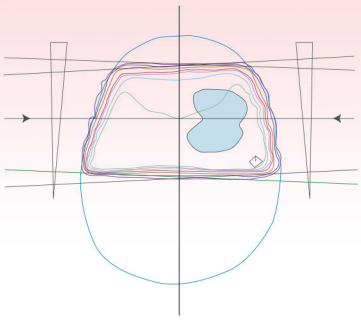
CHAPTER

9 Principles of Chemotherapy



Benjamin Izar, David P. Ryan, and Bruce A. Chabner

A BRIEF HISTORY

In the history of medicine, cancer was initially regarded as a localized disease, and engaged the primary attention of surgeons and radiation oncologists. Metastatic disease was regarded as untreatable. With the arrival of cancer chemotherapy in the form of alkylating agents, in the post–World War II era, it became possible for the first time to entertain the use of drugs to improve local control of cancer, to prevent distant recurrence after surgery, and even to cure systemic disease, as in childhood acute leukemia and choriocarcinoma. With the introduction of adjuvant therapy of breast cancer in the late 1960s the concept of even apparently localized cancer as a potentially systemic disease, which demands systemic therapy, was integrated into the thinking of cancer management in its earliest stages. However, progress in drug discovery was slow. At the time of the publication of the first trials of melphalan for adjuvant therapy of breast cancer in 1971, only a handful of agents were available for treatment of cancer and were primarily drugs developed for the treatment of leukemia. The addition of anthracylines, taxanes, and platinum analogs, and most recently targeted agents, has since that time vastly expanded the effectiveness and the range of uses of chemotherapy.

The history of cancer drug development begins with the initial trials of *alkylating agents* at Yale University, as first reported in 1946.[1] The Yale experiments proved that a systemically administered agent could cause regression of murine leukemia and ultimately a human tumor—in this case a mediastinal mass in a patient with Hodgkin disease. The conceptual basis for clinical studies of nitrogen mustard was the observation that mustard gases used in military campaigns produced lymph node depletion and bone marrow aplasia. Although it was known at the time that these compounds were highly reactive with proteins, nucleic acids, and other electron-rich molecules, the specific intracellular targets on nucleic acids were not identified until more than a decade later, and we are only now beginning to understand the reasons for the somewhat selective action of alkylating agents on tumor cells.

This early experience with alkylating agents led a number of bold investigators to undertake an alternative approach and search for compounds (antimetabolites) that would act as fraudulent counterparts of natural metabolites known to stimulate cancer cell growth. Among these metabolic targets, folic acid and nucleic acid bases proved most vulnerable (Figure 9-1). In the late 1940s, scientists from American Cyanamid synthesized analogs of folic acid, a vitamin known to stimulate the proliferation of cancer cells in culture and in humans. Sidney Farber, a pathologist, found striking but short-lived responses to the first antifolate, aminopterin, and its closely related analog, methotrexate, in children with acute lymphoblastic leukemia (ALL). Prospects for the treatment of leukemia with drugs escalated in the following decade, as new antimetabolites were discovered. Hitchings and Elion at Burroughs Wellcome successfully developed purine analogs, 6-thioguanine and 6-mercaptopurine. Soon afterward, corticosteroids, first used as antiinflammatory agents, were found to kill malignant lymphocytes in children with ALL and in adults and children with lymphoma. In response to the success of combination chemotherapy in childhood ALL, large-scale screening systems for anticancer drugs were initiated at the National Cancer Institute in 1955. These initial attempts at drug discovery tested random chemical libraries and natural product extracts against transplantable murine leukemias and later murine solid tumors, leading to the isolation of new cytotoxic compounds. The newly discovered compounds fell into four primary classes: agents that formed adducts with DNA, agents that blocked mitosis, agents that blocked the function of topoisomerases (DNA unwinding drugs), and antimetabolites.

Combinations of these drugs successfully overcome resistance to single agents and were able to cure childhood ALL and lymphomas. Solid tumor chemotherapy advanced more slowly. Through the discovery of 5-fluorouracil (5-FU), an analog of thymine and a highly potent inhibitor of thymidylate synthase, breast and colon cancer proved modestly responsive. Platinum analogs with potent DNA damaging activity were discovered to have antitumor activity in the early 1970s and, in combination, led to the cure of testicular cancer and to effective combination therapy of ovarian and colon cancer. In addition 5-FU and the platinum drugs were found to be radiosensitizers and improved local control in many tumors of the aero-digestive tracts.

Equally rewarding efforts in the field of natural product chemistry yielded valuable new anticancer drugs with unique mechanisms of action. Scientists at Eli Lilly discovered the antimitotic and antitumor properties of the vinca alkaloids. Shortly thereafter, analysis of fermentation broths led to the discovery of DNA damaging drugs such as mitomycin C, a novel alkylating agent; bleomycin, a DNA-cleaving peptide; and inhibitors of topoisomerase (anthracyclines, and camptothecins); and from natural product screening, even more potent antimitotic agents (paclitaxel and eribulin). Various drug combinations have become standard therapies for most metastatic tumors, and when combined with radiation treatment and surgery as adjuvant therapy, improved the 5-year survival of patients with cancer from 30% in 1950 to more than 60% in the year 2000.

The beginning of the 21st century has witnessed a major breakthrough for cancer treatment with the arrival of targeted therapies (Table 9-1).[2] The development of highly efficient technology for gene sequencing led to the identification of molecular alterations that cause malignancy and shifted the field of drug discovery from cytotoxics to therapies that selectively target oncogenic driver alterations. These driver mutations produce gene products that transform otherwise normal cells and create a tumor dependent for its survival on the specific activated pathway, a conditioned termed *oncogene addiction*. Inhibitors that shut down the target and its pathway lead to death of the cancer cell.

The goal of targeted therapy is not to induce DNA damage but is rather to inhibit specific signaling pathways that control proliferation, cell survival, invasion, metastases, or angiogenesis. A growing number of these agents are available and run the gamut from highly potent small molecules that inhibit

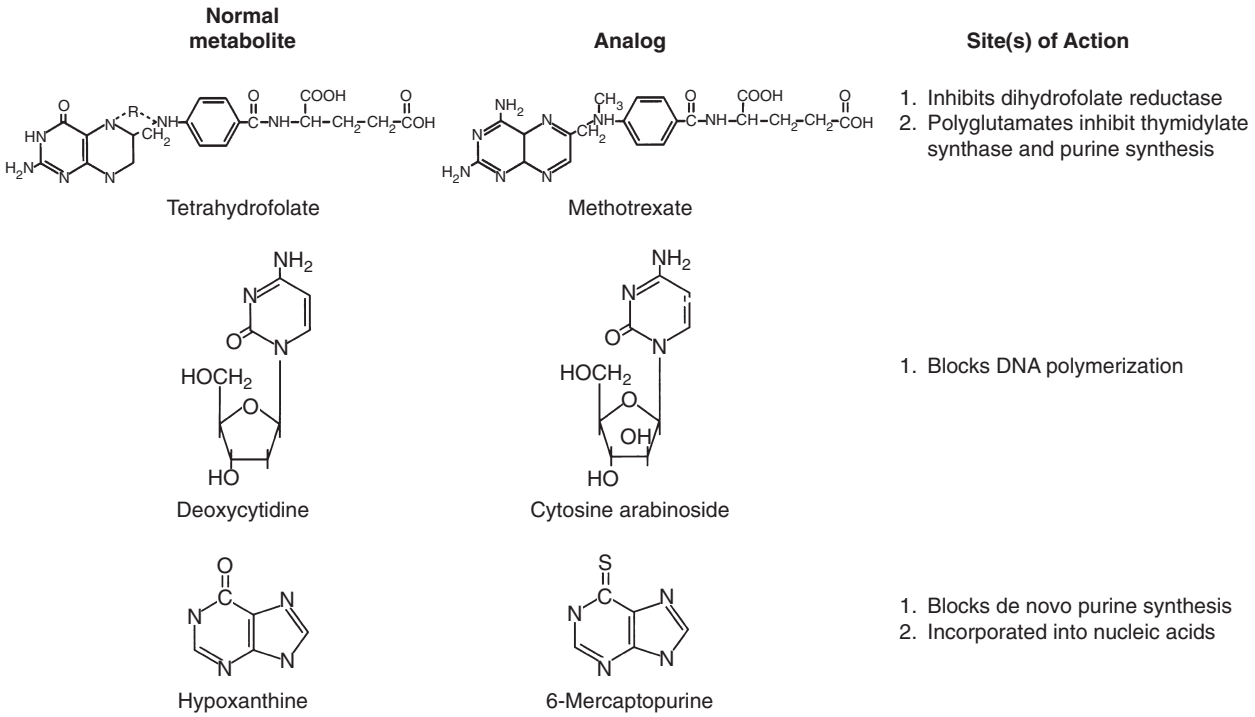


Figure 9-1 Examples of chemotherapy drugs that are analogs of natural metabolites.

