or that resistance can be reversed by giving higher doses of aspirin or by adding other antiplatelet drugs. Until such information is available, testing for aspirin resistance remains a research tool.

■ ADP RECEPTOR ANTAGONISTS

The ADP receptor antagonists include the thienopyridines (clopidogrel and prasugrel) as well as ticagrelor and cangrelor. All of these drugs target P2Y₁₂, the key ADP receptor on platelets.

Thienopyridines • MECHANISM OF ACTION The thienopyridines are structurally related drugs that selectively inhibit ADP-induced platelet aggregation by irreversibly blocking P2Y₁₂ (Fig. 118-3). Clopidogrel and prasugrel are prodrugs that require metabolic activation by the hepatic cytochrome P450 (CYP) enzyme system. Prasugrel is about 10-fold more potent than clopidogrel and has a more rapid onset of action because of better absorption and more streamlined metabolic activation.

INDICATIONS When compared with aspirin in patients with recent ischemic stroke, recent MI, or a history of peripheral arterial disease, clopidogrel reduced the risk of cardiovascular death, MI, and stroke by 8.7%. Therefore, clopidogrel is more effective than aspirin but is also more expensive. Clopidogrel and aspirin are often combined to capitalize on their capacity to block complementary pathways of platelet activation. For example, the combination of aspirin plus clopidogrel is recommended for at least 4 weeks after implantation of a bare metal stent in a coronary artery and for at least a year in those with a drug-eluting stent. Concerns about late in-stent thrombosis with drug-eluting stents have led some experts to recommend long-term use of clopidogrel plus aspirin for the latter indication.

The combination of clopidogrel and aspirin is also effective in patients with unstable angina. Thus, in 12,562 such patients, the risk of cardiovascular death, MI, or stroke was 9.3% in those randomized to the combination of clopidogrel and aspirin and 11.4% in those given aspirin alone. This 20% relative risk reduction with combination therapy was highly statistically significant. However, combining clopidogrel with aspirin increases the risk of major bleeding to about 2% per year. This bleeding risk persists even if the daily dose of aspirin is ≤100 mg. Therefore, the combination of clopidogrel and aspirin should only be used when there is a clear benefit. For example, this combination has not proven to be superior to clopidogrel alone in patients with acute ischemic stroke or to aspirin alone for primary prevention in those at risk for cardiovascular events.

Prasugrel was compared with clopidogrel in 13,608 patients with acute coronary syndromes who were scheduled to undergo percutaneous coronary intervention. The incidence of the primary efficacy endpoint, a composite of cardiovascular death, MI, or stroke, was significantly lower with prasugrel than with clopidogrel (9.9% and 12.1%, respectively), mainly reflecting a reduction in the incidence of nonfatal MI. The incidence of stent thrombosis also was significantly lower with prasugrel (1.1% and 2.4%, respectively). However, these advantages were at the expense of significantly higher rates of fatal bleeding (0.4% and 0.1%, respectively) and life-threatening bleeding (1.4% and 0.9%, respectively) with prasugrel. Because patients older than age 75 years and those with a history of prior stroke or transient ischemic attack have a particularly high risk of bleeding, prasugrel should generally be avoided in older patients, and the drug is contraindicated in those with a history of cerebrovascular disease. Caution is required if prasugrel is used in patients weighing less than 60 kg or in those with renal impairment.

When prasugrel was compared with clopidogrel in 7243 patients with unstable angina or MI without ST-segment elevation, prasugrel failed to reduce the rate of the primary efficacy endpoint, which was a composite of cardiovascular death, MI, and stroke. Because of the negative results of this study, prasugrel is reserved for patients undergoing percutaneous coronary intervention. In this setting, prasugrel is usually given in conjunction with aspirin. To reduce the risk of bleeding, the daily aspirin dose should be ≤100 mg.

For patients with noncardioembolic stroke or high-risk transient ischemic attack, the combination of clopidogrel or ticagrelor plus aspirin for 21–30 days followed by aspirin alone thereafter reduces the risk of stroke, MI, and vascular death by up to 30% compared with aspirin alone. Therefore, dual antiplatelet therapy is often administered for the first 3–4 weeks in such patients.

DOSING Clopidogrel is given once daily at a dose of 75 mg. Loading doses of clopidogrel are given when rapid ADP receptor blockade is desired. For example, patients undergoing coronary stenting are often given a loading dose of 300–600 mg, which produces inhibition of ADP-induced platelet aggregation in about 4–6 h. After a loading dose of 60 mg, prasugrel is given once daily at a dose of 10 mg. Patients older than age 75 years or weighing less than 60 kg should receive a lower daily prasugrel dose of 5 mg.

SIDE EFFECTS The most common side effect of clopidogrel and prasugrel is bleeding. Because of its greater potency, bleeding is more common with prasugrel than clopidogrel. To reduce the risk of bleeding, clopidogrel and prasugrel should be stopped 5–7 days before major surgery. In patients taking clopidogrel or prasugrel who present with serious bleeding, platelet transfusion may be helpful.

Hematologic side effects, including neutropenia, thrombocytopenia, and thrombotic thrombocytopenic purpura, are rare.

THIENOPYRIDINE RESISTANCE The capacity of clopidogrel to inhibit ADP-induced platelet aggregation varies among subjects. This variability reflects, at least in part, genetic polymorphisms in the CYP isoenzymes involved in the metabolic activation of clopidogrel. Most important of these is CYP2C19. Clopidogrel-treated patients with the loss-of-function *CYP2C19* 2 allele exhibit reduced platelet inhibition compared with those with the wild-type *CYP2C19* 1 allele and experience a higher rate of cardiovascular events. This is important because

estimates suggest that up to 25% of whites, 30% of African Americans, and 50% of Asians carry the loss-of-function allele, which would render them resistant to clopidogrel. Even patients with the reduced function *CYP2C19* 3, 4, or 5 alleles may derive less benefit from clopidogrel than those with the full-function *CYP2C19**1 allele. Concomitant administration of clopidogrel with proton pump inhibitors, which are inhibitors of *CYP2C19*, produces a small reduction in the inhibitory effects of clopidogrel on ADP-induced platelet aggregation. The extent to which this interaction increases the risk of cardiovascular events remains controversial.

In contrast to their effect on the metabolic activation of clopidogrel, *CYP2C19* polymorphisms appear to be less important determinants of the activation of prasugrel. Thus, no association was detected between the loss-of-function allele and decreased platelet inhibition or increased rate of cardiovascular events with prasugrel. The observation that genetic polymorphisms affecting clopidogrel absorption or metabolism influence clinical outcomes raises the possibilities that pharmacogenetic profiling may be useful to identify clopidogrel-resistant patients and that point-of-care assessment of the extent of clopidogrel-induced platelet inhibition may help detect patients at higher risk for subsequent cardiovascular events. Clinical trials designed to evaluate these possibilities have thus far been negative. Although administration of higher doses of clopidogrel can overcome a reduced response to clopidogrel, the clinical benefit of this approach is uncertain. Instead, prasugrel or ticagrelor may be better choices for these patients.

Ticagrelor As an orally active inhibitor of P2Y₁₂, ticagrelor differs from the thienopyridines in that ticagrelor does not require metabolic activation and it produces reversible inhibition of the ADP receptor.

MECHANISM OF ACTION Like the thienopyridines, ticagrelor inhibits P2Y₁₂. Because it does not require metabolic activation, ticagrelor has a more rapid onset and offset of action than clopidogrel, and it produces greater and more predictable inhibition of ADP-induced platelet aggregation than clopidogrel.

INDICATIONS Ticagrelor is indicated for the secondary prevention of atherothrombotic events in patients with an acute coronary syndrome treated medically or with percutaneous coronary intervention (PCI) with or without stent implantation or with coronary artery bypass graft (CABG) surgery. Ticagrelor also is indicated for up to 3 years for secondary prevention in patients with a prior history of MI at least one year ago who are at high risk for atherothrombotic events. For patients with acute coronary syndrome undergoing PCI, guidelines give preference to ticagrelor over clopidogrel. Guidelines give preference to ticagrelor over clopidogrel, particularly in higher risk patients.

DOSING Ticagrelor is initiated with an oral loading dose of 180 mg followed by 90 mg twice daily. The dose does not require adjustment in patients with renal impairment, but the drug should be used with caution in patients with hepatic disease and in those receiving potent inhibitors or inducers of CYP3A4 because ticagrelor is metabolized in the liver via CYP3A4. Ticagrelor is usually administered in conjunction with aspirin; the daily aspirin dose should not exceed 100 mg.

SIDE EFFECTS In addition to bleeding, the most common side effects of ticagrelor are dyspnea, which can occur in up to 15% of patients, and asymptomatic ventricular pauses. The dyspnea, which tends to occur soon after initiating ticagrelor, is usually self-limiting and mild in intensity. The mechanism responsible for this side effect is unknown.

To reduce the risk of bleeding, ticagrelor should be stopped at least 5 days before major surgery. Platelet transfusion is unlikely to be of benefit in patients with ticagrelor-related bleeding or in those requiring urgent surgery because the drug will bind to P2Y₁₂ on the transfused platelets. Bentracimab, an antibody fragment that binds ticagrelor and its metabolite with high affinity and rapidly reverses their platelet inhibitory effects, is under development for ticagrelor reversal prior to urgent surgery or intervention or for patients with serious bleeding.

Cangrelor Cangrelor is a rapidly acting reversible inhibitor of P2Y₁₂ that is administered intravenously. It has an immediate onset of action,

a half-life of 3–5 min, and an offset of action within an hour. Cangrelor is licensed for use in patients undergoing percutaneous coronary intervention and produces rapid ADP receptor blockade in those who have not received pretreatment with clopidogrel, prasugrel, or ticagrelor.

Cangrelor is administered as a 30 µg/kg IV bolus prior to percutaneous coronary intervention followed by an infusion of 4 µg/kg per minute for at least 2 h or for the duration of the procedure, whichever is longer. When transitioning to oral P2Y₁₂ inhibitor therapy, ticagrelor can be given at a loading dose of 180 mg at any time during the cangrelor infusion or immediately after discontinuation. In contrast, loading doses of prasugrel or clopidogrel (60 and 600 mg, respectively) should only be given after cangrelor is stopped because cangrelor blocks the interaction of their active metabolites with P2Y₁₂.

■ DIPYRIDAMOLE

Dipyridamole is a relatively weak antiplatelet agent on its own, but an extended-release formulation of dipyridamole combined with low-dose aspirin, a preparation known as Aggrenox, is sometimes used for secondary prevention in patients with transient ischemic attacks or ischemic stroke.

Mechanism of Action By inhibiting phosphodiesterase, dipyridamole blocks the breakdown of cyclic adenosine monophosphate (AMP). Increased levels of cyclic AMP reduce intracellular calcium and inhibit platelet activation. Dipyridamole also blocks the uptake of adenosine by platelets and other cells. This produces a further increase in local cyclic AMP levels because the platelet adenosine A₂ receptor is coupled to adenylate cyclase (Fig. 118-4).

Indications Dipyridamole plus aspirin was compared with aspirin or dipyridamole alone, or with placebo, in patients with an ischemic stroke or transient ischemic attack. The combination reduced the risk of stroke by 22.1% compared with aspirin and by 24.4% compared with dipyridamole. A second trial compared dipyridamole plus aspirin with aspirin alone for secondary prevention in patients with ischemic stroke. Vascular death, stroke, or MI occurred in 13% of patients given combination therapy and in 16% of those treated with aspirin alone. Another trial randomized 20,332 patients with noncardioembolic ischemic stroke to either Aggrenox or clopidogrel. The primary efficacy endpoint of recurrent stroke occurred in 9.0% of those given Aggrenox and in 8.8% of patients treated with clopidogrel. Although this difference was not statistically significant, the study failed to meet the prespecified margin to claim noninferiority of Aggrenox relative to clopidogrel. These results have dampened enthusiasm for the use of Aggrenox.