TABLE 9-1 FDA-Approved Targeted Cancer Therapies

Drug	Class	Target	Application
Imatinib (Gleevec)	Benzamide	BCR-ABL, C-KIT	CML, GIST
Dasatinib (Sprycel)	Carboxamide	BCR-ABL, SRC, C-KIT, PDGFRβ	CML, Ph + ALL
Nilotinib (Tasigna)	Benzamide	BCR-ABL, PDGFR, C-KIT	CML
Ponatinib (Iclusig)	Benzamide	BCR-ABL (including T151I), PDGFR, C-KIT, SRC, RET	CML, Ph+ ALL
Bosutinib (Bosulf)	Quinolinecarbonitrile	BCR-ABL, SRC, LYN, HCK	CML
Omacetaxine mepesuccinate (Synribo)	Cephalotaxine	Unclear	CML
Gefitinib (Iressa)	Quinazolinamine	EGFR-TKI	NSCLC
Erlotinib (Tarceva)	Quinazolinamine	EGFR-TKI	Colorectal, NSCLC
Afatinib (Gilotrif)	Butenamide	EGFR, HER2, HER4	NSCLC
Crizotinib (Xalkori)	Pyridin-2-amine	ALK, ROS1, MET	NSCLC
Dabrafenib (Tafinlar)	Sulfonamide	BRAF, CRAF	Melanoma
Trametinib (Mekinist)	Acetamide	MEK1 and MEK2	Melanoma
Vemurafenib (Zelboraf)	Carboxamide	BRAF	Melanoma
Ipilimumab (Yervoy)	Recombinant human monoclonal antibody	CTLA4	Melanoma
Cetuximab (Erbitux)	Chimeric monoclonal antibody	EGFR extracellular domain	Colorectal, ENT
Panitumumab (Vectibix)	Human monoclonal antibody	EGFR extracellular domain	Colorectal
Regorafenib (Stivagra)	Carboxamide	RET, VEGF receptor 1-3, PDGFR, FGFR, and others	Colorectal
Bevacizumab (Avastin)	Humanized monoclonal antibody	VEGF	Colorectal, NSCLC, breast, glioblastoma
Ziv-Afilbercept (Zaltrap)	Fusion protein (VEGF-binding portion of VEGF receptors 1 and 2 fused to human IgG1)	VEGF1 and 2	Colorectal
Trastuzumab (Herceptin)	Humanized monoclonal antibody	HER2 extracellular domain	Breast
Lapatinib (Tykerb)	Quinazolamine	HER2 and EGFR-TKI	Breast
Pertuzumab (Perjeta)	Humanized monoclonal antibody	HER2	Breast
Rituximab (Rituxan)	Chimeric monoclonal antibody	CD20	Lymphoma
Tositumomab (Bexxar)	131I murine monoclonal antibody	CD20	Lymphoma

TABLE 9-1 FDA-Approved Targeted Cancer Therapies—cont'd

Drug	Class	Target	Application
Ibritumomab (Zevalin)	111In murine monoclonal antibody	CD20	Lymphoma
Brentuximab (Adcetris)	Antibody-drug conjugate (IgG1, MMAE and a linker)	CD30	Lymphoma
Ibrutinib (Imbruvica)		BTK	Lymphoma
Vorinostat (Zolinza)	Phenyloctanediamide	HDAC	Lymphoma
Romidepsin (Istodax)	Bicyclic depsipeptide	HDAC	Lymphoma
Dentileukin Difitox (Ontak)	Recombinant fusion of diphtheria toxin and IL-2	IL2-receptor(s)	Lymphoma
Bortezomib (Velcade)	Modified boronic acid	26S proteasome	Myeloma
Carfilzomib (Kyprolis)	Carboxamide	26S proteasome	Myeloma
Obinutuzumab (Gazyva)	Humanized monoclonal antibody	CD20	CLL
Ofatumumab (Arzerra)	Human monoclonal antibody	CD20	CLL
Alemtuzumab (Campath)	Humanized monoclonal antibody	CD52	B-CLL
Gemtuzumab (Mylotarg)	Calicheamicin linked to human monoclonal antibody	CD33	AML (CD33+)
Sunitinib (Sutent)	Butanedioic acid	VEGFR, C-KIT, PDGFR, RET, FLT3	Renal, GIST
Sorafenib (Nexavar)	Carboxamide	BRAF, FLT3, C-KIT, VEGFR, RET, PDGFRβ	Hepatocellular, renal
Everolimus (Afinitor)	Rapamycin derivative	mTOR	Renal
Temsirolimus (Torisel)	Macrolide	mTOR	Renal
Axitinb (Inlyta)	Benzamide	VEGF receptor 1-3	Renal
Pazopanib (Votrient)	Methylbenzenesulfonamide	VEGF receptor 1-3, PDGFR, FGFR1, C-KIT	Renal, sarcoma
Vandetanib (Vandetanib)	Quinazolin-4-amine	EGFR and VEGF	Thyroid
Vismodegib (Erivedge)	Benzamide	Smoothened (Hedgehog pathway)	Basal cell
Capozantinib (Cometriq)	Carboxamide	RET, MET, PDGFR, FGFR	Thyroid

AML, *Acute myelocytic leukemia;* B-CLL, *B-cell chronic lymphocytic leukemia;* CML, *chronic myelogenous leukemia;* GIST, *gastrointestinal stromal tumor;* NSCLC, *non–small cell lung cancer.*

specific targets such as receptor tyrosine kinases (RTKs), to monoclonal antibodies that block the same receptors, initiate immune responses against tumor antigens, carry cytotoxic payloads, or block tumor blood vessel formation. Monoclonal antibodies such as trastuzumab, cetuximab, and bevacizumab also enhance the effectiveness of chemotherapy, and cetuximab, an inhibitor of the epidermal growth factor receptor (EGFR), synergizes with irradiation (see Chapters 4 and 5). The role of targeted drugs in the overall management of cancers is continuously evolving. Targeted drugs used in sequence with or in conjunction with irradiation and chemotherapy are under active investigation in most solid tumors, lymphomas, and leukemia.

THE BASIS OF CHEMOTHERAPY: CANCER CELL BIOLOGY

Every phase of chemotherapeutic research, from discovery to clinical application, is based on our improved understanding of cancer cell biology (see Chapter 2). Cancer cells have unique properties that distinguish them from their normal counterparts and that form the basis for treatment. Among these properties are continuous and excessive proliferation, defective repair of DNA leading to high mutation rates and great population diversity, reduced rates of apoptosis (i.e., programmed cell death), altered metabolism to enhance lipids and nucleic acid synthesis, induction of nutrient vessels, ability to escape immune surveillance, and ability to invade neighboring tissues and to metastasize.[2] Many of these properties have been the starting point for successful drug discovery efforts.

Most cancer cells display defects in DNA repair that allow the rapid generation of a diversity of subclones, thus increasing their adaptation to adverse environments (low pH, poor nutrients), and increasing the probability of drug resistance. With a few notable exceptions, the successful cytotoxic drugs have attacked only the first of these properties, proliferation. The new generation of targeted agents is expanding the horizon for cancer treatment by addressing the full circle of biological changes in human tumors.

MODELS FOR CHEMOTHERAPY

In parallel with the discoveries of new drugs in the period from 1946 to 1970, Skipper and colleagues[3] at the Southern Research Institute and at the National Cancer Institute developed and characterized transplantable murine leukemias, notably L1210 and P388, as well as murine solid tumors such as sarcoma 180 and B16 melanoma. Their model systems allowed reproducible, quantitative experiments with chemotherapy and radiation therapy in mice. They established a rational basis for understanding the kinetics of cell kill, evaluating efficacy of drug combinations, and studying mechanisms of drug resistance. From their experiments emerged the theoretical basis for combination chemotherapy:

1. *Fractional cell kill.* Each dose of chemotherapy kills a constant fraction of the tumor cell population. Pharmacokinetic parameters correlate with cell killing. For example cell kill by alkylating agents increases linearly with dose and

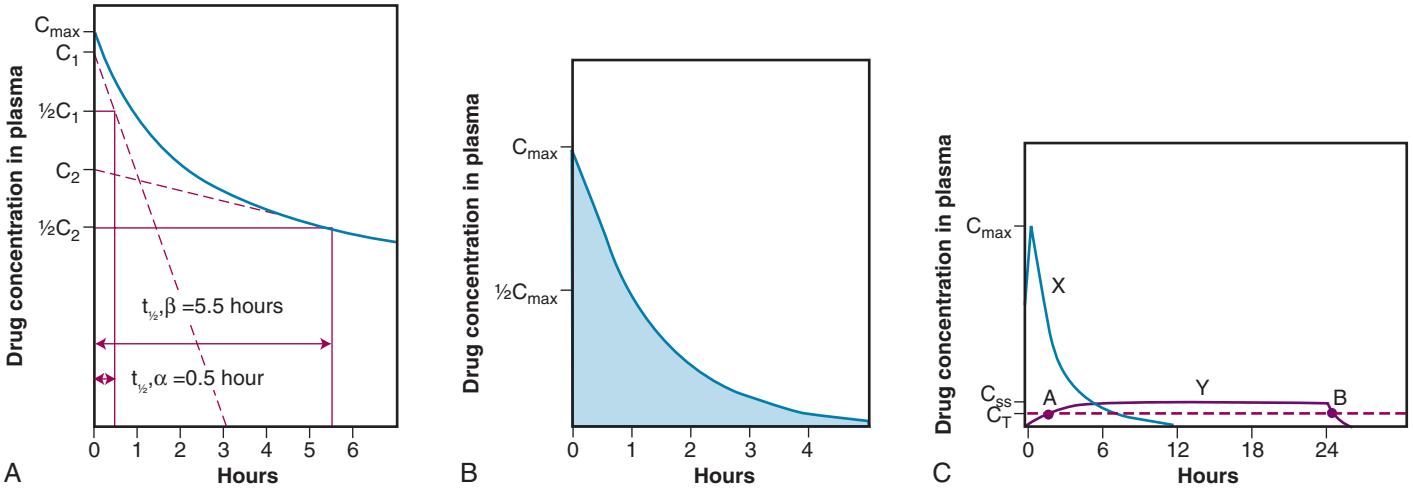


Figure 9-2 Drug elimination from plasma. **A,** The *solid blue curve* illustrates a semilogarithmic plot of drug concentration versus time after a rapid intravenous injection. The *dashed red line* intercepting the *y*-axis at C_2 represents an extrapolation of the log-linear terminal phase. The *dashed red line* that intersects the *y*-axis at C_1 is obtained by subtracting the extrapolated values of the log-linear terminal phase from the observed drug concentrations. Maximum drug concentration in plasma (C_{max}) = $C_1 + C_2$. The initial (α) phase half-life ($t_{1/2,\alpha}$) is the time for C_1 to decay to $\frac{1}{2}C_1$. The terminal (β) phase half-life ($t_{1/2,\beta}$) is the time for C_2 to decay to $\frac{1}{2}C_2$. This biphasic behavior results from distribution of the drug among rapidly and slowly perfused regions of the body, as well as its elimination. **B,** Drug concentration in plasma versus time is plotted on linear axes. The *blue shaded area* is the area under the curve (AUC); it represents the integral of drug concentration over time. The AUC is a measure of total systemic exposure to the drug. **C,** Linear plots of drug concentration versus time are illustrated for a rapid intravenous injection *(X)* and a 24-hour continuous infusion *(Y)* of the same total dose of drug (the AUCs are equivalent). Notice that the duration of drug concentrations above the threshold for cytotoxicity (C_T) is much longer with the continuous infusion (represented as B-A) than with the bolus administration. Conversely, the maximum plasma concentration achieved by bolus administration (C_{max}) is much larger than that of the continuous infusion (C_{SS}).

peak drug concentration, whereas for most other drugs, kill depends on the area under the curve describing drug concentration over time (or $C \times T$) (Figure 9-2). For other drugs, such as paclitaxel and for most of the targeted therapies, the *time of exposure above a threshold concentration* determines the cytotoxicity for tumor and for normal target tissues. For drugs that depend on continuous inhibition of the target, such as the receptor tyrosine kinase inhibitors, it is important to maintain a constant receptor-inhibiting drug concentration in plasma.

2. *Importance of dose intensity (dose per unit time).* During the time period between cycles of treatment, tumor cells resume proliferation. Short rest periods between treatment cycles, and higher doses of drug produce the best results.
3. *Drug resistance.* Exposure of tumors to single-agent chemotherapy rapidly results in outgrowth of drug-resistant cells. Biochemical studies of these drug-resistant cells disclosed a number of changes, including decreased drug uptake (methotrexate), increased drug export (anthracyclines and taxanes), enhanced DNA repair (alkylating agents and platinum analogs), mutations or amplification in the drug target (methotrexate and 5-FU), or loss of intracellular pathways required for drug activation (many nucleoside and base analogs such as 5-FU and fludarabine require phosphorylation). Combinations of drugs with different mechanisms of action and different mechanisms of resistance were able to cure lymphomas and leukemia that readily become resistant to single agents.
4. *Cell cycle dependency of cell kill.* Most anticancer drugs, particularly the antimetabolites, have their greatest effect on actively proliferating cells. Drugs that act on DNA synthesis damage cells during periods of DNA synthesis (S phase), whereas mitotic inhibitors produce cell kill through exposure of cells during mitosis (M phase) (Figure 9-3). The rate of cell proliferation slows and the number of nonproliferating cells increases with expansion of the tumor mass. Other factors such as poor perfusion and increased oncotic pressure discourage nutrient and drug entry into large, poorly vascularized tumor masses. Chemotherapy is most effective when the tumor burden is lowest and cell proliferation is most active, as in the adjuvant setting.

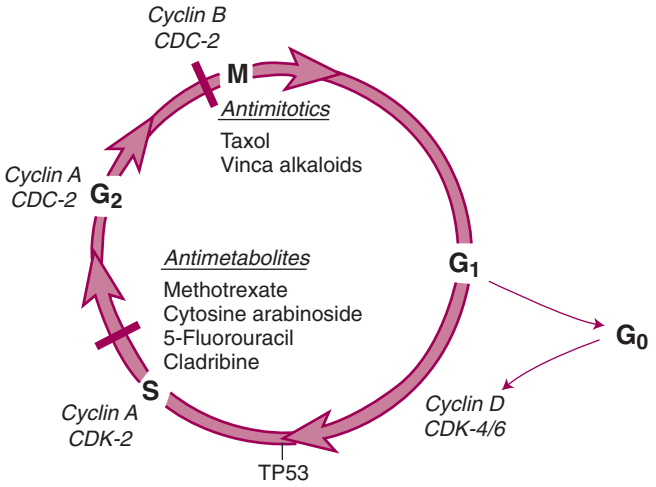


Figure 9-3 The cell cycle, its controls and checkpoints, and the site of action of cell cycle phase-specific drugs. The cell cycle phases are G_0 (nondividing cells), G_1 (resting phase), S (DNA synthesis), G_2 (gap between S and M), and M (mitosis). Transitions between phases are controlled by the appearance of specific cyclin proteins that complex with and activate cyclin-dependent kinases. The G_1/S transition is also controlled at a checkpoint by proteins such as TP53, which monitor DNA integrity. Other proteins monitor the G_2/M checkpoint.

These principles expounded in mouse models profoundly influenced all aspects of clinical chemotherapy, including regimen design, the use of drugs in combination, adjuvant

chemotherapy, and high-dose chemotherapy. Relying on these principles, the cure of ALL was accomplished through development of effective combination therapy. However, other measures were required to prevent relapse: the institution of intrathecal methotrexate and neuro-axis irradiation to eliminate central nervous system (CNS) leukemia, refinement of drug dosage and schedule to maximize drug efficacy, the implementation of maintenance methotrexate and 6 mercaptopurine,[4] and supportive care with platelets and antibiotics. Each of these insights, further refined by the use of new antinausea medications and bone marrow colony-stimulating factors, has become a basic component of modern chemotherapy.

SOLID TUMOR CHEMOTHERAPY

The principles enumerated have been applied with greatest success to the treatment of aggressive and rapidly proliferating tumors such as leukemias and lymphomas. The development of drug therapy for the more common solid tumors has taken a slower and more tortuous course. Drugs identified in mouse leukemia screening systems have been less effective against most solid tumors, notable exceptions being choriocarcinoma and testicular cancer, which can be cured with repeated cycles of intensive chemotherapy. However, new chemotherapies have improved survival of metastatic cancer: 5-FU, beginning in the 1950s, doxorubicin and cisplatin in the early 1970s, etoposide and paclitaxel in the late 1980s, the antimetabolites pemetrexed and gemcitabine in 1990s, and since 2000, a new antimitotic, eribulin[5]; a unique alkylator, bendamustine[6]; and an albumin encased taxane, abraxane.[7]

An important breakthrough in solid tumor chemotherapy was the proposal to employ drugs in the adjuvant setting after removal of the primary tumor in patients at high risk for relapse. Drugs that produced only partial responses in advanced disease could prevent disease recurrence in a significant fraction of women with Stage II (node positive) localized breast cancer. The conceptual basis for this strategy derived from experimental chemotherapy models that revealed that tumors are most susceptible to chemotherapy when the tumor burden is small and cells are actively proliferating. Cytotoxic drugs now have a firmly established role in the adjuvant treatment of lung, breast, and colorectal cancers either before or after surgery (see Chapter 4).

Newer concepts aimed at improving local control of otherwise inoperable tumors have led to so-called neoadjuvant, or induction, therapy.[8] In this strategy, drugs are used alone or in combination with radiation therapy, before surgery, in the initial treatment of locally advanced tumors of the breast, head and neck, bladder, rectum, and lung (see Chapter 4, Tables 4-2 to 4-7). This preoperative therapy reduces the size of otherwise unresectable tumors to a point where total surgical removal is feasible, less morbid and organ preserving, or, in some cases, such as anal cancer, even unnecessary. A new paradigm for drug approval has been established with the Food and Drug Administration's (FDA's) accelerated approval of the monoclonal antibody, pertuzumab, for neoadjuvant therapy of HER2+ breast cancer, based on improvements in the pathological complete remission rate at the time of surgery.[9]

Advances in bone marrow stem cell harvesting from peripheral blood, their storage, and reinfusion after high-dose chemotherapy has allowed escalating drug dosage and increasing dose intensity. In this setting, high-dose chemotherapy followed by marrow stem cell infusion is remarkably safe and reliably cures a significant minority of patients with relapsed lymphomas and relapsed or high-risk leukemia and a fraction of patients with relapsed testicular cancer. As they become safer to use, high-dose regimens may be employed in earlier stages of disease to enhance the cure rate of otherwise incurable cancers in patients, although the value of this approach for most solid tumors is unproven

DRUG INTERACTIONS WITH IRRADIATION

Because most patients with cancer now require multimodality therapy, even for early-stage tumors, it is important to understand the potential benefits and risks of drug interaction with irradiation.[10] The biological basis for these interactions and the rationale for current regimens are presented in Chapters 4 and 5. To be effective, irradiation requires the presence of oxygen to produce toxic oxygen radicals and is countered by the cell's attempts to scavenge the radicals and repair DNA damage. Radiosensitizers can act by increasing oxygenation, creating new DNA breaks, depleting scavengers of oxygen free radicals, or blocking repair of DNA breaks. Each of these properties has been the subject of intensive clinical investigation. The most favorable of these interactions identified thus far stem from the radiosensitizing properties of three drugs, often used in combination with irradiation: 5-FU, a drug that inhibits thymidylate synthase and thereby blocks DNA synthesis; platinum analogs, which form adducts with DNA, create DNA breaks, and deplete free radical scavengers such as sulfhydrils and glutathione; and mitomycin C, which forms free radicals and DNA adducts in hypoxic environments. Other drugs, particularly gemcitabine, doxorubicin, and bleomycin, are extremely potent radiation sensitizers, and generally should not be used simultaneously with irradiation for fear of serious toxicity to heart, lungs, and other normal tissues. The potential value of combining irradiation with antivascular endothelial growth factor (anti-VEGF) therapies, which normalize tumor blood vessels and improve oxygenation and drug deliver, is reviewed in Chapter 5 and is again intriguing, but unproven.

Most recently, inhibitors of DNA repair have entered clinical trial. Oliparib blocks the function of polyadenosyl ribose polymerase (PARP), a central component of base repair; it has antitumor activity in patients harboring breast cancer or serous ovarian cancer with defects in homologous DNA repair due to BRCA 1 or BRCA 2 mutations, and has interesting, but unproven potential for enhancing radiation damage to DNA in tumors with DNA repair defects such as BRCA 1 or 2 mutations (see Chapter 4).