Because of its vasodilatory effects and the paucity of data supporting the use of dipyridamole in patients with symptomatic coronary artery disease, Aggrenox should not be used for stroke prevention in such patients. Clopidogrel is a better choice in this setting.

Dosing Aggrenox is given twice daily. Each capsule contains 200 mg of extended-release dipyridamole and 25 mg of aspirin.

Side Effects Because dipyridamole has vasodilatory effects, it must be used with caution in patients with coronary artery disease. Gastrointestinal complaints, headache, facial flushing, dizziness, and hypotension can also occur. These symptoms often subside with continued use of the drug.

■ GP IIb/IIIa RECEPTOR ANTAGONISTS

As a class, parenteral Gp IIb/IIIa receptor antagonists have a niche in patients with acute coronary syndrome. The three agents in this class are abciximab, eptifibatide, and tirofiban.

Mechanism of Action A member of the integrin family of adhesion receptors, Gp IIb/IIIa is found on the surface of platelets and megakaryocytes. With about 80,000 copies per platelet, Gp IIb/IIIa is the most abundant receptor. Consisting of a noncovalently linked heterodimer, Gp IIb/IIIa is inactive on resting platelets. When platelets

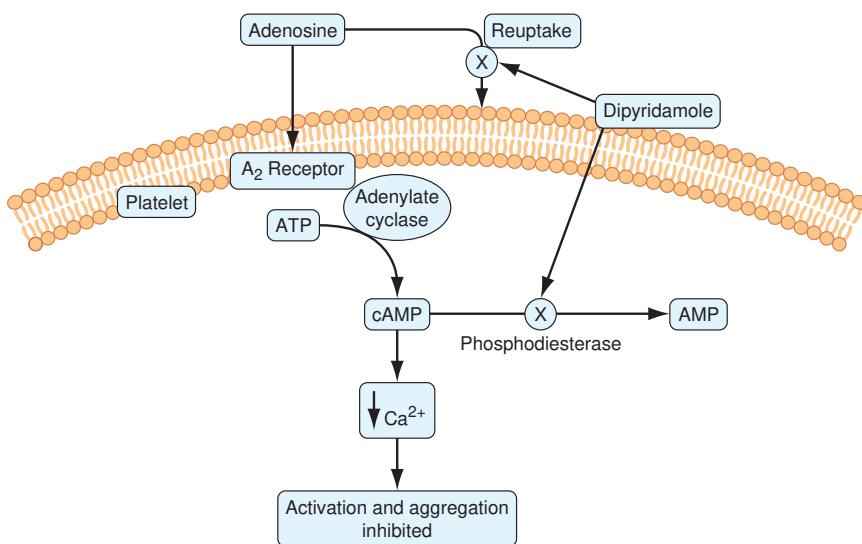


FIGURE 118-4 Mechanism of action of dipyridamole. Dipyridamole increases levels of cyclic AMP (cAMP) in platelets by (1) blocking the reuptake of adenosine and (2) inhibiting phosphodiesterase-mediated cyclic AMP degradation. By promoting calcium uptake, cyclic AMP reduces intracellular levels of calcium. This, in turn, inhibits platelet activation and aggregation.

are activated, inside-outside signal transduction pathways trigger a conformational activation of the receptor. Once activated, Gp IIb/IIIa binds adhesive molecules, such as fibrinogen and, under high shear conditions, VWF. Binding is mediated by the Arg-Gly-Asp (RGD) sequence found on the α chains of fibrinogen and on VWF, and by the Lys-Gly-Asp (KGD) sequence located within a unique dodecapeptide domain on the γ chains of fibrinogen. Once bound, fibrinogen and/or VWF bridge adjacent platelets together to induce platelet aggregation.

Although abciximab, eptifibatide, and tirofiban all target the Gp IIb/IIIa receptor, they are structurally and pharmacologically distinct (Table 118-1). Abciximab is a Fab fragment of a humanized murine monoclonal antibody directed against the activated form of Gp IIb/IIIa. Abciximab binds to the activated receptor with high affinity and blocks the binding of adhesive molecules. In contrast, eptifibatide and tirofiban are synthetic small molecules. Eptifibatide is a cyclic heptapeptide that binds Gp IIb/IIIa because it incorporates the KGD motif, whereas tirofiban is a nonpeptidic tyrosine derivative that acts as an RGD mimetic. Abciximab has a long half-life and can be detected on the surface of platelets for up to 2 weeks; eptifibatide and tirofiban have short half-lives.

Indications Abciximab and eptifibatide are used in patients undergoing percutaneous coronary interventions, particularly those who have not been pretreated with an ADP receptor antagonist. Tirofiban is used in high-risk patients with unstable angina. Eptifibatide also can be used for this indication.

TABLE 118-1 Features of Gp IIb/IIIa Antagonists

FEATURE	ABCIXIMAB	EPTIFIBATIDE	TIROFIBAN
Description	Fab fragment of humanized mouse monoclonal antibody	Cyclic KGD-containing heptapeptide	Nonpeptidic RGD mimetic
Specific for Gp IIb/IIIa	No	Yes	Yes
Plasma half-life	Short (min)	Long (2.5 h)	Long (2.0 h)
Platelet-bound half-life	Long (days)	Short (s)	Short (s)
Renal clearance	No	Yes	Yes

Abbreviation: Gp, glycoprotein.

Dosing All of the Gp IIb/IIIa antagonists are given as an IV bolus followed by an infusion. The recommended dose of abciximab is a bolus of 0.25 mg/kg followed by an infusion of 0.125 $\mu\text{g}/\text{kg}$ per minute to a maximum of 10 $\mu\text{g}/\text{kg}$ for 12 h. In patients undergoing percutaneous coronary intervention, eptifibatide is given as two 180 $\mu\text{g}/\text{kg}$ boluses given 10 min apart, followed by an infusion of 2.0 $\mu\text{g}/\text{kg}$ per minute for 18–24 h. For patients with acute coronary syndrome, the second eptifibatide bolus is withheld. Tirofiban is started at a rate of 0.4 $\mu\text{g}/\text{kg}$ per minute for 30 min; the drug is then continued at a rate of 0.1 $\mu\text{g}/\text{kg}$ per minute for up to 18 h. Because eptifibatide and tirofiban are cleared by the kidneys, the doses must be reduced in patients with renal insufficiency. Thus, the eptifibatide infusion is reduced to 1 $\mu\text{g}/\text{kg}$ per minute in patients with a creatinine clearance below 50 mL/min, whereas the dose of tirofiban is cut in half for patients with a creatinine clearance below 30 mL/min.

Side Effects In addition to bleeding, thrombocytopenia is the most serious

complication. Thrombocytopenia is immune-mediated and is caused by antibodies directed against neoantigens on Gp IIb/IIIa that are exposed upon antagonist binding. With abciximab, thrombocytopenia occurs in up to 5% of patients. Thrombocytopenia is severe in ~1% of these individuals. Thrombocytopenia is less common with the other two agents, occurring in ~1% of patients.

VORAPAXAR

An orally active PAR-1 antagonist, vorapaxar blocks thrombin-induced platelet activation. Vorapaxar has a half-life of about 200 h.

Indications When compared with placebo in 12,944 patients with acute coronary syndrome without ST-segment elevation, vorapaxar failed to significantly reduce the primary efficacy endpoint, a composite of cardiovascular death, MI, stroke, recurrent ischemia requiring rehospitalization, and urgent coronary revascularization. Moreover, vorapaxar was associated with increased rates of bleeding, including intracranial bleeding.

In a second trial, vorapaxar was compared with placebo for secondary prevention in 26,449 patients with prior MI, ischemic stroke, or peripheral arterial disease. Overall, vorapaxar reduced the risk for cardiovascular death, MI, or stroke by 13%, but doubled the risk of intracranial bleeding. In the prespecified subgroup of 17,779 patients with prior MI, however, vorapaxar reduced the risk for cardiovascular death, MI, or stroke by 20% compared with placebo (from 9.7% to 8.1%, respectively). The rate of intracranial hemorrhage was higher with vorapaxar than with placebo (0.6% and 0.4%, respectively; $p = .076$) as was the rate of moderate or severe bleeding (3.4% and 2.1%, respectively; $p < .0001$). Based on these data, vorapaxar is licensed for patients younger than 75 years with MI or peripheral artery disease who have no history of stroke, transient ischemic attack, or intracranial bleeding and weigh more than 60 kg.

Dosing Vorapaxar is given at a dose of 2.08 mg once daily.

Side Effects The major side effect is bleeding. Platelet transfusion may be of benefit for vorapaxar reversal.

ANTICOAGULANTS

There are both parenteral and oral anticoagulants. The parenteral anti-coagulants include heparin, low-molecular-weight heparin (LMWH), fondaparinux (a synthetic pentasaccharide), lepirudin, desirudin, bivalirudin, and argatroban. Currently available oral anticoagulants

include warfarin; dabigatran etexilate, an oral thrombin inhibitor; and rivaroxaban, apixaban, and edoxaban, which are oral factor Xa inhibitors.

PARENTERAL ANTICOAGULANTS

Heparin A sulfated polysaccharide, heparin is isolated from mammalian tissues rich in mast cells. Most commercial heparin is derived from porcine intestinal mucosa and is a polymer of alternating $\text{-}\text{glucuronic acid}$ and $N\text{-acetyl-}\text{-}\text{glucosamine}$ residues.

MECHANISM OF ACTION Heparin acts as an anticoagulant by activating antithrombin (previously known as antithrombin III) and accelerating the rate at which antithrombin inhibits clotting enzymes, particularly thrombin and factor Xa. Antithrombin, the obligatory plasma cofactor for heparin, is a member of the serine protease inhibitor (serpin) superfamily. Synthesized in the liver and circulating in plasma at a concentration of $2.6 \pm 0.4 \mu\text{M}$, antithrombin acts as a suicide substrate for its target enzymes.

To activate antithrombin, heparin binds to the serpin via a unique pentasaccharide sequence that is found on one-third of the chains of commercial heparin (Fig. 118-5). Heparin chains without this pentasaccharide sequence have little or no anticoagulant activity. Once bound to antithrombin, heparin induces a conformational change in the reactive center loop of antithrombin that renders it more readily accessible to its target proteases. This conformational change enhances the rate at which antithrombin inhibits factor Xa by at least two orders of magnitude but has little effect on the rate of thrombin inhibition. To catalyze thrombin inhibition, heparin serves as a template that binds antithrombin and thrombin simultaneously. Formation of this ternary complex brings the enzyme in close apposition to the inhibitor, thereby promoting the formation of a stable covalent thrombin-antithrombin complex.

Only pentasaccharide-containing heparin chains composed of at least 18 saccharide units (which correspond to a molecular weight of 5400) are of sufficient length to bridge thrombin and antithrombin together. With a mean molecular weight of 15,000, and a range of 5000–30,000, almost all of the chains of unfractionated heparin are long enough to do so. Consequently, by definition, heparin has equal capacity to promote the inhibition of thrombin and factor Xa by antithrombin and is assigned an anti-factor Xa to anti-factor IIa (thrombin) ratio of 1:1.

Heparin causes the release of tissue factor pathway inhibitor (TFPI) from the endothelium. A factor Xa-dependent inhibitor of tissue factor-bound factor VIIa, TFPI may contribute to the antithrombotic activity of heparin. Longer heparin chains induce the release of more TFPI than shorter ones.

PHARMACOLOGY Heparin must be given parenterally. It is usually administered SC or by continuous IV infusion. When used for therapeutic purposes, the IV route is most often employed. If heparin is given SC for treatment of thrombosis, the dose of heparin must be high enough to overcome the limited bioavailability associated with this method of delivery.

In the circulation, heparin binds to the endothelium and to plasma proteins other than antithrombin. Heparin binding to endothelial

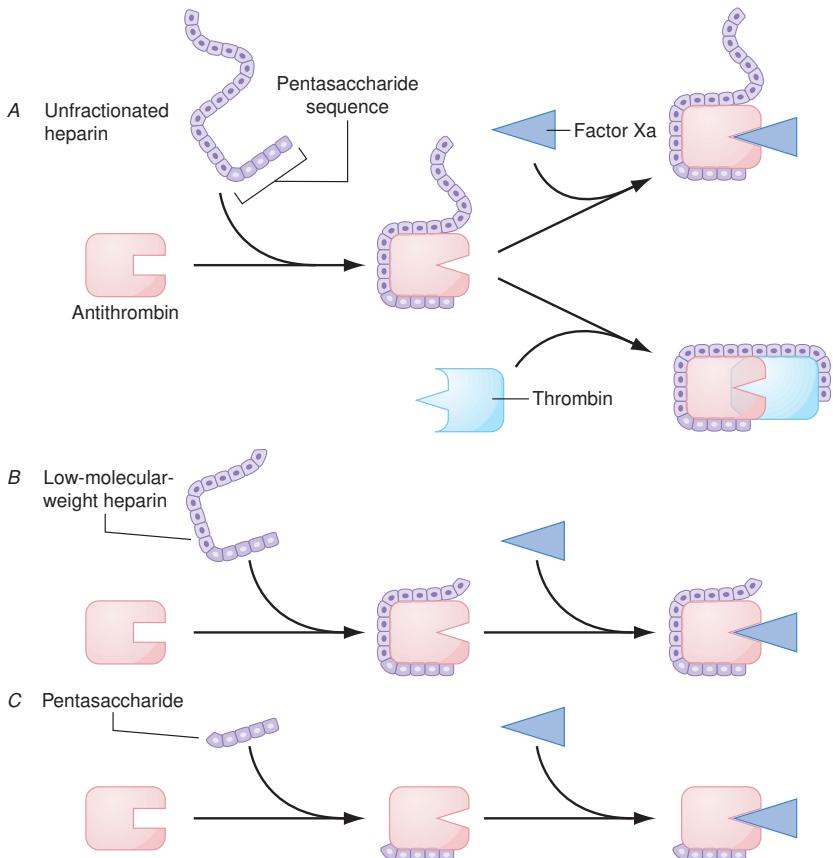


FIGURE 118-5 Mechanism of action of heparin, low-molecular-weight heparin (LMWH), and fondaparinux, a synthetic pentasaccharide. **A.** Heparin binds to antithrombin via its pentasaccharide sequence. This induces a conformational change in the reactive center loop of antithrombin that accelerates its interaction with factor Xa. To potentiate thrombin inhibition, heparin must simultaneously bind to antithrombin and thrombin. Only heparin chains composed of at least 18 saccharide units, which corresponds to a molecular weight of 5400, are of sufficient length to perform this bridging function. With a mean molecular weight of 15,000, all of the heparin chains are long enough to do this. **B.** LMWH has greater capacity to potentiate factor Xa inhibition by antithrombin than thrombin because, with a mean molecular weight of 4500–5000, at least half of the LMWH chains are too short to bridge antithrombin to thrombin. **C.** The pentasaccharide only accelerates factor Xa inhibition by antithrombin because the pentasaccharide is too short to bridge antithrombin to thrombin.

cells explains its dose-dependent clearance. At low doses, the half-life of heparin is short because it binds rapidly to the endothelium. With higher doses of heparin, the half-life is longer because heparin is cleared more slowly once the endothelium is saturated. Clearance is mainly extra renal; heparin binds to macrophages, which internalize and depolymerize the long heparin chains and secrete shorter chains back into the circulation. Because of its dose-dependent clearance mechanism, the plasma half-life of heparin ranges from 30 to 60 min with bolus IV doses of 25 and 100 units/kg, respectively.

Once heparin enters the circulation, it binds to plasma proteins other than antithrombin, a phenomenon that reduces its anticoagulant activity. Some of the heparin-binding proteins found in plasma are acute-phase reactants whose levels are elevated in ill patients. Others, such as high-molecular-weight multimers of VWF, are released from activated platelets or endothelial cells. Activated platelets also release platelet factor 4 (PF4), a highly cationic protein that binds heparin with high affinity. The large amounts of PF4 found in the vicinity of platelet-rich arterial thrombi can neutralize the anticoagulant activity of heparin. This phenomenon may attenuate heparin's capacity to suppress thrombus growth.

Because the levels of heparin-binding proteins in plasma vary from person to person, the anticoagulant response to fixed or weight-adjusted doses of heparin is unpredictable. Consequently, coagulation

monitoring is essential to ensure that a therapeutic response is obtained. This is particularly important when heparin is administered for treatment of established thrombosis because a subtherapeutic anticoagulant response may render patients at risk for recurrent thrombosis, whereas excessive anticoagulation increases the risk of bleeding.

MONITORING THE ANTICOAGULANT EFFECT Heparin therapy can be monitored using the activated partial thromboplastin time (aPTT) or anti-factor Xa level. Although the aPTT is the test most often used for this purpose, there are problems with this assay. aPTT reagents vary in their sensitivity to heparin, and the type of coagulometer used for testing can influence the results. Consequently, laboratories must establish a therapeutic aPTT range with each reagent-coagulometer combination by measuring the aPTT and anti-factor Xa level in plasma samples collected from heparin-treated patients. For most of the aPTT reagents and coagulometers in current use, therapeutic heparin levels are achieved with a two- to threefold prolongation of the aPTT. Anti-factor Xa levels also can be used to monitor heparin therapy. With this test, therapeutic heparin levels range from 0.3 to 0.7 units/mL.

Up to 25% of heparin-treated patients with venous thromboembolism require >35,000 units/d to achieve a therapeutic aPTT. These patients are considered heparin resistant. It is useful to measure anti-factor Xa levels in heparin-resistant patients because many will have a therapeutic anti-factor Xa level despite a subtherapeutic aPTT. This dissociation in test results occurs because elevated plasma levels of fibrinogen and factor VIII, both of which are acute-phase proteins, shorten the aPTT but have no effect on anti-factor Xa levels. Heparin therapy in patients who exhibit this phenomenon is best monitored using anti-factor Xa levels instead of the aPTT. Patients with congenital or acquired antithrombin deficiency and those with elevated levels of heparin-binding proteins may also need high doses of heparin to achieve a therapeutic aPTT or anti-factor Xa level. If there is good correlation between the aPTT and the anti-factor Xa levels, either test can be used to monitor heparin therapy.

DOSING For prophylaxis, heparin is usually given in fixed doses of 5000 units SC two or three times daily. With these low doses, coagulation monitoring is unnecessary. In contrast, monitoring is essential when the drug is given in therapeutic doses. Fixed-dose or weight-based heparin nomograms are used to standardize heparin dosing and to shorten the time required to achieve a therapeutic anticoagulant response. At least two heparin nomograms have been validated in patients with venous thromboembolism and reduce the time required to achieve a therapeutic aPTT. Weight-adjusted heparin nomograms have also been evaluated in patients with acute coronary syndromes. After an IV heparin bolus of 5000 units or 70 units/kg, a heparin infusion rate of 12–15 units/kg per hour is usually administered. In contrast, weight-adjusted heparin nomograms for patients with venous thromboembolism use an initial bolus of 5000 units or 80 units/kg, followed by an infusion of 18 units/kg per hour. Thus, patients with venous thromboembolism appear to require higher doses of heparin to achieve a therapeutic aPTT than do patients with acute coronary syndromes. This may reflect differences in the thrombus burden. Heparin binds to fibrin, and the amount of fibrin in patients with extensive DVT is greater than that in those with coronary thrombosis.

LIMITATIONS Heparin has pharmacokinetic and biophysical limitations (Table 118-2). The pharmacokinetic limitations reflect heparin's propensity to bind in a pentasaccharide-independent fashion to cells and plasma proteins. Heparin binding to endothelial cells explains its dose-dependent clearance, whereas binding to plasma proteins results in a variable anticoagulant response and can lead to heparin resistance.

The biophysical limitations of heparin reflect the inability of the heparin-antithrombin complex to inhibit factor Xa when it is incorporated into the prothrombinase complex, the complex that converts prothrombin to thrombin, and to inhibit thrombin bound to fibrin. Consequently, factor Xa bound to activated platelets within platelet-rich thrombi has the potential to generate thrombin, even in the face of heparin. Once this thrombin binds to fibrin, it too is protected

TABLE 118-2 Pharmacokinetic and Biophysical Limitations of Heparin	
LIMITATIONS	MECHANISM
Poor bioavailability at low doses	Binds to endothelial cells and macrophages
Dose-dependent clearance	Binds to macrophages
Variable anticoagulant response	Binds to plasma proteins whose levels vary from patient to patient
Reduced activity in the vicinity of platelet-rich thrombi	Neutralized by platelet factor 4 released from activated platelets
Limited activity against factor Xa incorporated in the prothrombinase complex and thrombin bound to fibrin	Reduced capacity of heparin-antithrombin complex to inhibit factor Xa bound to activated platelets and thrombin bound to fibrin

from inhibition by the heparin-antithrombin complex. Clot-associated thrombin can then trigger thrombus growth by locally activating platelets and amplifying its own generation through feedback activation of factors V, VIII, and XI. Further compounding the problem is the potential for heparin neutralization by the high concentrations of PF4 released from activated platelets within the platelet-rich thrombus.

SIDE EFFECTS The most common side effect of heparin is bleeding. Other complications include thrombocytopenia, osteoporosis, and elevated levels of transaminases.

Bleeding The risk of bleeding rises as the dose of heparin is increased. Concomitant administration of drugs that affect hemostasis, such as antiplatelet or fibrinolytic agents, increases the risk of bleeding, as does recent surgery or trauma. Heparin-treated patients with serious bleeding can be given protamine sulfate to neutralize the heparin. Protamine sulfate, a mixture of basic polypeptides isolated from salmon sperm, binds heparin with high affinity, and the resultant protamine-heparin complexes are then cleared. Typically, 1 mg of protamine sulfate neutralizes 100 units of heparin. Protamine sulfate is given IV. Anaphylactoid reactions to protamine sulfate can occur, and drug administration by slow IV infusion is recommended to reduce the risk.

Thrombocytopenia Heparin can cause thrombocytopenia. Heparin-induced thrombocytopenia (HIT) is an antibody-mediated process that is triggered by antibodies directed against neoantigens on PF4 that are exposed when heparin binds to this protein. These antibodies, which are usually of the IgG isotype, bind simultaneously to the heparin-PF4 complex and to platelet Fc receptors. Such binding activates the platelets and generates platelet microparticles. Circulating microparticles are prothrombotic because they express anionic phospholipids on their surface and can bind clotting factors and promote thrombin generation.

The clinical features of HIT are illustrated in Table 118-3. Typically, HIT occurs 5–14 days after initiation of heparin therapy, but it can manifest earlier if the patient has received heparin within the past 3 months. A platelet count <100,000/ μ L or a 50% decrease in the platelet count from the pretreatment value should raise the suspicion of HIT. HIT is more common in surgical patients than in medical patients and, like many autoimmune disorders, occurs more frequently in females than in males.