Drugs and irradiation may share common mechanisms of tumor cell resistance. Increased proficiency of repair of double strand breaks may impair the response to both irradiation and to several classes of antitumor drugs, including alkylating agents, platinum analogs, and topoisomerase inhibitors (see Chapter 4). Cells with defects in apoptosis and checkpoint function (TP53 mutation) fail to initiate apoptosis in radiation resistant tumors. Stem cells, both cancer related and normal varieties, are inherently resistant to reactive oxygen damage and overexpress the multidrug resistance (MDR) transporter.[11] Tumor stem cells are quiescent regarding cell cycle progression and may reside in hypoxic niches within tumors. The epidermal-to-mesenchymal phenotypic transition (EMT), with distinctive cell surface markers[2] and a drug-resistant profile similar to that seen in stem cells, appears to be a common event in drug- and radiation-resistant tumors. New drugs designed to reactivate apoptotic pathways (BCL2 inhibitors) and cell checkpoint function (HMD2 inhibitors), and inhibitors of the PI3 kinase cell survival pathway are of particular interest in reversing EMT changes.

Because drugs have become an integral part of the initial therapy of many patients with cancer, it is essential that the medical oncologist, surgeon, and radiation oncologist understand the principles of chemotherapy and the specific features of the commonly used agents.

THE CYTOTOXIC DRUGS: TARGETING DNA SYNTHESIS AND MITOSIS

The cytotoxic drugs in current clinical use act directly on the synthesis or integrity of DNA. These drugs may inhibit synthesis of DNA or its precursors, block cell division, inhibit necessary changes in DNA topology, or covalently bind to DNA, causing strand breaks or miscoding. All such drugs affect the integrity of DNA, and in the presence of the normal machinery (TP53) for monitoring DNA integrity, they induce apoptosis. Unfortunately, they are also cytotoxic toward normal cells. The reasons for their somewhat selective toxicity for malignant versus normal cells, as is apparent in the cure of lymphomas and leukemias, are poorly understood. The process of malignant transformation may enhance sensitivity to DNA damage by virtue of defects in DNA repair, although at the same time these same defects expand tumor cell diversity and encourage drug resistance. Many of the defective genes responsible for inherited cancer syndromes, such as *BRCA1* and *BRCA2* genes, mismatch repair genes, *TP53*, and nucleotide excision repair defects, create sensitivity to irradiation and to agents that inhibit aspects of DNA repair.

In designing clinical regimens, the relationship of drug action to the cell cycle is of particular importance because this knowledge serves as the basis for combining drugs and sequences in clinical practice and influences their use in combination with radiation therapy. The cell cycle and its primary controls are shown in Figure 9-3. Antimetabolites such as methotrexate, 5-FU, cytosine arabinoside, gemcitabine, and the purine antagonists kill cells that are actively synthesizing DNA; therefore, they are cell cycle dependent. Nucleoside analogs, including cytosine arabinoside, fludarabine phosphate, and cladribine, must be incorporated into newly synthesized DNA to be cytotoxic. Alternately, other agents such as the camptothecins, etoposide, and doxorubicin produce DNA strand breaks at any stage of the cell cycle, and are less dependent on cell cycle events for cytotoxicity, although these breaks may become lethal only as the cell crosses a checkpoint and enters DNA synthesis. Antimitotic agents, constituting a separate class of drugs, block the formation of the mitotic spindle and thereby prevent separation of chromosomes to the daughter cells. Drugs of this class are therefore effective against cells during the mitotic phase of the cell cycle. Still others, such as alkylating agents and platinum compounds, bind covalently to DNA and produce strand cross-links and strand breaks. Their toxicity seems less dependent on cell cycle stage.

Once it has been damaged through its encounter with a cytotoxic drug, the cancer cell has several options, and its eventual viability depends on which pathway it takes. If the normal monitors for genomic integrity (including most prominently the product of the *TP53* gene) are intact, the cell may halt further progression in the cell cycle while its DNA is repaired. If the damage is sufficiently extensive, TP53 may initiate apoptosis. If, however, TP53 function is absent, cell cycle progression may continue past the G1-S checkpoint despite drug-induced DNA damage, and the cell may prove viable. In most experimental settings, lack of wild-type TP53 is associated with drug and irradiation resistance. Paradoxically, some DNA repair defects, as found in mismatch repair (colon cancer), are associated with resistance to thio-purine analogs, platinum analogs, and alkylating agents, perhaps as a result of a failure to respond to DNA adducts and to initiate apoptosis.

From a theoretical viewpoint, it is understandable that rapidly dividing tumor cells, such as those found in acute leukemia, high-grade lymphoma, and choriocarcinoma, may be exquisitely sensitive to antimetabolites and cell cycle–specific drugs. How do these drugs kill the more slowly dividing solid tumors? Many of these tumors have long cell cycles (4 to 5 days). Many cells are in the G0 phase (nondividing state), and at any moment, only 1% to 3% of cells are in S phase. Cell kinetic factors diminish the effectiveness of chemotherapy, and the disordered vascularization of tumors may also contribute by limiting drug entry into areas of slow blood flow. Despite these disadvantages, solid tumor chemotherapy produces meaningful responses. Several factors, including pharmacologic, pharmacokinetic, and tumor cell-specific factors likely contribute to solid tumor killing by cycle specific or cycle sensitive drugs:

1. Drugs such as paclitaxel and doxorubicin clear slowly from the blood stream and the extracellular space. Alternative regimens, such as prolonged drug infusion, seem to increase the activity of 5-FU and paclitaxel, compared with the results of bolus administration.
2. Other drugs, such as methotrexate, which forms an intracellular polyglutamated species, and gemcitabine, which forms a long-lived intracellular triphosphate, persist inside the cell long after their disappearance from plasma.
3. As mentioned, tumor cells may be less able to repair DNA and more susceptible to cell death induced by DNA damage than their normal counterparts. For example, transformed mouse embryo fibroblasts have a heightened sensitivity to cancer drugs compared with their nontransformed parent, provided they have wild-type *TP53* genes.[12]
4. Underlying the tumor proliferation rate is a significant death rate that results from a number of factors, such as hypoxia, nutrient deprivation, disordered DNA synthesis, and mitosis, and, in general, a high-background rate of mutation that affects genes essential for cell integrity and survival. A small shift in the balance between cell proliferation and cell death may lead to regression of a tumor, despite its low growth fraction

DRUGS THAT INDUCE DIFFERENTIATION

Although differentiation inducers have long attracted interest for cancer drug development, only a small number of compounds have reached clinical evaluation. 5-aza-cytidine and its close congener, 5-aza-2′deoxycytidine are both approved for treatment of myelodysplastic syndrome. They induce differentiation by irreversibly inhibiting DNA methyl transferase. They are modestly myelosuppressive but induce the production of both myeloid and erythroid lineages and reduce transfusion dependence in MDS.[13]

A more compelling example of differentiation induction is provided by all trans-retinoic acid (ATRA), which binds to the mutated retinoic acid receptor created by the RAR-PML translocation in acute promyelocytic leukemia (APL), thereby inducing differentiation and remission. Its primary toxicity is a syndrome of pulmonary failure resulting from clogging of small vessels by the mature leukemic granulocytes. Arsenic trioxide is a second agent capable of induction of differentiation in APL. It promotes degradation of the translocated fusion protein, produces free radicals, and has antiangiogenic properties as well. Its toxicities include the pulmonary failure syndrome seen with ATRA, prolongation of the PR interval, arrhythmias that are accentuated by potassium (K+) and magnesium (Mg+) depletion, and hyperglycemia.

IMMUNOTHERAPY

Immunotherapies have been the object of much research attention, but only in the past decade have drugs of this kind proved useful. Monoclonal antibodies have clearly won a place in the standard regimens for treatment of lymphoma

(brentuximab for Hodgkin disease and rituximab for B-cell lymphomas), breast cancer (traztuzumab, pertuzumab, and TDM1 for Her-2 positive tumors), and cetuximab and panitumumab for colorectal cancer. Their mechanisms of action may simply be engagement and inhibition of a receptor necessary for survival, but auxiliary actions such as antibody dependent cellular cytotoxicity may play a role. The newest additions to immunotherapy include anti CTLA-4 and anti-PD1 and antiPDL-1 antibodies, which release autoimmune antitumor responses; these have shown remarkable long-term responses in melanoma, kidney cancers, and adenocarcinoma of the lung.[14-17] Even more impressive are the massive antitumor effects of chimeric antigen receptor T cells in which T-cell receptors are engineered to recognize the CD-19 auto-antigen antigen and destroy all B cells, physiological and malignant, including B-cell lymphomas and leukemias.[18]

TARGETED THERAPIES: SIGNAL TRANSDUCTION INHIBITORS

Expanding knowledge of the biology of cancer cell growth and death and the processes that regulate differentiation has provided a new set of targets for cancer treatment. Excessive proliferation of tumor cells has many causes; observations of tumor biology in model systems and in well-studied human cancers have shown that the proliferative drive may result from mutations that stimulate cell division as, for example, mutated growth factor receptors, whereas other mutations free the cancer cell from normal cell cycle controls through loss of suppressor functions such as mutations in the cell cycle checkpoint, TP53, or mutations in the retinoblastoma pathway. Still other mutations promote cell survival as occurs with activating mutations in the PI3-kinase pathway and activation of antiapoptotic proteins such as BCL-2 (see Chapter 2).

Drug discovery efforts have targeted specific activated pathways and mutations that promote proliferation and cell survival (see Table 9-1).[19] Inhibiting the function of a dominant gene product is a classic problem in cancer drug discovery. In the late 1980s, the first efforts at targeted inhibition led to development of inhibitors of the K-RAS protein, a signal transducer that links growth factor receptors to the MEK-ERK downstream cascade. K-RAS is mutated in 80% to 90% of pancreatic cancers and provides the proliferative drive in subsets of patients with non–small cell lung cancer (NSCLC), acute myelogenous leukemia, and colorectal cancers. The K-RAS protein is activated by an enzymatic step that attaches a lipid tail (a farnesyl group) to its near-terminal cysteine. Although inhibitors of farnesylation caused regression of experimental K-RAS-driven tumors, these drugs failed in clinical trials because the inhibitors lacked specificity for farnesylation of K-RAS, and alternative pathways for lipid modification of K-RAS circumvented the inhibition. New K-RAS strategies now focus on blocking a unique binding pocket in the G12C mutation, or downstream inhibition of the MEK-ERK pathway activated by mutant K-RAS.[20]

The first successful targeted small molecule emerged from studies of the BCR-ABL kinase in chronic myeloid leukemia (CML). In 1998, Druker introduced imatinib, an adeno triphosphate (ATP) competitive inhibitor that produced molecular remissions in a high percentage of patients who had progressed on standard chemotherapy. Imatinib produces long-term disease control, with normalization of both blood counts and cytogenetics in more than 90% of previously untreated patients in the chronic phases of the disease. Newer inhibitors, nilotinib, busotinib, and dasatinib, kill CML cells with mutations that confer resistance to imatinib. The newest and most potent BCR-ABL inhibitor, ponatinib, has sustained activity against virtually all resistant cell lines, including T315I, and was approved for the treatment of resistant CML and Philadelphia chromosome positive ALL.[19]

The success of imatinib has spawned an ever-increasing number of successful targeted agents (see Table 9-1). A parallel development in chronic lymphocytic leukemia (CLL) has led to the success of ibrutinib, an inhibitor of the Bruton tyrosine kinase, a key mediator of B-cell receptor signaling and early B-cell proliferation. The drug is approved for second line CLL and mantle cell lymphoma.[21] Its toxicities are limited to diarrhea, rash, and mild hepatic enzyme elevations. It does not produce profound immunodeficiency, as compared to the highly immunosuppressive anti-CD20 antibody, rituximab.

Imatinib also inhibits the KIT (CD117) receptor and proved to be the first targeted small molecule effective in the management of a solid tumor, in this case metastatic gastrointestinal stromal tumor (GIST). The presence of specific activating mutations within the *KIT* gene predicts for response in patients with GIST (see Table 9-1). The multitargeted tyrosine kinase inhibitor, sunitinib, has demonstrated benefit in patients with imatinib-refractory GIST, even in those with *KIT* mutations that are typically resistant to imatinib.[19]

Downstream of RTKs, other phosphorylation steps, when constitutively activated by mutation, may lead to malignancy and may be successfully targeted by small molecules. A striking example is presented by B-RAF inhibitors for melanoma. More than 50% of advanced stage melanomas carry a mutation of *B-RAF*, most commonly resulting from V600E or V600K mutations (BRAFV600E or BRAFV600K), which are transforming alterations that produce constitutive activation of the downstream MEK/ERK pathway and cancer cell proliferation. Vemurafenib, a selective inhibitor of the mutated BRAF protein, with limited inhibition of the wild type enzyme, produced an unparalleled 80% antitumor response rate in patients with advanced melanomas. A subsequent study showed that the combination of another BRAF inhibitor, dabrafenib, with a MEK inhibitor, trametinib, was superior to monotherapy with dabrafenib. The response rate in the cohort receiving combination therapy was 76% and a progression-free survival of 9.8 months compared with 5.8 months in the monotherapy groups, leading to approval of both new drugs in 2013.[22] It is noteworthy that the same mutation in *B-RAF*, V600E, found in melanoma also occurs in subsets of colorectal cancer, thyroid cancer, and NSCLC. Although lung and thyroid cancers with B-RAF mutations do show sensitivity to B-RAF inhibition, colorectal cancers of this type are unresponsive, indicating that the tissue context of the mutation may be important in determining response.[19]

RECEPTOR TYROSINE KINASES AS TARGETS

Mutations or translocations in genes coding for RTKs cause epithelial cancers arising in subsets of patients with lung, colon, thyroid, and breast tumors. These RTK mutations constitutively activate the kinase function of EGFR, EML4-ALK, and ROS-1 in NSCLC, C-KIT in melanoma and gastrointestinal stromal tumors, and RET in medullary thyroid cancer. The net effect is to create a cell under constant proliferative stimulus. Cells with these mutations become addicted to the aberrant signal and die when the signal is interrupted. Most clinically effective kinase inhibitors target the ATP binding pocket of the RTK.

EGFR inhibitors have proved useful in NSCLC. EGFR is overexpressed in many solid tumors and plays a critical role in activating downstream molecules that control cell cycle

progression, proliferation, angiogenesis, and survival. Subsequent trials have revealed that this new class of drugs is highly effective for the 10% of patients with NSCLC with tumors that harbor *activating mutations* in EGFR, but there is no clear benefit in the 40% to 80% of NSCLCs that overexpress *nonmutated* EGFR. Activating mutations caused by deletions or base substitutions clustered around the ATP-binding pocket of the tyrosine kinase domain of EGFR. Seventy percent of EGFR-mutated tumors respond initially to erlotinib. The median duration of response is 10 months, exceeding the results of chemotherapy by 3 to 4 months. The benefits of erlotinib and gefitinib are offset by important toxicities, including acneform rash and diarrhea. Resistance arises through further mutations in the ATP-binding pocket of the receptor, or by activation of the c-MET pathway. A second-generation EGFR inhibitor, afatinib, with greater potency of binding, produced a highly significant improvement in response rate and progression-free survival, compared to conventional chemotherapy for relapsed patients. Third-generation inhibitors from Astra-Zeneca and Clovis, which irreversibly bind to mutant EGFR and specifically block the mutant receptor, remain active against EGFR-resistance mutations such as T790M, have much reduced toxicity to bowel and skin, and produce clinical responses in patients resistant to erlotinib, according to early clinical experience.

Cetuximab, a chimeric IgG1 monoclonal antibody targeting the extracellular portion of the EGFR, is not active against lung adenocarcinoma with wild type or mutated EGFR, but is approved for the treatment of chemotherapy-refractory colorectal cancer. The addition of cetuximab to irinotecan results in nearly doubled response rates, as compared to irinotecan alone.[19] Panitumumab, another monoclonal antibody targeting the EGFR, improved progression-free survival rates compared with placebo in patients with chemotherapy refractory. Responses are more frequent in patients who develop the typical acneform rash associated with these agents. More importantly, patients who have activating mutations of K-RAS or BRAF derive no benefit from these EGFR antibodies. Cetuximab enhances local disease control with irradiation in squamous cell carcinoma of the head and neck.[23]

Other subsets of NSCLC contain unique RTK translocations that have been successfully targeted. The *EML4-ALK* translocation activates the anaplastic large-cell kinase RTK in 4% of patients with NSCLC. In a phase I/II trial of the MET/ALK inhibitor, crizotinib, patients possessing the ALK fusion protein exhibited a 53% clinical response rate, with an additional 20% of patients having stable disease for prolonged periods, findings confirmed in a phase II trial. This agent also has activity against other *ALK* driven tumors, including anaplastic large cell lymphoma and inflammatory myeofibroblastic sarcomas.[19] Resistance to crizotinib develops in the form of mutations in the ATP-binding site of the fusion kinase. A series of second-generation inhibitors, insensitive to these crizotinib-resistance mutations, have strikingly high response rates in *EML4-ALK* mutated tumors and will likely displace crizotinib as primary therapy (Shaw, A. New Engl J Med, in press). Crizotinib is also active against kinases associated with an activating translocation of the ROS-1 RTK,[24] found in 1% of NSCLC.

HER2-DIRECTED THERAPY

HER2 (EGFR 2 or ERB B-2), is a RTK activated by heregulin. It lacks the ability to signal downstream but promotes proliferation and survival through dimerization with one of the other EGFR receptor family members, preferentially HER3. As a dimer, it signals through both the MAP kinase and PI3K/AKT pathways. About 25% of breast cancers demonstrate amplification of the *HER2* gene, resulting in ligand-independent cancer cell proliferation and survival. *HER2* amplification is associated with a more aggressive phenotype and worse clinical outcomes. Trastuzumab, a monoclonal antibody against HER2, is approved for the treatment of metastatic HER2-positive breast cancer. Although monotherapy with traztuzumab shows modest efficacy, its combination with taxanes or other chemotherapies in HER+ breast cancer results in improved response rate, disease-free survival, and overall survival and decreased recurrence in adjuvant therapy, as compared with chemotherapy alone.[25] Trastuzumab also shows efficacy with chemotherapy for *HER2* amplified gastric adenocarcinomas.[26]

A newer antibody, pertuzumab, blocks HER2 dimerization with HER 3 and prevents signaling through HER3. In combination with trastuzumab and docetaxel, pertuzumab improved progression-free survival (18.5 months versus 12.4 months) and overall survival in metastatic disease[27] and yielded an improved pathological complete response rate in presurgical treatment of breast cancer (neoadjuvant therapy). A growing number of targeted therapies are being tested, with the aim of gaining accelerated approval by demonstrating improvements in pathological CR rates in neoadjuvant trials of early breast cancers.

Small molecules that compete with ATP are also effective in blocking HER2 signaling. Lapatinib, a selective HER2 and EGFR inhibitor, proved effective with capecitabine in the treatment of HER2-positive breast cancer, after progression on traztuzumab.[19] In combination with traztuzumab and a taxane, lapatinib enhances time to progression in first-line therapy.[25]

Trastuzumab has also been used as a carrier of a highly toxic maytansine derivative, emtansine. Trastuzumab-DM-1 (TDM-1), approved in 2013 for the treatment of HER2-positive breast cancer after progression on trastuzumab, produced superior progression-free survival (9.6 months versus 6.4 months) and overall survival (30.9 months versus 25.1 months), as compared with lapatinib plus capecitabine.[9]

PI3 KINASE AS A TARGET FOR CANCER THERAPY

Intracellular regulatory pathways have attracted the interest of drug discovery programs. The phosphatidylinositol-3-kinase (PI3K) pathway (Figure 9-4) receives signals from a number of RTKs; these signals activate proliferation and exert prosurvival effects through down-regulation of the intrinsic pathway of apoptosis. Multiple components of this pathway, especially the PI3K alpha (breast, endometrial, lung, and colon cancer) and delta (lymphomas) iso-enzymes, display amplification or mutations in human cancer. The PTEN phosphorylase, a suppressor of the pathway, is frequently inactive or deleted in breast cancer and other tumors. Inhibitors of sequential components of this pathway, including PI3CA inhibitors for metastatic breast cancer and the PI3K delta inhibitor idelalisib, used with rituximab for chronic lymphocytic leukemia,[28] have shown promising activity in early trials.