HIT can be associated with thrombosis, either arterial or venous. Venous thrombosis, which manifests as DVT and/or PE, is more

TABLE 118-3 Features of Heparin-Induced Thrombocytopenia	
FEATURES	DETAILS
Thrombocytopenia	Platelet count of ≤100,000/ μ L or a decrease in platelet count of ≥50%
Timing	Platelet count falls 5–14 days after starting heparin
Type of heparin	More common with unfractionated heparin than low-molecular-weight heparin
Type of patient	More common in surgical patients and patients with cancer than general medical patients; more common in women than in men
Thrombosis	Venous thrombosis more common than arterial thrombosis

TABLE 118-4 Management of Heparin-Induced Thrombocytopenia

Stop all heparin.
Give an alternative anticoagulant, such as argatroban, bivalirudin, fondaparinux, or rivaroxaban.
Do not give platelet transfusions.
Do not give warfarin until the platelet count returns to its baseline level. If warfarin was administered, give vitamin K to restore the INR to normal.
Evaluate for thrombosis, particularly deep vein thrombosis.

Abbreviation: INR, international normalized ratio.

common than arterial thrombosis. Arterial thrombosis can manifest as ischemic stroke or acute MI. Rarely, platelet-rich thrombi in the distal aorta or iliac arteries can cause critical limb ischemia.

The diagnosis of HIT is established using enzyme-linked assays to detect antibodies against heparin-PF4 complexes or with platelet activation assays. Enzyme-linked assays are sensitive but can be positive in the absence of any clinical evidence of HIT. The most specific diagnostic test for HIT is the serotonin release assay. This test is performed by quantifying serotonin release when washed platelets loaded with labeled serotonin are exposed to patient serum in the absence or presence of varying concentrations of heparin. If the patient serum contains the HIT antibody, heparin addition induces platelet activation and serotonin release.

Management of HIT is outlined in Table 118-4. Heparin should be stopped in patients with suspected or documented HIT, and an alternative anticoagulant should be administered to prevent or treat thrombosis. The agents most often used for this indication are parenteral direct thrombin inhibitors, such as argatroban or bivalirudin, or factor Xa inhibitors, such as fondaparinux or rivaroxaban. A HIT-like syndrome known as vaccine induced thrombotic thrombocytopenia is a rare complication after vaccination with adenovirus COVID-19 vaccines. Characterized by thrombosis and thrombocytopenia that occur 4 to 28 days after vaccination, patients can present with cerebral or splanchnic vein thrombosis as well as DVT or PE. The diagnosis is established by evidence of antibodies against PF4 and a positive serotonin release assay with added PF4. Treatment can include intravenous immunoglobulin, steroids, and plasma exchange to offset the effects of the antibodies against PF4 and anticoagulants such as argatroban, fondaparinux or rivaroxaban to treat the thrombosis.

Patients with HIT, particularly those with associated thrombosis, often have evidence of increased thrombin generation that can lead to consumption of protein C. If these patients are given warfarin without a concomitant anticoagulant that inhibits thrombin or thrombin generation, the further decrease in protein C levels induced by the vitamin K antagonist can trigger skin necrosis. To avoid this problem, patients with HIT should be treated with a direct thrombin inhibitor or with fondaparinux until the platelet count returns to normal levels. At this point, low-dose warfarin therapy can be introduced, and the parenteral anticoagulant can be discontinued when the international normalized ratio (INR) has been therapeutic for at least 2 days. Alternatively, a direct oral anticoagulant can be given.

Osteoporosis Treatment with therapeutic doses of heparin for >1 month can cause a reduction in bone density. This complication has been reported in up to 30% of patients given long-term heparin therapy, and symptomatic vertebral fractures occur in 2–3% of these individuals.

Heparin causes bone loss both by decreasing bone formation and by enhancing bone resorption. Thus, heparin affects the activity of both osteoblasts and osteoclasts.

Elevated Levels of Transaminases Therapeutic doses of heparin are frequently associated with modest elevations in the serum levels of hepatic transaminases without a concomitant increase in the level of bilirubin. The levels of transaminases rapidly return to normal when the drug is stopped. The mechanism responsible for this phenomenon is unknown.

Low-Molecular-Weight Heparin Consisting of smaller fragments of heparin, LMWH is prepared from unfractionated heparin by

TABLE 118-5 Advantages of LMWH Over Heparin

ADVANTAGE	CONSEQUENCE
Better bioavailability and longer half-life after subcutaneous injection	Can be given subcutaneously once or twice daily for both prophylaxis and treatment
Dose-independent clearance	Simplified dosing
Predictable anticoagulant response	Coagulation monitoring is unnecessary in most patients
Lower risk of heparin-induced thrombocytopenia	Safer than heparin for short- or long-term administration
Lower risk of osteoporosis	Safer than heparin for extended administration

Abbreviation: LMWH, low-molecular-weight heparin.

controlled enzymatic or chemical depolymerization. The mean molecular weight of LMWH is about 5000, one-third the mean molecular weight of unfractionated heparin. LMWH has advantages over heparin (Table 118-5) and has replaced heparin for most indications.

MECHANISM OF ACTION Like heparin, LMWH exerts its anticoagulant activity by activating antithrombin. With a mean molecular weight of 5000, which corresponds to about 17 saccharide units, at least half of the pentasaccharide-containing chains of LMWH are too short to bridge thrombin to antithrombin (Fig. 118-5). However, these chains retain the capacity to accelerate factor Xa inhibition by antithrombin because this activity is largely the result of the conformational changes in antithrombin evoked by pentasaccharide binding. Consequently, LMWH catalyzes factor Xa inhibition by antithrombin more than thrombin inhibition. Depending on their unique molecular weight distributions, LMWH preparations have anti-factor Xa to anti-factor IIa ratios ranging from 2:1 to 4:1.

PHARMACOLOGY Although usually given SC, LMWH also can be administered IV if a rapid anticoagulant response is needed. LMWH has pharmacokinetic advantages over heparin. These advantages reflect the fact that shorter heparin chains bind less avidly to endothelial cells, macrophages, and heparin-binding plasma proteins. Reduced binding to endothelial cells and macrophages eliminates the rapid, dose-dependent, and saturable mechanism of clearance that is a characteristic of unfractionated heparin. Instead, the clearance of LMWH is dose-independent and its plasma half-life is longer. Based on measurement of anti-factor Xa levels, LMWH has a plasma half-life of ~4 h. LMWH is cleared almost exclusively by the kidneys, and the drug can accumulate in patients with renal insufficiency.

LMWH exhibits about 90% bioavailability after SC injection. Because LMWH binds less avidly to heparin-binding proteins in plasma than heparin, LMWH produces a more predictable dose response, and resistance to LMWH is rare. With a longer half-life and more predictable anticoagulant response, LMWH can be given SC once or twice daily without coagulation monitoring, even when the drug is given in treatment doses. These properties render LMWH more convenient than unfractionated heparin. Capitalizing on this feature, studies in patients with venous thromboembolism have shown that home treatment with LMWH is as effective and safe as in-hospital treatment with continuous IV infusions of heparin. Outpatient treatment with LMWH streamlines care, reduces health care costs, and increases patient satisfaction.

MONITORING In the majority of patients, LMWH does not require coagulation monitoring. If monitoring is necessary, anti-factor Xa levels must be measured because most LMWH preparations have little effect on the aPTT. Therapeutic anti-factor Xa levels once daily and twice daily doses of LMWH range from 0.5 to 1.2 units/mL and 1.0 to 2.0 units/mL, respectively, when measured 3–4 h after drug administration. When LMWH is given in prophylactic doses, peak anti-factor Xa levels of 0.2–0.5 units/mL are desirable.

Indications for LMWH monitoring include renal impairment and obesity. LMWH monitoring in patients with a creatinine clearance of ≤ 30 mL/min is advisable to ensure that there is no drug accumulation.

Although weight-adjusted LMWH dosing appears to produce therapeutic anti-factor Xa levels in patients who are overweight, this approach has not been extensively evaluated in those with morbid obesity. It may also be advisable to monitor the anticoagulant activity of LMWH during pregnancy because dose requirements can change, particularly in the third trimester. Monitoring should also be considered in high-risk settings, such as in pregnant women with mechanical heart valves who are given LMWH for prevention of valve thrombosis, and when LMWH is used in treatment doses in infants or children.

DOSING The doses of LMWH recommended for prophylaxis or treatment vary depending on the LMWH preparation. For prophylaxis, once-daily SC doses of 4000–5000 units are often used, whereas doses of 2500–3000 units are given when the drug is administered twice daily. For treatment of venous thromboembolism, a dose of 150–200 units/kg is given if the drug is administered once daily. If a twice-daily regimen is used, a dose of 100 units/kg is given. In patients with unstable angina, LMWH is given SC on a twice-daily basis at a dose of 100–120 units/kg.

SIDE EFFECTS The major complication of LMWH is bleeding. Meta-analyses suggest that the risk of major bleeding is lower with LMWH than with unfractionated heparin. HIT and osteoporosis are less common with LMWH than with unfractionated heparin.

Bleeding Like the situation with heparin, bleeding with LMWH is more common in patients receiving concomitant therapy with anti-platelet or fibrinolytic drugs. Recent surgery, trauma, or underlying hemostatic defects also increase the risk of bleeding with LMWH.

Although protamine sulfate can be used as an antidote for LMWH, protamine sulfate incompletely neutralizes the anticoagulant activity of LMWH because it only binds the longer chains of LMWH. Because longer chains are responsible for catalysis of thrombin inhibition by antithrombin, protamine sulfate completely reverses the anti-factor IIa activity of LMWH. In contrast, protamine sulfate only partially reverses the anti-factor Xa activity of LMWH because the shorter pentasaccharide-containing chains of LMWH do not bind to protamine sulfate. Consequently, patients at high risk for bleeding may be more safely treated with continuous IV unfractionated heparin than with SC LMWH.

Thrombocytopenia The risk of HIT is about fivefold lower with LMWH than with heparin. LMWH binds less avidly to platelets and causes less PF4 release. Furthermore, with lower affinity for PF4 than heparin, LMWH is less likely to induce the conformational changes in PF4 that trigger the formation of HIT antibodies.

LMWH should not be used to treat HIT patients because most HIT antibodies exhibit cross-reactivity with LMWH. This in vitro cross-reactivity is not simply a laboratory phenomenon because there are case reports of thrombosis when HIT patients were switched from heparin to LMWH.

Osteoporosis Because the risk of osteoporosis is lower with LMWH than with heparin, LMWH is a better choice for extended treatment.

Fondaparinux A synthetic analogue of the antithrombin-binding pentasaccharide sequence, fondaparinux differs from LMWH in several ways (Table 118-6). Fondaparinux is licensed for thromboprophylaxis

in general medical or surgical patients and in high-risk orthopedic patients and as an alternative to heparin or LMWH for initial treatment of patients with established venous thromboembolism. Although fondaparinux is used in Europe as an alternative to heparin or LMWH in patients with acute coronary syndrome, the drug is not licensed for this indication in the United States.

MECHANISM OF ACTION As a synthetic analogue of the antithrombin-binding pentasaccharide sequence found in heparin and LMWH, fondaparinux has a molecular weight of 1728. Fondaparinux binds only to antithrombin (Fig. 118-5) and is too short to bridge thrombin to antithrombin. Consequently, fondaparinux catalyzes factor Xa inhibition by antithrombin and does not enhance the rate of thrombin inhibition.

PHARMACOLOGY Fondaparinux exhibits complete bioavailability after SC injection. With no binding to endothelial cells or plasma proteins, the clearance of fondaparinux is dose independent, and its plasma half-life is 17 h. The drug is given SC once daily. Because fondaparinux is cleared unchanged via the kidneys, it is contraindicated in patients with a creatinine clearance <30 mL/min and should be used with caution in those with a creatinine clearance <50 mL/min.