mTOR occupies a central position in the downstream signaling pathway for PI3K. It forms multiprotein complexes that signal for proliferation, survival, and angiogenesis and modulate intermediary metabolism. mTOR inhibitors specific for the TORC1 complex have been approved for cancer indications, including the immunosuppressive rapalogs, temsirolimus for patients with high-risk renal cell carcinoma, and everolimus for the same disease refractory to immunotherapies. Everolimus is also approved for mantle cell lymphoma and for carcinoid and peripheral neuro-ectodermal tumors, and in combination with examestane for hormone refractory breast cancer. The mTOR inhibitors cause interstitial lung

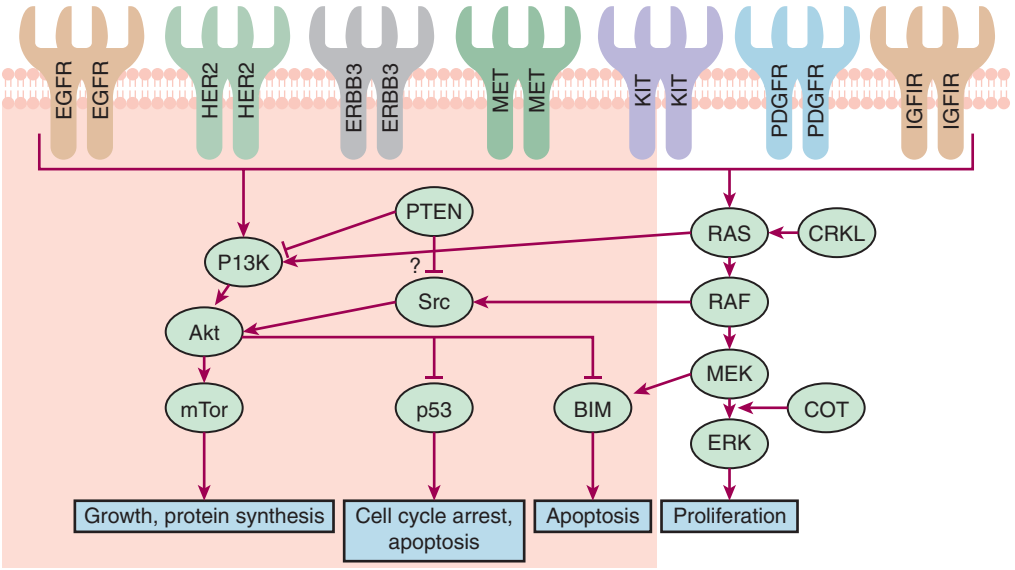


Figure 9-4 Physiology of major transmembrane tyrosine-kinase receptors and associated intracellular pathways. Receptors are displayed as homodimers. Note that most of these receptors may form heterodimers to activate the same intracellular pathways. The blue background highlights intracellular tyrosine kinases of the PI3K/AKT pathway; the yellow background highlights the RAS/RAF/ERK pathway. There is crosstalk between both pathways on multiple levels, three of which are displayed, including RAF-dependent Src-activation, MEK-dependent BIM-activation and RAS-dependent PI3K activation.

disease as a major complications, along with rash, diarrhea, and hepatic toxicity and may augment myelosuppression as a result of chemotherapy.[19] Newer mTOR inhibitors block both TORC-1 and TORC-2 complexes, as well as upstream kinases, and may show improved therapeutic effects.

PROTEASOME INHIBITION

In 2003, the FDA approved bortezomib, a proteasome inhibitor, for patients who have progressed while receiving prior therapy for multiple myeloma. It blocks the chymotryptic activity of proteasome degradation of key proteins and thereby blocks activation of nuclear factor kappa B (NFκB), a potent survival factor for cancer cells. It is currently approved for first-line therapy for multiple myeloma in combination with dexamethasone, and second-line therapy of mantle cell lymphoma. A more potent successor molecular, carfilzomib, also a proteasome inhibitor, has impressive antimyeloma activity and less neurotoxicity in second-line treatment.[19] Their other toxicities are occasional myelosuppression and diarrhea.

ANGIOGENESIS ANTAGONISTS

Although most drug discovery efforts are aimed at pathways and targets that govern the balance of cell proliferation and cell death, other unique aspects of cancer biology have attracted notice, particularly tumor blood vessel formation, Angiogenesis has been traced to tumor cell secretion of factors such as basic fibroblast growth factor, angiopoeitins, and VEGF. Specific inhibitors of this process, such as anti-VEGF antibody (bevacizumab) (see Table 9-1) and anti-VEGFR small molecules (sunitinib, pozatinib, vandetanib, and sorafenib), are now approved for multiple clinical indications.[19]

In 2004, the humanized monoclonal antibody bevacizumab was approved for the first-line treatment of metastatic colorectal cancer in combination with chemotherapy and subsequently with chemotherapy for first-line treatment of ovarian and lung cancer and for recurrent glioblastoma multiforme after first-line therapy. Compared with chemotherapy alone, the addition of bevacizumab to chemotherapy in metastatic colorectal cancer and metastatic cervical cancer improved the response rate, progression-free survival rate, and overall survival rate.[29,30] Bevacizumab also extends progression-free survival as a single agent in renal cell carcinoma, and in combination with temozolamide and radiation therapy in glioblastoma multiforme but does not extend overall survival for those indications. Bevacizumab has multiple effects on tumor perfusion and drug access. A study of neoadjuvant 5-FU combined with radiation therapy for T3 rectal cancer demonstrated a decrease in tumor perfusion, vascular volume, microvascular density, and interstitial fluid pressure after administration of bevacizumab.[31] As a class effect, the antiangiogenic drugs reduce interstitial pressure and improve oxygenation to enhance irradiation effects, and improve delivery of cytotoxic chemotherapy to the tumor, enhancing the radio- and chemosensitizing properties of the drug.

Other antibodies target EGFR by using a decoy receptor. Ziv-afilbercept, a recombinant fusion protein of IgG and VEGFR, binds circulating VEGF. Added to FOLFIRI, it doubled the response rate and marginally improved survival in refractory metastatic colorectal cancer, as compared to FOLFIRI alone.[19]

Small molecules effectively inhibit VEGFR by competing with ATP for the intracellular kinase site on the receptor molecule. Many of these compounds inhibit other tyrosine kinases. Sunitinib inhibits VEGFR2, c-KIT, and platelet-derived growth factor receptor (PDGFR). In a phase III trial comparing sunitinib with interferon-alfa in patients with metastatic renal cell carcinoma, sunitinib produced a superior response rate and progression-free survival. Sunitinib improved time to tumor progression in patients with GIST resistant to imatinib therapy and lengthened survival in patients with unresectable or metastatic peripheral neuro-ectodermal tumors. It received approval for these three indications.[19]

Other antiangiogenic compounds have been approved for clinical use. Sorafenib, an inhibitor of B-RAF kinase and VEGFR2, is approved for unresectable hepatocellular carcinoma on the basis of an improvement in overall survival.

Sorafenib also improved survival rates when compared with placebo in patients with metastatic clear cell carcinoma of the kidney. Axitinib produced longer progression-free survival than did sorafenib as second-line therapy for renal cell carcinomas.[32] Pazopanib has also won approval for renal cancers and soft-tissue sarcomas.[19]

It is notable that the antiangiogenic drugs produce significant toxicity: hypertension with occasional congestive heart failure, proteinuria, rare episodes of intestinal perforation (usually in patients who have undergone abdominal surgery), thrombosis, and hemorrhage, and for the oral compounds, diarrhea, fatigue, and hepatic enzyme elevations.[19]

Thalidomide and its analogs, also called immunomodulatory derivatives (IMIDs) have defied categorization with respect to their mechanism of antitumor activity. Originally used as a sedative and then as a the treatment for erythema nodosum leprosum, thalidomide and its IMID analogs have antiangiogenic and anticytokine properties, suppressing TNFα, IL-12, IL-6, and NFκB. Analogs are known to destabilize cyclin D complexes and promote ubiquitination of key lymphocyte transcription factors, IKZF1 and IKZF.[33] More potent and less sedating derivatives, lenalidomide and pomalidomide, are highly active in multiple myeloma and mantle cell lymphoma, and have notable activity in other lymphomas and chronic leukemias. Lenalidomide produces hematologic remissions in the 5q-forms of myelodysplasia. The side effects of the IMIDS include peripheral neuropathy, diarrhea or constipation, and venous thrombosis, especially when used in combination with dexamethasone, and lenalidamide is associated with an increased rate of second malignancies when used with melphalan.[34]

EPIGENETIC MODIFIERS

Epigenetics is a rapidly expanding area of research that examines the control of gene expression by histones and by DNA methylation. Histones couple with DNA and, in their unmodified state, block access by polymerases and transcription factors. Methylation, acetylation, or phosphorylation of histones decompacts chromatin, increases gene expression, and can induce differentiation and apoptosis in tumor cells. Histone deacetylase inhibitors (HDACs) are a new class of antitumor drugs that can induce cell cycle arrest and differentiation. They may cause hyperacetylation of nonhistone proteins as well, including TP53, HSP90, RAF, AKT, HER2, and BCR-ABL. Through their effects on acetylation of histones and key oncoproteins, they exert valuable antitumor activity in cutaneous and peripheral T-cell lymphomas. They may also serve as radiation sensitizers.[35] Two agents of this class, vorinostat and romidepsin, are FDA approved for patients with cutaneous T-cell lymphoma. Their most common side effects are diarrhea, fatigue, nausea, and loss of appetite, but they may also prolong the cardiac QT interval and may induce arrhythmias. They are currently being studied in combination therapy of multiple solid tumors.