Dosing Fondaparinux produces a predictable anticoagulant response after administration in fixed doses because it does not bind to plasma proteins. The drug is given at a dose of 2.5 mg once daily for prevention of venous thromboembolism. For initial treatment of established venous thromboembolism, fondaparinux is given at a dose of 7.5 mg once daily. The dose can be reduced to 5 mg once daily for those weighing <50 kg and increased to 10 mg for those >100 kg. When given in these doses, fondaparinux is as effective as heparin or LMWH for initial treatment of patients with DVT or PE and produces similar rates of bleeding.

Fondaparinux is used at a dose of 2.5 mg once daily in patients with acute coronary syndrome. When this prophylactic dose of fondaparinux was compared with treatment doses of enoxaparin in patients with non-ST-segment elevation acute coronary syndrome, there was no difference in the rate of cardiovascular death, MI, or stroke at 9 days. However, the rate of major bleeding was 50% lower with fondaparinux than with enoxaparin, a difference that likely reflects the fact that the dose of fondaparinux was lower than that of enoxaparin. In acute coronary syndrome patients who require percutaneous coronary intervention, there is a risk of catheter thrombosis with fondaparinux unless adjunctive heparin is given at the time of the procedure.

SIDE EFFECTS Fondaparinux does not cause HIT because it does not bind to PF4. In contrast to LMWH, there is no cross-reactivity of fondaparinux with HIT antibodies. Consequently, fondaparinux appears to be effective for treatment of HIT patients, although large clinical trials supporting its use are lacking.

The major side effect of fondaparinux is bleeding. Fondaparinux has no antidote. Protamine sulfate has no effect on the anticoagulant activity of fondaparinux because it fails to bind to the drug. Recombinant activated factor VII reverses the anticoagulant effects of fondaparinux in volunteers, but it is unknown whether this agent controls fondaparinux-induced bleeding.

Parenteral Direct Thrombin Inhibitors Direct thrombin inhibitors bind directly to thrombin and block its interaction with its substrates. Approved parenteral direct thrombin inhibitors include recombinant hirudins (lepirudin and desirudin), argatroban, and bivalirudin (Table 118-7). Lepirudin and desirudin are no longer available. Argatroban is licensed for treatment of patients with HIT, and bivalirudin is approved as an alternative to heparin in patients undergoing percutaneous coronary intervention, including those with HIT.

ARGATROBAN A univalent inhibitor that targets the active site of thrombin, argatroban is metabolized in the liver. Consequently, this drug must be used with caution in patients with hepatic insufficiency. Argatroban is not cleared via the kidneys, so this drug is safer than fondaparinux for HIT patients with renal impairment.

TABLE 118-6 Comparison of LMWH and Fondaparinux

FEATURES	LMWH	FONDAPARINUX
Number of saccharide units	15–17	5
Catalysis of factor Xa inhibition	Yes	Yes
Catalysis of thrombin inhibition	Yes	No
Bioavailability after subcutaneous administration (%)	90	100
Plasma half-life (h)	4	17
Renal excretion	Yes	Yes
Induces release of tissue factor pathway inhibitor	Yes	No
Neutralized by protamine sulfate	Partially	No

TABLE 118-7 Comparison of the Properties of Lepirudin, Bivalirudin, and Argatroban

	LEPIRUDIN/ DESIRUDIN	BIVALIRUDIN	ARGATROBAN
Molecular mass	7000	1980	527
Site(s) of interaction with thrombin	Active site and exosite 1	Active site and exosite 1	Active site
Renal clearance	Yes	No	No
Hepatic metabolism	No	No	Yes
Plasma half-life (min)	60 (IV) 120–180 (SC)	25	45

Argatroban is administered by continuous IV infusion and has a plasma half-life of ~45 min. The aPTT is used to monitor its anticoagulant effect, and the dose is adjusted to achieve an aPTT 1.5–3 times the baseline value, but not to exceed 100 s. Argatroban also prolongs the INR, a feature that can complicate the transitioning of patients to warfarin. This problem can be circumvented by using the levels of factor X to monitor warfarin instead of the INR. Alternatively, argatroban can be stopped for 2–3 h before INR determination.

BIVALIRUDIN A synthetic 20-amino-acid analogue of hirudin, bivalirudin is a divalent thrombin inhibitor. Thus, the N-terminus of bivalirudin interacts with the active site of thrombin, whereas its C-terminus binds to exosite 1. Bivalirudin has a plasma half-life of 25 min, the shortest half-life of all the parenteral direct thrombin inhibitors. Bivalirudin is degraded by peptidases and is partially excreted via the kidneys. When given in high doses in the cardiac catheterization laboratory, the anticoagulant activity of bivalirudin is monitored using the activated clotting time. With lower doses, its activity can be assessed using the aPTT.

Bivalirudin is licensed as an alternative to heparin in patients undergoing percutaneous coronary intervention. Bivalirudin also has been used successfully in HIT patients who require percutaneous coronary intervention or cardiac bypass surgery.

■ ORAL ANTICOAGULANTS

For many years, vitamin K antagonists such as warfarin were the only available oral anticoagulants. This situation changed with the introduction of the direct oral anticoagulants, which include dabigatran, rivaroxaban, apixaban, and edoxaban.

Warfarin A water-soluble vitamin K antagonist initially developed as a rodenticide, warfarin is the coumarin derivative most often prescribed in North America. Like other vitamin K antagonists, warfarin interferes with the synthesis of the vitamin K-dependent clotting proteins, which include prothrombin (factor II) and factors VII, IX, and X. The synthesis of the vitamin K-dependent anticoagulant proteins, proteins C and S, is also reduced by vitamin K antagonists.

MECHANISM OF ACTION All of the vitamin K-dependent clotting factors possess glutamic acid residues at their N termini. A posttranslational modification adds a carboxyl group to the γ -carbon of these residues to generate γ -carboxyglutamic acid. This modification is essential for expression of the activity of these clotting factors because it permits their calcium-dependent binding to negatively charged phospholipid surfaces. The γ -carboxylation process is catalyzed by a vitamin K-dependent carboxylase. Thus, vitamin K from the diet is reduced to vitamin K hydroquinone by vitamin K reductase (Fig. 118-6). Vitamin K hydroquinone serves as a cofactor for the carboxylase enzyme, which in the presence of carbon dioxide replaces the hydrogen on the γ -carbon of glutamic acid residues with a carboxyl group. During this process, vitamin K hydroquinone is oxidized to vitamin K epoxide, which is then reduced to vitamin K by vitamin K epoxide reductase.

Warfarin inhibits vitamin K epoxide reductase (VKOR), thereby blocking the γ -carboxylation process. This results in the synthesis of vitamin K-dependent clotting proteins that are only partially γ -carboxylated. Warfarin acts as an anticoagulant because these

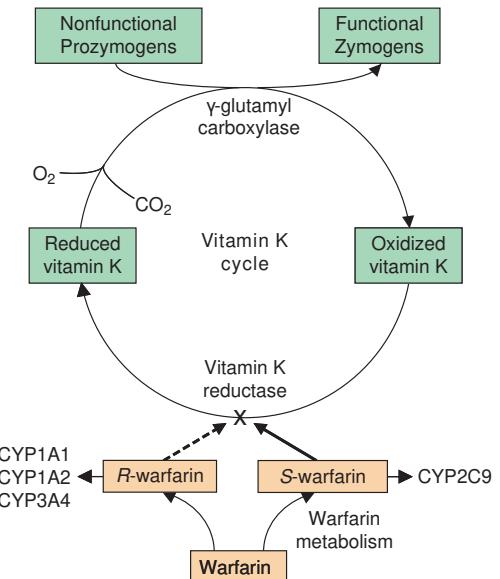


FIGURE 118-6 Mechanism of action of warfarin. A racemic mixture of *S*- and *R*-enantiomers, *S*-warfarin is most active. By blocking vitamin K epoxide reductase, warfarin inhibits the conversion of oxidized vitamin K into its reduced form. This inhibits vitamin K-dependent γ -carboxylation of factors II, VII, IX, and X because reduced vitamin K serves as a cofactor for a γ -glutamyl carboxylase that catalyzes the γ -carboxylation process, thereby converting prozymogens to zymogens capable of binding calcium and interacting with anionic phospholipid surfaces. *S*-warfarin is metabolized by CYP2C9. Common genetic polymorphisms in this enzyme can influence warfarin metabolism. Polymorphisms in the C1 subunit of vitamin K reductase (*VKORC1*) also can affect the susceptibility of the enzyme to warfarin-induced inhibition, thereby influencing warfarin dosage requirements.

partially γ -carboxylated proteins have little or no biological activity. The onset of action of warfarin is delayed until the newly synthesized clotting factors with reduced activity gradually replace their fully active counterparts.

The antithrombotic effect of warfarin depends on a reduction in the functional levels of factor X and prothrombin, clotting factors that have half-lives of 24 and 72 h, respectively. Because the antithrombotic effect of warfarin is delayed, patients with established thrombosis or at high risk for thrombosis require concomitant treatment with a rapidly acting parenteral anticoagulant, such as heparin, LMWH, or fondaparinux, for at least 5 days.

PHARMACOLOGY Warfarin is a racemic mixture of *R* and *S* isomers. Warfarin is rapidly and almost completely absorbed from the gastrointestinal tract. Levels of warfarin in the blood peak about 90 min after drug administration. Racemic warfarin has a plasma half-life of 36–42 h, and >97% of circulating warfarin is bound to albumin. Only the small fraction of unbound warfarin is biologically active.

Warfarin accumulates in the liver where the two isomers are metabolized via distinct pathways. CYP2C9 mediates oxidative metabolism of the more active *S* isomer (Fig. 118-6). Two relatively common variants, *CYP2C9*2* and *CYP2C9*3*, encode an enzyme with reduced activity. Patients with these variants require lower maintenance doses of warfarin. Approximately 25% of Caucasians have at least one variant allele of *CYP2C9*2* or *CYP2C9*3*, whereas those variant alleles are less common in African Americans and Asians (Table 118-8). Heterozygosity for *CYP2C9*2* or *CYP2C9*3* decreases the warfarin dose requirement by 20–30% relative to that required in subjects with the wild-type *CYP2C9*1/*1* alleles, whereas homozygosity for the *CYP2C9*2* or *CYP2C9*3* alleles reduces the warfarin dose requirement by 50–70%.

Consistent with their decreased warfarin dose requirement, subjects with at least one *CYP2C9* variant allele are at increased risk for bleeding. Compared with individuals with no variant alleles, the risk of warfarin-associated bleeding is almost 2-fold higher in *CYP2C9*2* or *CYP2C9*3* carriers.

TABLE 118-8 Frequencies of *CYP2C9* Genotypes and *VKORC1* Haplotypes in Different Populations and their Effect on Warfarin Dose Requirements

GENOTYPE/ HAPLOTYPE	FREQUENCY, %		DOSE REDUCTION COMPARED WITH WILD-TYPE
	CAUCASIANS	AFRICAN AMERICANS (A/A)	
<i>CYP2C9</i>			
'1/'1	70	90	95
'1/'2	17	2	0
'1/'3	9	3	4
'2/'2	2	0	0
'2/'3	1	0	0
'3/'3	0	0	1
<i>VKORC1</i>			
Non-A/non-A	37	82	7
Non-A/A	45	12	30
A/A	18	6	63

Polymorphisms in *VKORC1* also can influence the anticoagulant response to warfarin. Several genetic variations of *VKORC1* are in strong linkage disequilibrium and have been designated as non-A haplotypes. *VKORC1* variants are more prevalent than variants of *CYP2C9*. Asians have the highest prevalence of *VKORC1* variants, followed by Caucasians and African Americans (Table 118-8). Polymorphisms in *VKORC1* likely explain 30% of the variability in warfarin dose requirements. Compared with *VKORC1* non-A/non-A homozygotes, the warfarin dose requirement decreases by 25 and 50% in A haplotype heterozygotes and homozygotes, respectively. These findings prompted the U.S. Food and Drug Administration (FDA) to amend the prescribing information for warfarin to indicate that lower initiation doses should be considered for patients with *CYP2C9* and *VKORC1* genetic variants. In addition to genotype data, other pertinent patient information has been incorporated into warfarin dosing algorithms. Although such algorithms help predict suitable warfarin doses, it remains unclear whether better dose identification improves patient outcome in terms of reducing hemorrhagic complications or recurrent thrombotic events.

In addition to genetic factors, the anticoagulant effect of warfarin is influenced by diet, drugs, and various disease states. Fluctuations in dietary vitamin K intake affect the activity of warfarin. A wide variety of drugs can alter absorption, clearance, or metabolism of warfarin. Because of the variability in the anticoagulant response to warfarin, coagulation monitoring is essential to ensure that a therapeutic response is obtained.

MONITORING Warfarin therapy is most often monitored using the prothrombin time, a test that is sensitive to reductions in the levels of prothrombin, factor VII, and factor X. The test is performed by adding thromboplastin, a reagent that contains tissue factor, phospholipid, and calcium, to citrated plasma and determining the time to clot formation. Thromboplastins vary in their sensitivity to reductions in the levels of the vitamin K-dependent clotting factors. Thus, less sensitive thromboplastins will trigger the administration of higher doses of warfarin to achieve a target prothrombin time. This is problematic because higher doses of warfarin increase the risk of bleeding.

The INR was developed to circumvent many of the problems associated with the prothrombin time. To calculate the INR, the patient's prothrombin time is divided by the mean normal prothrombin time, and this ratio is then multiplied by the international sensitivity index (ISI), which is an index of the sensitivity of the thromboplastin used for prothrombin time determination to reductions in the levels of the vitamin K-dependent clotting factors. Sensitive thromboplastins have an ISI near 1.0. Most current thromboplastins have ISI values that range from 0.9 to 1.4.

Although the INR has helped to standardize anticoagulant practice, problems persist. The precision of INR determination varies depending on reagent-coagulometer combinations. This leads to variability in the INR results. Also complicating INR determination is unreliable reporting of the ISI by thromboplastin manufacturers. Furthermore, every laboratory must establish the mean normal prothrombin time with each new batch of thromboplastin reagent. To accomplish this, the prothrombin time must be measured in fresh plasma samples from at least 20 healthy volunteers using the same coagulometer that is used for patient samples.

For most indications, warfarin is administered in doses that produce a target INR of 2.0–3.0. An exception is patients with mechanical heart valves, particularly those in the mitral position or older ball and cage valves in the aortic position, where a target INR of 2.5–3.5 is recommended. Studies in atrial fibrillation demonstrate an increased risk of cardioembolic stroke when the INR falls below 1.7 and an increase in bleeding with INR values >4.5. These findings highlight the fact that vitamin K antagonists have a narrow therapeutic window. In support of this concept, a study in patients receiving long-term warfarin therapy for unprovoked venous thromboembolism demonstrated a higher rate of recurrent venous thromboembolism with a target INR of 1.5–1.9 compared with a target INR of 2.0–3.0.

DOSING Warfarin is usually started at a dose of 5–10 mg. Lower doses are used for patients with *CYP2C9* or *VKORC1* polymorphisms, which affect the pharmacodynamics or pharmacokinetics of warfarin and render patients more sensitive to the drug. The dose is then titrated to achieve the desired target INR. Because of its delayed onset of action, patients with established thrombosis or those at high risk for thrombosis are given concomitant initial treatment with a rapidly acting parenteral anticoagulant, such as heparin, LMWH, or fondaparinux. Early prolongation of the INR reflects reduction in the functional levels of factor VII. Consequently, concomitant treatment with the parenteral anticoagulant should be continued until the INR has been therapeutic for at least 2 consecutive days. A minimum 5-day course of parenteral anticoagulation is recommended to ensure that the levels of factor Xa and prothrombin have been reduced into the therapeutic range with warfarin.

Because warfarin has a narrow therapeutic window, frequent coagulation monitoring is essential to ensure that a therapeutic anticoagulant response is maintained. Even patients with stable warfarin dose requirements should have their INR determined every 3–4 weeks although there are studies suggesting that less frequent monitoring is feasible. More frequent monitoring is necessary when new medications are introduced because so many drugs enhance or reduce the anticoagulant effects of warfarin.

SIDE EFFECTS Like all anticoagulants, the major side effect of warfarin is bleeding. A rare complication is skin necrosis. Warfarin crosses the placenta and can cause fetal abnormalities. Consequently, warfarin should not be used during pregnancy.

Bleeding At least half of the bleeding complications with warfarin occur when the INR exceeds the therapeutic range. Bleeding complications may be mild, such as epistaxis or hematuria, or more severe, such as retroperitoneal or gastrointestinal bleeding. Life-threatening intracranial bleeding can also occur.

To minimize the risk of bleeding, the INR should be maintained in the therapeutic range. In asymptomatic patients whose INR is between 3.5 and 10, warfarin should be withheld until the INR returns to the therapeutic range. If the INR is over 10, oral vitamin K can be administered at a dose of 2.5–5 mg, although there is no evidence that doing so reduces the bleeding risk. Higher doses of oral vitamin K (5–10 mg) produce more rapid reversal of the INR but may render patients temporarily resistant to warfarin when the drug is restarted.

Patients with serious bleeding need more aggressive treatment. These patients should be given 5–10 mg of vitamin K by slow IV infusion. Additional vitamin K should be given until the INR is in the normal range. Treatment with vitamin K should be supplemented with four-factor prothrombin complex concentrate, which contains all

four vitamin K-dependent clotting proteins. Prothrombin complex concentrate normalizes the INR more rapidly than transfusion of fresh frozen plasma.

Warfarin-treated patients who experience bleeding when their INR is in the therapeutic range require investigation into the cause of the bleeding. Those with gastrointestinal or genitourinary bleeding often have an underlying lesion.

Skin Necrosis A rare complication of warfarin, skin necrosis usually is seen 2–5 days after initiation of therapy. Well-demarcated erythematous lesions form on the thighs, buttocks, breasts, or toes. Typically, the center of the lesion becomes progressively necrotic. Examination of skin biopsies taken from the border of these lesions reveals thrombi in the microvasculature.

Warfarin-induced skin necrosis is seen in patients with congenital or acquired deficiencies of protein C or protein S. Initiation of warfarin therapy in these patients produces a precipitous fall in plasma levels of proteins C or S, thereby eliminating this important anticoagulant pathway before warfarin exerts an antithrombotic effect through lowering of the functional levels of factor X and prothrombin. The resultant procoagulant state triggers thrombosis. Why the thrombosis is localized to the microvasculature of fatty tissues is unclear.

Treatment involves discontinuation of warfarin and reversal with vitamin K, if needed. An alternative anticoagulant, such as heparin or LMWH, should be given in patients with thrombosis. Protein C concentrate can be given to protein C-deficient patients to accelerate healing of the skin lesions; fresh-frozen plasma may be of value if protein C concentrate is unavailable and for those with protein S deficiency. Occasionally, skin grafting is necessary when there is extensive skin loss.

Because of the potential for skin necrosis, patients with known protein C or protein S deficiency require overlapping treatment with a parenteral anticoagulant when initiating warfarin therapy. Warfarin should be started in low doses in these patients, and the parenteral anticoagulant should be continued until the INR is therapeutic for at least 2–3 consecutive days. Alternatively, treatment with rivaroxaban or apixaban could be given, although there is limited information about their efficacy and safety in patients with severe protein C or S deficiency.

Pregnancy Warfarin crosses the placenta and can cause fetal abnormalities or bleeding. The fetal abnormalities include a characteristic embryopathy, which consists of nasal hypoplasia and stippled epiphyses. The risk of embryopathy is highest if warfarin is given in the first trimester of pregnancy. Central nervous system abnormalities can also occur with exposure to warfarin at any time during pregnancy. Finally, maternal administration of warfarin produces an anticoagulant effect in the fetus that can cause bleeding. This is of particular concern at delivery when trauma to the head during passage through the birth canal can lead to intracranial bleeding. Because of these potential problems, warfarin is contraindicated in pregnancy, particularly in the first and third trimesters. Instead, heparin, LMWH, or fondaparinux can be given during pregnancy for prevention or treatment of thrombosis.

Warfarin does not pass into the breast milk. Consequently, warfarin can safely be given to nursing mothers.

Special Problems Patients with a lupus anticoagulant and those who need urgent or elective surgery present special challenges. Although observational studies suggested that patients with thrombosis complicating the antiphospholipid antibody syndrome required higher intensity warfarin regimens to prevent recurrent thromboembolic events, two randomized trials showed that targeting an INR of 2.0–3.0 is as effective as higher intensity treatment and produces less bleeding. Monitoring warfarin therapy can be problematic in patients with antiphospholipid antibody syndrome if the lupus anticoagulant prolongs the baseline INR; factor X levels can be used instead of the INR in such patients.

There is no need to stop warfarin before procedures associated with a low risk of bleeding; these include dental cleaning, simple dental extraction, cataract surgery, or skin biopsy. For procedures associated with a moderate or high risk of bleeding, warfarin should be stopped 5 days before the procedure to allow the INR to return to normal levels. Patients at high risk for thrombosis, such as those with mechanical heart valves, can be bridged with once- or twice-daily SC injections of LMWH when the INR falls to <2.0. The last dose of LMWH should be given 12–24 h before the procedure, depending on whether LMWH is administered twice or once daily. After the procedure, treatment with warfarin can be restarted.

Direct Oral Anticoagulants The direct oral anticoagulants (DOACs) include dabigatran, which inhibits thrombin, and rivaroxaban, apixaban, and edoxaban, which inhibit factor Xa. These drugs have a rapid onset and offset of action and have half-lives that permit once- or twice-daily administration. Designed to produce a predictable level of anticoagulation, the DOACs are more convenient to administer than warfarin because they are given in fixed doses without routine coagulation monitoring.

MECHANISM OF ACTION The DOACs are small molecules that bind reversibly to the active site of their target enzyme. Table 118-9 summarizes the distinct pharmacologic properties of these agents.

INDICATIONS All four DOACs are licensed for stroke prevention in patients with nonvalvular atrial fibrillation, which encompasses patients without mechanical heart valves or severe rheumatic mitral valve disease, and for treatment of venous thromboembolism (VTE). Dabigatran, rivaroxaban, and apixaban are licensed for thromboprophylaxis after elective hip or knee arthroplasty; edoxaban is only licensed for this indication in Japan. Finally, low-dose rivaroxaban is licensed for use with aspirin for secondary prevention in patients with coronary or peripheral artery disease.