OPTIMIZING CLINICAL CHEMOTHERAPY: PHARMACOKINETICS AND PHARMACODYNAMICS

In the treatment of patients with cancer, the ultimate effectiveness of drug therapy is determined by three factors[1]: the inherent sensitivity of the tumor,[2] the ability to deliver drug to its site of action in therapeutic concentrations, and the limitations of host toxicity.[3] It is generally not possible to measure drug concentrations in tumor, although new imaging techniques such as positron emission tomography (PET) and nuclear magnetic resonance imaging (MRI) do allow noninvasive tracking of the distribution and transformation of some drugs in humans in experimental settings.[36] The pharmacokinetic profile of drug in plasma, or change in drug concentration over time, represents the closest correlate of tumor exposure accessible to measurement in the usual clinical setting. In the initial clinical trials of new agents, pharmacokinetic studies are critical for determining whether cytotoxic drug concentrations are achieved and for adjusting the route and schedule of drug administration to achieve an optimal profile, as suggested by preclinical models. In phase II and III trials, pharmacokinetics can yield important additional information on the effects of age, gender, drug interactions, and organ dysfunction on drug clearance.

Anticancer drugs are cleared from the body by one mechanism or a combination of several mechanisms, including renal excretion, hepatic metabolism, chemical decomposition, or metabolic alteration at extrahepatic sites (Table 9-2). Considerable variation is often found among individuals in their ability to clear anticancer drugs, which may necessitate dose adjustment for patients with renal or hepatic dysfunction. Other factors, such as gender, age, serum albumin concentration, lean body mass, nutritional status, and performance status, can influence the pharmacokinetic behavior and pharmacologic effect of a drug, but their quantitative impact on drug disposition is difficult to predict in individual patients. Inherited defects (polymorphisms) in metabolic capability may lead to severe unexpected toxicity for drugs such as 5-FU (i.e., dihydropyrimidine dehydrogenase deficiency) and 6-mercaptopurine (i.e., thiopurine methyltransferase deficiency).[37] Genetic variation in the UDP*-glucuronosyltransferase 1A1 gene (UGT1A1 gene), which is associated with Gilbert syndrome, results in severe toxicity, primarily neutropenia, in patients receiving irinotecan because of the inability to conjugate and clear the active compound, SN-38.

Virtually all targeted small molecules and natural products (taxanes, vincas, and anthracyclines) are cleared through metabolism by hepatic microsomes and require dose reduction in the presence of abnormal liver function. A smaller number of cytotoxic drugs, including methotrexate, pemetrexed, fludarabine, hydroxyurea, and bleomycin, are excreted unchanged in the urine (see Table 9-2) and depend primarily on renal function. Adjustments in dose are required in the presence of renal dysfunction.

Correlations between pharmacokinetic and pharmacodynamic endpoints, such as organ toxicity and therapeutic outcome, are more difficult to establish, but some important insights into these relationships have been identified for several anticancer drugs (Table 9-3). For antimetabolites, most natural product drugs, and platinum compounds, pharmacodynamic endpoints such as antitumor effects and toxicity to bone marrow and intestinal epithelium correlate best with the area under the plasma concentration time curve (see Figure 9-2). For other drugs, such as paclitaxel, the duration of time above a threshold plasma concentration (0.05 to 0.1 μM) correlates best with toxicity to marrow, and there is preliminary evidence that duration above a threshold level also correlates with antitumor effectiveness (Figure 9-2).[49] For alkylating agents, which display a relatively simple relationship between peak plasma concentration and toxicity, dose rather than drug concentration over time correlates best with toxicity and tumor cell killing.

Pharmacokinetic studies can also reveal the existence of drug interactions, which are common for drugs that depend on hepatic microsomal clearance. Inducers of metabolism such as phenylhydantoin, or inhibitors such as imidazole antifungal drugs, can alter clearance, leading to toxicity or ineffective therapy (Table 9-4).

TABLE 9-2 Clearance Mechanisms of Anticancer Drugs

Primary Clearance Mechanism	Drug	Dose Modification for Organ Dysfunction
Hepatic metabolism	Busulfan, taxanes, vinca alkaloids, irinotecan, anthracyclines, imatinib, nilotinib, bosutinib, dabrafenib, pazopanib, axitinib, lapatinib, vandentanib, bortezomib	Y
	Chlorambucil, cyclophosphamide,* ifosfamide,* thiotepa, ponatinib, dasatinib, erlotinib, gefitinib, crizotinib, sorafenib, sunitinib, ibrutinib, regorafenib, cabozantinib, vismodegib, carfilzomib, romidepsin	N
Reduction	Mitomycin C*	N
Deacetylation	Trametinib	N
Conjugation	Etoposide, vorinostat	N
	6-Mercaptopurine	Y†
Soluble enzymes	Ara-C, gemcitabine, omacetaxine mepesuccinate, cyclophosphamide metabolites, ifosfamide metabolites	N
	5-Fluorouracil	Y‡
Nonenzymatic hydrolysis	Camptothecins, BCNU,* mechlorethamine, melphalan	N
Renal excretion	Bleomycin, carboplatin, deoxycoformycin, etoposide, fludarabine, hydroxyurea, methotrexate, topotecan, imatinib, vandetanib	Y

Y, *Yes;* N, *No.*
**Enzymatic and spontaneous chemical reactions required for drug activation, aldehyde dehydrogenase inactivates.*
†Methyltransferase deficiency.
‡Dihydropyrimidine dehydrogenase deficiency.

TABLE 9-3 Pharmacodynamic Relationships of Chemotherapeutic Agents in Cancer Patients

Drug	Clinical Effect	Pharmacokinetic correlate	References
Busulfan	Hepatotoxicity	AUC	38
Carboplatin	Thrombocytopenia	AUC	39
Cisplatin	Nephrotoxicity	C_{max} (unchanged drug)	40
Doxorubicin	Cardiotoxicity	C_{max}	41
Etoposide phosphate	Myelosuppression	AUC (etoposide)	42
Fludarabine phosphate	Leukopenia	AUC	43
5-Fluorouracil	Risk of toxicity	AUC	44
Methotrexate	Response Bone marrow suppression	$C_{max,}$ C × T: (C over 10 μM)	45,46
Paclitaxel	Neutropenia	Time above threshold C_p	47
Topotecan	Neutropenia	AUC (total drug)	48

AUC, *Area under the plasma concentration–time profile;* C_{max}, *maximum plasma concentration;* C_p, *plasma concentration;* GSH, *glutathione.*

TABLE 9-4 Examples of Pharmacokinetic Drug Interactions Involving Anticancer Agents

Chemotherapeutic Agent	Interacting Drugs	Effect on Anticancer Drug Clearance	Probable Mechanism	References
Cyclophosphamide	Phenobarbital	↑	CYP450 enzyme induction	50,51
Doxorubicin	Cyclosporine	↓	Inhibit P-glycoprotein–mediated biliary excretion	52,53
Methotrexate	Aspirin, penicillins	↓	Inhibit tubular secretion	54-56
	Cephalosporins	↑	Inhibit tubular resorption	
Paclitaxel	Verapamil	↓	Inhibit CYP450 metabolism or biliary excretion, block MDR pump	57
Vinblastine	Erythromycin, imidazole antifungal drugs	↓	Inhibit CYP450 metabolism	58

CLINICAL SCHEDULES OF DRUG ADMINISTRATION

Cancer drugs are given in repetitive cycles of administration interrupted by rest periods that facilitate the recovery of normal tissues, particularly bone marrow and gastrointestinal mucosa. With the availability of myeloid stimulatory factors such as granulocyte colony-stimulating factor, it is possible to shorten the period between cycles from the traditional 4-week interval to 2 weeks. The ability to provide such dose-dense therapy with the aid of growth colony-stimulating factor may result in improved outcome without an increase

in toxicity.[59] The theoretical basis for cyclic chemotherapy was established by animal models, which showed that for any given drug schedule and dose, a specific fraction of tumor cells is killed. Repeated cycles of treatment are therefore required for curative therapy. Whether it is necessary to eliminate the last tumor cell to cure the disease is still in doubt. Molecular markers for residual disease may be present for years after remission induction in children with acute leukemia.

Although peak drug plasma concentration, area under the curve (AUC), and time over a threshold level have been correlated with response and toxicity for selected chemotherapeutic agents, clinical investigators lack pharmacokinetic data and further, lack correlations of pharmacokinetics with pharmacodynamic effects for most combination therapy regimens. A greater effort has been made to develop such correlations for targeted drugs, but the information is usually imprecise. The practical endpoint in combination clinical studies is typically to maximize dose intensity (drug administered per unit time) of the individual agents. For cytotoxic drugs, clinical data support a strong relationship between dose intensity and response in the treatment of lymphomas, breast, ovarian, and colon tumors.[60] Taken to its extreme, high-dose chemotherapy with bone marrow stem cell replacement yields the highest dose intensity and the highest response rates in patients who have otherwise refractory tumors, but at a high dollar cost and at the risk of fatal toxicity. In clinical practice, most oncologists administer full doses of drug, aiming at achieving reversible and readily tolerated toxicity as their clinical endpoint.

In designing a combination chemotherapy regimen, the clinical investigator must choose from a range of options regarding route, schedule, and sequence of drug administration. A number of factors determine the final choice of schedule of administration.