DOSING For prevention of stroke in patients with nonvalvular atrial fibrillation, rivaroxaban is given at a dosage of 20 mg once daily, with a reduction to 15 mg once daily in patients with a creatinine clearance of 15–49 mL/min; dabigatran is given at a dosage of 150 mg twice daily, with a reduction to 75 mg twice daily in those with a creatinine clearance of 15–30 mL/min; apixaban is given at a dosage of 5 mg twice daily, with a reduction to 2.5 mg twice daily for patients with at least two of the “ABC” criteria (i.e., age >80 years, body weight <60 kg, and creatinine >1.5 g/dL); and edoxaban is given at a dosage of 60 mg once daily for patients with a creatinine clearance of 50–95 mL/min and with a

TABLE 118-9 Comparison of the Pharmacologic Properties of the Direct Oral Anticoagulants

CHARACTERISTIC	RIVAROXABAN	APIXABAN	EDOXABAN	DABIGATRAN
Target	Factor Xa	Factor Xa	Factor Xa	Thrombin
Prodrug	No	No	No	Yes
Bioavailability	80%	60%	50%	6%
Dosing	qd (bid)	bid	qd	bid (qd)
Half-life	7–11 h	12 h	9–11 h	12–17 h
Renal excretion	33% (66%)	25%	35%	80%
Interactions	3A4/P-gp	3A4/P-gp	P-gp	P-gp

Abbreviations: bid, twice a day; P-gp, P-glycoprotein; qd, once a day.

reduction to 30 mg once daily for patients with any one of the following criteria: creatinine clearance of 15–50 mL/min, body weight of 60 kg or less, or use of potent P-glycoprotein inhibitors, such as verapamil or quinidine. At doses of 15 or 20 mg once daily, rivaroxaban must be administered with food to enhance absorption. Apixaban and edoxaban can be given with or without food. Administration of dabigatran with food may reduce dyspepsia.

For treatment of VTE, dabigatran and edoxaban are started after patients have received at least a 5-day course of treatment with a parenteral anticoagulant such as LMWH; dabigatran is given at a dose of 150 mg twice daily provided the creatinine clearance is >30 mL/min, and the dosage regimen for edoxaban is identical to that used in patients with atrial fibrillation. In contrast, rivaroxaban and apixaban can be given in all-oral regimens; rivaroxaban is started at a dose of 15 mg twice daily for 21 days and is then reduced to 20 mg once daily thereafter, whereas apixaban is started at a dose of 10 mg twice daily for 7 days and is then reduced to 5 mg twice daily thereafter. For secondary VTE prevention, the dosage of apixaban can be lowered to 2.5 mg twice daily while the dose of rivaroxaban can be lowered to 10 mg once daily, doses that have safety profiles like those of placebo and aspirin, respectively.

Thromboprophylaxis after elective hip or knee replacement surgery is started after surgery and is often continued for 30 days in patients undergoing hip replacement and for 10–14 days in patients undergoing knee replacement. Dabigatran is given at a dose of 220 mg once daily, whereas rivaroxaban and apixaban are given at doses of 10 mg once daily and 2.5 mg twice daily, respectively. In lower risk patients undergoing hip or knee replacement surgery, a 5-day course of rivaroxaban followed by a 30-day course of aspirin at a dose of 81 mg daily appears to be as effective and safe as extended thromboprophylaxis with rivaroxaban.

For secondary prevention of adverse cardiac or limb events in patients with coronary or peripheral artery disease, rivaroxaban is given at a dose of 2.5 mg twice daily on top of aspirin (81 or 100 mg once daily).

MONITORING Although designed to be administered without routine monitoring, there are situations where determination of the anticoagulant activity of the new oral anticoagulants can be helpful. These include assessment of adherence, detection of accumulation or overdose, identification of bleeding mechanisms, and determination of activity prior to surgery, intervention, or reversal. For qualitative assessment of anticoagulant activity, the prothrombin time can be used for factor Xa inhibitors and the aPTT for dabigatran. Rivaroxaban and edoxaban prolong the prothrombin time more than apixaban. In fact, because apixaban has such a limited effect on the prothrombin time, anti-factor Xa assays are needed to assess its activity. The effect of the drugs on tests of coagulation varies depending on the time that the blood is drawn relative to the timing of the last dose of the drug and the reagents used to perform the tests. Chromogenic anti-factor Xa assays and the diluted thrombin clotting time or ecarin clot time with appropriate calibrators provide quantitative assays to measure the plasma levels of the factor Xa inhibitors and dabigatran, respectively.

SIDE EFFECTS Like all anticoagulants, bleeding is the most common side effect of the DOACs. The DOACs are associated with less intracranial bleeding than warfarin, but the higher dose regimens of dabigatran, rivaroxaban, and edoxaban are associated with more gastrointestinal bleeding.

Dyspepsia occurs in up to 10% of patients treated with dabigatran; this problem improves with time and can be minimized by administering the drug with food. Dyspepsia is rare with rivaroxaban, apixaban, and edoxaban.

PERIPROCEDURAL MANAGEMENT Like warfarin, the DOACs must be stopped before procedures associated with a moderate or high risk of bleeding. The drugs should be held for 1–2 days, or longer if renal function is impaired. Assessment of residual anticoagulant activity before procedures associated with a high bleeding risk is prudent.

MANAGEMENT OF BLEEDING With minor bleeding, withholding one or two doses of drug is usually sufficient. With more serious bleeding, the approach is similar to that with warfarin, except that vitamin K administration is of no benefit; the anticoagulant and any antiplatelet drugs should be withheld, the patient should be resuscitated with fluids and blood products as necessary, and the bleeding site should be identified and managed. Coagulation testing or measurement of DOAC level will determine the extent of anticoagulation, and renal function should be assessed so that the half-life of the drug can be calculated. Timing of the last dose of anticoagulant is important; oral activated charcoal may help prevent absorption of drug administered in the past 4 h, particularly in cases of overdose. If >24 h have elapsed since the last intake, the DOAC is unlikely to be responsible for the bleeding unless there is marked impairment of renal function.

Anticoagulant reversal should be considered if bleeding continues despite supportive measures or if the bleeding is life-threatening or occurs in a critical organ (e.g., intracranial) or in a closed space (e.g., the pericardium or retroperitoneum). Idarucizumab is licensed for dabigatran reversal in such patients or in those requiring urgent surgery or intervention. A humanized antibody fragment, idarucizumab, binds dabigatran with high affinity to form an essentially irreversible complex that is cleared by the kidneys. Idarucizumab is given intravenously as a 5-g bolus and is supplied in a box containing two 50-mL vials, each containing 2.5 g of idarucizumab. Idarucizumab rapidly reverses the anticoagulant effects of dabigatran and normalizes the aPTT, diluted thrombin time, or ecarin clot time.

Andexanet alfa is available for reversal of rivaroxaban, apixaban, and edoxaban. A recombinant variant of factor Xa without catalytic activity, andexanet serves as a decoy to sequester oral factor Xa inhibitors until they are cleared from the circulation. Low- or high-dose IV andexanet regimens are used. The low-dose regimen starts with a bolus of 400 mg followed by an infusion of 4 mg/min for up to 120 min, whereas the high-dose regimen starts with a bolus of 800 mg followed by an infusion of 8 mg/min for up to 120 min. The low-dose regimen is used for reversal of doses of rivaroxaban or apixaban of 10 mg or 5 mg or less, respectively, or for any dose of rivaroxaban or apixaban if the last dose was taken >8 h prior to presentation. The high-dose regimen is used to reverse rivaroxaban or apixaban doses over 10 and 5 mg, respectively, if the last dose was taken <8 h since presentation, or for reversal if the dose of rivaroxaban or apixaban or the timing of the last dose is unknown.

Andexanet alfa is expensive and is not available in all hospitals. Because of its cost, andexanet alfa is often reserved for reversal in patients with life-threatening bleeds such as intracranial hemorrhage or bleeds into a closed space such as retroperitoneal or pericardial bleeds. If andexanet is unavailable, the results of prospective cohort studies suggest that four-factor prothrombin complex concentrate (25–50 units/kg) also is effective at restoring hemostasis. If there is continued bleeding, activated prothrombin complex concentrate (50 units/kg) or recombinant factor VIIa (90 µg/kg) can be considered.

Neither andexanet alfa nor four-factor prothrombin complex concentrate has been evaluated for reversal in patients requiring urgent surgery or intervention. Furthermore, andexanet alfa not only reverses oral factor Xa inhibitors but also reverses heparin and LMWH. This could be problematic in patients who require cardiac surgery or vascular surgery, procedures where heparin is used routinely. To circumvent this problem, most surgical procedures and interventions can be undertaken without reversal, and four-factor prothrombin complex concentrate can be given if necessary. For patients requiring surgery to stop bleeding such as those with a ruptured aortic aneurysm or with bleeding secondary to polytrauma, upfront four-factor prothrombin concentrate administration can be considered.

PREGNANCY As small molecules, the DOACs pass through the placenta. Consequently, these agents are contraindicated in pregnancy, and when used by women of childbearing potential, appropriate contraception is important. The DOACs should be avoided in nursing mothers.

FIBRINOLYTIC DRUGS

■ ROLE OF FIBRINOLYTIC THERAPY

Fibrinolytic drugs are used to degrade thrombi and are administered systemically or can be delivered via catheters directly into the substance of the thrombus. Systemic delivery is used for treatment of acute MI, acute ischemic stroke, and most cases of massive PE. The goal of therapy is to produce rapid thrombus dissolution, thereby restoring blood flow. In the coronary circulation, restoration of blood flow reduces morbidity and mortality rates by limiting myocardial damage, whereas in the cerebral circulation, rapid thrombus dissolution decreases the neuronal death and brain infarction that produce irreversible brain injury. For patients with massive PE, the goal of thrombolytic therapy is to restore pulmonary artery perfusion.

Peripheral arterial thrombi and thrombi in the proximal deep veins of the leg are most often treated using catheter-directed thrombolytic therapy. Catheters with multiple side holes can be used to enhance drug delivery. In some cases, intravascular devices that fragment and extract the thrombus are used to hasten treatment. These devices can be used alone or in conjunction with fibrinolytic drugs.

■ MECHANISM OF ACTION

Currently approved fibrinolytic agents include streptokinase; acylated plasminogen streptokinase activator complex (anistreplase); urokinase; recombinant tissue-type plasminogen activator (rtPA), which is also known as alteplase or activase; and two recombinant derivatives of rtPA, tenecteplase and reteplase. All these agents act by converting plasminogen, the zymogen, to plasmin, the active enzyme (Fig. 118-7). Plasmin then degrades the fibrin matrix of thrombi and produces soluble fibrin degradation products.

Endogenous fibrinolysis is regulated at two levels. Plasminogen activator inhibitors, particularly the type 1 form (PAI-1), prevent excessive plasminogen activation by regulating the activity of tPA and urokinase-type plasminogen activator (uPA). Once plasmin is generated, it is regulated by plasmin inhibitors, the most important of which is α_2 -antiplasmin. The plasma concentration of plasminogen is twofold higher than that of α_2 -antiplasmin. Consequently, with pharmacologic doses of plasminogen activators, the concentration of plasmin that is generated can exceed that of α_2 -antiplasmin. In addition to degrading fibrin, unregulated plasmin can also degrade fibrinogen and other clotting factors. This process, which is known as the *systemic lytic state*, reduces the hemostatic potential of the blood and increases the risk of bleeding.

The endogenous fibrinolytic system is geared to localize plasmin generation to the fibrin surface. Both plasminogen and tPA bind to fibrin to form a ternary complex that promotes efficient plasminogen activation. In contrast to free plasmin, plasmin generated on the fibrin surface is relatively protected from inactivation by α_2 -antiplasmin, a feature that promotes fibrin dissolution. Furthermore, C-terminal lysine residues, exposed as plasmin degrades fibrin, serve as binding sites for additional plasminogen and tPA molecules. This creates a

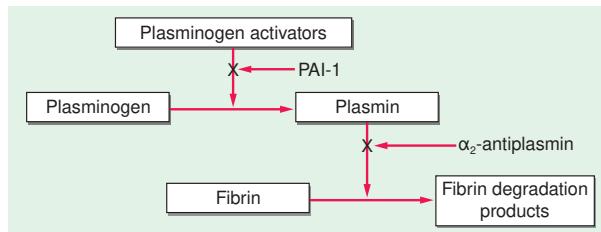


FIGURE 118-7 The fibrinolytic system and its regulation. Plasminogen activators convert plasminogen to plasmin. Plasmin then degrades fibrin into soluble fibrin degradation products. The system is regulated at two levels. Type 1 plasminogen activator inhibitor (PAI-1) regulates the plasminogen activators, whereas α_2 -antiplasmin serves as the major inhibitor of plasmin.

positive feedback that enhances plasmin generation. When used pharmacologically, the various plasminogen activators capitalize on these mechanisms to a lesser or greater extent.

Plasminogen activators that preferentially activate fibrin-bound plasminogen are considered fibrin-specific. In contrast, nonspecific plasminogen activators do not discriminate between fibrin-bound and circulating plasminogen. Activation of circulating plasminogen results in the generation of unopposed plasmin that can trigger the systemic lytic state. Alteplase and its derivatives are fibrin-specific plasminogen activators, whereas streptokinase, anistreplase, and urokinase are non-specific agents.

■ STREPTOKINASE

Unlike other plasminogen activators, streptokinase is not an enzyme and does not directly convert plasminogen to plasmin. Instead, streptokinase forms a 1:1 stoichiometric complex with plasminogen. Formation of this complex induces a conformational change in plasminogen that exposes its active site (Fig. 118-8). The streptokinase-plasminogen complex then converts additional plasminogen to plasmin.

Streptokinase has no affinity for fibrin, and the streptokinase-plasminogen complex activates both free and fibrin-bound plasminogen. Activation of circulating plasminogen generates sufficient amounts of plasmin to overwhelm α_2 -antiplasmin. Unopposed plasmin not only degrades fibrin in the occlusive thrombus but also induces a systemic lytic state.

When given systemically to patients with acute MI, streptokinase reduces mortality. For this indication, the drug is usually given as an IV infusion of 1.5 million units over 30–60 min. Patients who receive streptokinase can develop antibodies against the drug, as can patients with prior streptococcal infection. These antibodies can reduce the effectiveness of streptokinase.

Allergic reactions occur in ~5% of patients treated with streptokinase. These may manifest as a rash, fever, chills, and rigors. Although anaphylactic reactions can occur, these are rare. Transient hypotension is common with streptokinase and has been attributed to plasmin-mediated release of bradykinin from kininogen. The hypotension usually responds to leg elevation and administration of IV fluids and low doses of vasoconstrictors, such as dopamine or norepinephrine.

■ ANISTREPLASE

To generate this drug, streptokinase is combined with equimolar amounts of Lys-plasminogen, a plasmin-cleaved form of plasminogen

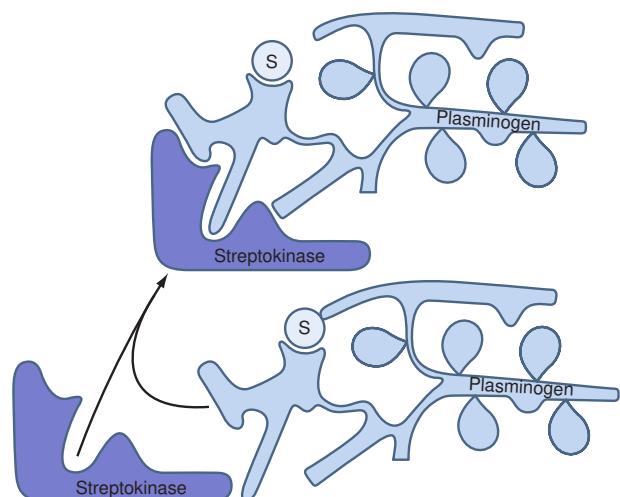


FIGURE 118-8 Mechanism of action of streptokinase. Streptokinase binds to plasminogen and induces a conformational change in plasminogen that exposes its active site. The streptokinase/plasminogen complex then serves as the activator of additional plasminogen.

with a Lys residue at its N terminal. The active site of Lys-plasminogen that is exposed upon combination with streptokinase is then masked with an anisoyl group. After IV infusion, the anisoyl group is slowly removed by deacylation, giving the complex a half-life of ~100 min. This allows drug administration via a single bolus infusion.

Although it is more convenient to administer, anistreplase offers few mechanistic advantages over streptokinase. Like streptokinase, anistreplase does not distinguish between fibrin-bound and circulating plasminogen. Consequently, it too produces a systemic lytic state. Likewise, allergic reactions and hypotension are just as frequent with anistreplase as they are with streptokinase.

When anistreplase was compared with alteplase in patients with acute MI, reperfusion was obtained more rapidly with alteplase than with anistreplase. Improved reperfusion was associated with a trend toward better clinical outcomes and reduced mortality rate with alteplase. These results and the high cost of anistreplase have dampened the enthusiasm for its use.

■ UROKINASE

Urokinase is a two-chain serine protease derived from cultured fetal kidney cells with a molecular weight of 34,000. Urokinase converts plasminogen to plasmin directly by cleaving the Arg560-Val561 bond. Unlike streptokinase, urokinase is not immunogenic and allergic reactions are rare. Urokinase produces a systemic lytic state because it does not discriminate between fibrin-bound and circulating plasminogen.

Despite many years of use, urokinase has never been systemically evaluated for coronary thrombolysis. Instead, urokinase is often employed for catheter-directed lysis of thrombi in the deep veins or the peripheral arteries. Because of production problems, urokinase is no longer available.

■ ALTEPLASE

A recombinant form of single-chain tPA, alteplase has a molecular weight of 68,000. Alteplase is rapidly converted into its two-chain form by plasmin. Although single- and two-chain forms of tPA have equivalent activity in the presence of fibrin, in its absence, single-chain tPA has tenfold lower activity.

Alteplase consists of five discrete domains (Fig. 118-9); the N-terminal A chain of two-chain alteplase contains four of these domains. Residues 4 through 50 make up the finger domain, a region that resembles the finger domain of fibronectin; residues 50 through 87 are homologous with epidermal growth factor, whereas residues 92 through 173 and 180 through 261, which have homology to the kringle

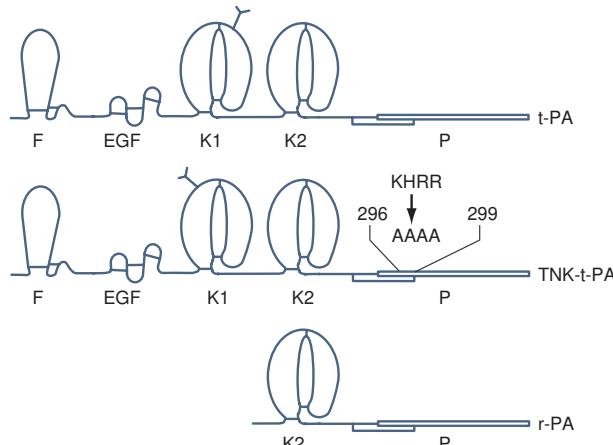


FIGURE 118-9 Domain structures of alteplase (t-PA), tenecteplase (TNK-tPA), and reteplase (r-PA). The finger (F), epidermal growth factor (EGF), first and second kringles (K1 and K2, respectively), and protease (P) domains are illustrated. The glycosylation site (Y) on K1 has been repositioned in tenecteplase to endow it with a longer half-life. In addition, a tetra-alanine substitution in the protease domain renders tenecteplase resistant to type 1 plasminogen activator inhibitor (PAI-1) inhibition. Reteplase is a truncated variant that lacks the F, EGF, and K1 domains.

domains of plasminogen, are designated as the first and second kringle, respectively. The fifth alteplase domain is the protease domain; it is located on the C-terminal B chain of two-chain alteplase.

The interaction of alteplase with fibrin is mediated by the finger domain and, to a lesser extent, by the second kringle domain. The affinity of alteplase for fibrin is considerably higher than that for fibrinogen. Consequently, the catalytic efficiency of plasminogen activation by alteplase is two to three orders of magnitude higher in the presence of fibrin than in the presence of fibrinogen. This phenomenon helps to localize plasmin generation to the fibrin surface.

Although alteplase preferentially activates plasminogen in the presence of fibrin, alteplase is not as fibrin-selective as was first predicted. Its fibrin specificity is limited because like fibrin, (DD)E, the major soluble degradation product of cross-linked fibrin, binds alteplase and plasminogen with high affinity. Consequently, (DD)E is as potent as fibrin as a stimulator of plasminogen activation by alteplase. Whereas plasmin generated on the fibrin surface results in thrombolysis, plasmin generated on the surface of circulating (DD)E degrades fibrinogen. Fibrinogen degradation results in the accumulation of fragment X, a high-molecular-weight clottable fibrinogen degradation product. Incorporation of fragment X into hemostatic plugs formed at sites of vascular injury renders them susceptible to lysis. This phenomenon may contribute to alteplase-induced bleeding.

A trial comparing alteplase with streptokinase for treatment of patients with acute MI demonstrated significantly lower mortality with alteplase than with streptokinase, although the absolute difference was small. The greatest benefit was seen in patients age <75 years with anterior MI who presented <6 h after symptom onset.

For treatment of acute MI or acute ischemic stroke, alteplase is given as an IV infusion over 60–90 min. The total dose of alteplase usually ranges from 90 to 100 mg. Allergic reactions and hypotension are rare, and alteplase is not immunogenic.

■ TENECTEPLASE

Tenecteplase is a genetically engineered variant of tPA and was designed to have a longer half-life than tPA and to be resistant to inactivation by PAI-1. To prolong its half-life, a new glycosylation site was added to the first kringle domain (Fig. 118-9). Because addition of this extra carbohydrate side chain reduced fibrin affinity, the existing glycosylation site on the first kringle domain was removed. To render the molecule resistant to inhibition by PAI-1, a tetra-alanine substitution was introduced at residues 296–299 in the protease domain, the region responsible for the interaction of tPA with PAI-1.

Tenecteplase is more fibrin-specific than tPA. Although both agents bind to fibrin with similar affinity, the affinity of tenecteplase for (DD)E is significantly lower than that of tPA. Consequently, (DD)E does not stimulate systemic plasminogen activation by tenecteplase to the same extent as tPA. As a result, tenecteplase produces less fibrinogen degradation than tPA.

For coronary thrombolysis, tenecteplase is given as a single IV bolus. In a large phase III trial that enrolled >16,000 patients, the 30-day mortality rate with single-bolus tenecteplase was similar to that with accelerated-dose tPA. Although rates of intracranial hemorrhage were also similar with both treatments, patients given tenecteplase had fewer noncerebral bleeds and a reduced need for blood transfusions than those treated with tPA. The improved safety profile of tenecteplase likely reflects its enhanced fibrin specificity.

■ RETEPLASE

Reteplase is a single-chain, recombinant tPA derivative that lacks the finger, epidermal growth factor, and first kringle domains (Fig. 118-9). This truncated derivative has a molecular weight of 39,000. Reteplase binds fibrin more weakly than tPA because it lacks the finger domain. Because it is produced in *Escherichia coli*, reteplase is not glycosylated. This endows it with a plasma half-life longer than that of tPA. Consequently, reteplase is given as two IV boluses, which are separated by 30 min. Clinical trials have demonstrated that reteplase is at least as effective as streptokinase for treatment of acute MI, but the agent is not superior to tPA.

CONCLUSIONS AND FUTURE DIRECTIONS

Thrombosis involves a complex interplay among the vessel wall, platelets, the coagulation system, and the fibrinolytic pathways. Activation of coagulation also triggers inflammatory pathways that may exacerbate thrombosis. A better understanding of the biochemistry of blood coagulation and advances in structure-based drug design have identified new targets and resulted in the development of novel antithrombotic drugs. Well-designed clinical trials have provided detailed information on which drugs to use and when to use them. Despite these advances, however, thromboembolic disorders remain a major cause of morbidity and mortality. Therefore, the search for better and safer targets continues.

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