1. *Pharmacokinetics of the individual agents in humans.* Specific factors, such as peak drug concentration and clearance from plasma, desired AUC, and duration of drug concentration above a threshold, are central considerations for individual agents (see Figure 9-2). In general, clinical investigators attempt to reproduce a profile of drug concentration and duration of exposure that mimics optimal conditions in experimental systems.
2. *Cell cycle kinetics and cell cycle phase specificity of the agent.* If the agent in question exerts its effects only against cells in a specific phase of the cell cycle, the schedule must be planned to ensure a maximum duration of drug concentration above the threshold. In practice, the best example is the prolonged infusion of antimetabolites (i.e., 5-FU or cytosine arabinoside given by continuous infusion). Most dividing cells in a tumor that is growing relatively rapidly, such as acute myelocytic leukemia, are exposed to drug during their DNA synthetic phase if the infusion of cytosine arabinoside lasts for 7 days.
3. *Drug interactions.* Interactions that alter pharmacokinetic behavior or enhance or inhibit cytotoxicity (Table 9-4) may dictate the use of a specific schedule or sequence of drug administration.
4. *Drug interactions with irradiation.* The growing interest in combined-modality treatment for solid tumors stems from the expectation that many cancer drugs act as radiosensitizers. In most instances, the maximum radiosensitization is realized if the drug is present during the exposure of tumor to irradiation. Continuous drug infusion or daily administration is favored, although rigorous proof that one schedule is better than another is often lacking.
5. *Toxicity.* Most chemotherapy regimens are developed to allow rapid and complete recovery of normal tissues between cycles of treatment. Ultimately, toxicity limits the dose of drug employed and the duration of administration. If drugs are used in combination with other treatment modalities, further limitations of schedule and dose may be imposed. For example, the radiosensitizing effect of 5-FU or cisplatin-based chemotherapy on mucosa or bone marrow may limit the dose of radiation therapy and chemotherapy if the two modalities are given concurrently and particularly if the field of irradiation includes the marrow, gut, or other epithelial surfaces.
6. *Convenience and cost.* Oral drug administration is the easiest and least expensive way to give drugs. However, because of variable and often limited oral bioavailability of cancer drugs, few cytotoxic drugs are given by this route. Intravenous administration guarantees entry of the full dose into the bloodstream. Intermittent bolus schedules are usually favored, although pharmacokinetic or cell cycle kinetic considerations can pose strong arguments for prolonged infusion schedules. An example of such a cell cycle phase-specific drug is the antimetabolite 5-FU. An improved response rate and less toxicity result when 5-FU is administered as a continuous infusion for 48 hours, rather than the daily times five schedule.[61] Oral therapies represent a further advance in terms of convenience and cost, provided they are equally effective. Capecitabine, an oral prodrug of 5-FU, appears to be equally active as infusional 5-FU when combined with oxaliplatin in patients with metastatic colorectal cancer.[62]
7. *Targeted drugs* are usually administered on a schedule that ensures long-term, continuous exposure to the agent. Monoclonal antibodies administered every 3 to 4 weeks provide prolonged exposure because of their very long plasma half-life (2 to 3 weeks), whereas the daily oral dosing of targeted drugs is required to produce trough levels of drug in plasma above the minimum inhibitory concentration.

The Special Problem of Drug Sanctuaries

Although drug distribution from the bloodstream into most tissue compartments occurs with relative ease, certain "sanctuary" sites present special barriers to penetration. In general, tumor blood vessels proliferate in response to the needs imposed by rapid tissue proliferation, but the resulting vessels tend to be unusually permeable, allowing permeation of protein into the extracellular space and resulting in an increased interstitial pressure, sluggish flow, and poor drug penetration. These changes are corrected by antiangiogenic therapies such as bevacizumab and small-molecule VEGFR inhibitors (see later discussion) and may allow better penetration of oxygen and small molecules.

Other tissue sites tend to exclude many drugs. Most chemotherapy agents achieve low concentrations in the testes, vitreous humor of the eye, and the brain when administered systemically. The brain is protected by blood-brain barrier, a compact alignment of microvascular endothelium, which limits the passage of large molecules and hydrophilic drugs. In addition, there are active MDR export pumps in the vascular endothelium of the brain.[63] Disruption in the barrier by tumor vessels associated with metastases or primary brain tumors allows for greater penetration of drugs into the CNS, but the barrier seems to be maintained at the infiltrating edge of tumor. Although radiation therapy is the most effective means of treating many types of brain metastases, systemic chemotherapy or immunotherapy may benefit patients with CNS tumors. Temozolomide, a highly lipophilic alkylating agent, extends the survival time of patients with malignant glioma when it is used in conjunction with irradiation. Other drugs clearly reach intracranial tumors.[64]

EGFR inhibitors and second-generation ALK inhibitors have demonstrated activity against brain metastases resulting from NSCLC.[19] High-dose methotrexate is the preferred mode of treatment for primary CNS lymphomas, although the ratio of cerebrospinal fluid (CSF) to blood concentration of drug is less than 0.1.[65,66] Even antibodies, such as ipilimumab may be effective against intracranial tumors. The VEGF inhibitor bevacizumab depletes a systemically circulating growth factor. It is approved for second-line treatment for patients with glioblastoma multiforme with tumor progression after temozolomide and radiotherapy.[67]

PREDICTING TUMOR RESPONSE

Pharmacogenomics may enable the clinician to determine which drugs will be poorly tolerated by an individual and may allow more rational selection of a particular antineoplastic agent in the individual patient. For example, a high level of thymidylate synthase, in some tumors, the result of a polymorphism in tandem repeats in the gene's promoter region, predicts for resistance to 5-FU in patients with colorectal cancer.[68] Genomic polymorphisms in the DNA repair genes *ERCC1* (formerly designated *XPD*) and *XRCC1* may be prognostic factors in patients with NSCLC treated with cisplatin.

Transcriptional silencing may alter gene expression and affect the outcome of chemotherapy. O-6-methylguanine-DNA methyltransferase (MGMT) is an enzyme responsible for DNA repair following alkylating agent chemotherapy. The gene may be silenced in tumors by methylation of its promoter, creating vulnerability of the tumor cell to DNA damage. MGMT gene silencing occurs in about 20% of glioblastomas and is associated with a high response rate to the alkylating agent temozolomide in patients with glioblastoma and neuroendocrine tumors.[69]

As discussed previously, it is now possible to predict which patients with NSCLC, melanoma, or colorectal cancer are more likely to benefit from a particular targeted therapy. In the case of NSCLC, those patients whose tumors possess an activating mutation in the ATP-binding pocket of the RTK are more likely to benefit from the use of an EGFR-TKI or an inhibitor of the ALK receptor tyrosine kinase. Similarly, patients with colorectal cancer whose tumors possess a mutation in either KRAS or BRAF, resulting in their constitutive activation, are unlikely to benefit from a monoclonal antibody inhibiting EGFR.

DRUG RESISTANCE

The fractional cell kill hypothesis must be modified to incorporate concepts of drug resistance. In practice, the response to an initial cycle of chemotherapy may be much greater than to subsequent doses, and in many solid tumors, such as breast cancer and lung cancer, tumors may again grow rapidly after an initial response. The explanation for this finding lies in the problem of drug resistance and the ability of cancer drugs to select for drug-resistant cells. Mechanisms of resistance may affect any of the steps necessary for drug action: entry of drug into tumor cells by means of active transporters, enzymatic drug activation, action at a molecular target within cells, and even the processes in the cell that trigger cell death. Although many examples of drug resistance have been characterized in model systems, the understanding of resistance in clinical practice remains incomplete. Some of the more common mechanisms in model systems, and evidence for the same mechanism in the treatment of human tumors are given in Table 9-5.

TABLE 9-5 Drug Resistance Mechanisms in Cancer Therapies

Molecular Mechanism	Example	Drugs Affected
Decrease in cellular uptake	Deletion of folate transporter Deletion of nucleoside transporter	Methotrexate, pemetrexed Ara-C, gemcitabine
Increase in cellular efflux	Increased multidrug resistance (CD116) expression or gene amplification	Vinca alkaloids, anthracyclines, taxanesl, etoposide, topotecan
Alteration of target protein	Mutant dihydrofolate reductase Mutant topoisomerase I Mutant tubulin BCR-ABL mutations or amplification EGFR mutations EML4-ALK mutations BRAF splicing variants or BRAF amplification KIT mutation, KIT amplification, or PDGFR alterations	Methotrexate Topotecan Vinca alkaloids, paclitaxel Imatinib, dasatinib, nilotinib Gefitinb, erlotinib, cetuximab, crizotinib Crizotinib Vemurafenib Imatinib
Alternative pathway activation	Multiple mechanisms (see reference 19 for details)	Gefitinib, erlotinib, crizotinib, cetuximab, panitumumab, imatinib (GIST), vemurafenib, bevacizumab, trastuzumab
Deletion of target protein	Deletion of topoisomerase I Deletion of topoisomerase II	Topotecan Anthracyclines, etoposide
Decreased activation of drug in tumor	Deoxycytidine kinase deficiency Decreased folylpolyglutamate synthetase	Ara-C, fludarabine, cladribine Methotrexate, pemetrexed
Increased detoxification	Increased glutathione or glutathione transferase	Alkylating agents, platinum analogs
Enhanced DNA repair	Increased nucleotide excision repair Increased O-6-alkyl-guanine alkyl transferase	Platinum compounds, alkylating agents, Procarbazine, temozolamide Nitrosoureas
Defective recognition of DNA adducts	Mismatch repair defect	Platinum compounds, 6-mercaptopurine, nitrosoureas
Alteration of apoptosis pathways	Mutant TP53, BCL2	Alkylating agents, antimetabolites, EGFR inhibitors, RAS/RAF/MEK pathway inhibitors

With few exceptions, single-agent treatment of cancer produces only temporary responses. This rule applies as well to the targeted agents, where resistance to agents such as EGFR inhibitors develops through mutation in the binding site of the target protein or through activation of an alternative proliferation pathway (C-MET). Resistance likely antedates exposure to the drugs but is a spontaneous product of the underlying genetic instability of tumors. Although the sensitive fraction of cells is killed with each dose of drug, drug-resistant mutants proliferate and replace their sensitive counterparts under the selective pressure of drug. Based on the experience of antibiotic treatment of bacterial infections, it was logical to combine drugs that had different mechanisms of resistance. If the frequency of mutation to resistance is 1 cell of 10^{-6}, the chances of any cell being simultaneously resistant to two drugs would be the product of these probabilities, or 10^{-12}. Goldie et al have argued convincingly that the best strategy for chemotherapy is to employ as many non–cross-resistant agents as early as possible in the treatment regimen.[70] In addition, the use of radiosensitizing chemotherapy with irradiation can have major benefits for local control of tumor and for reducing local morbidity related to surgery in tumors of the aerodigestive tract and have the added potential of synergistic DNA damage.[71-74]

In using drug combinations, certain qualifying considerations have to be kept in mind:

1. Drug resistance mechanisms, such as the overexpression of the MDR transport system, may confer resistance to multiple drugs, as may defects in cell death pathways, enhanced DNA repair, poor tumor blood flow, or other biological factors[2]
2. Simultaneous administration of multiple agents may lead to overwhelming toxicity, unfavorable drug interactions at the molecular target, or unwanted pharmacokinetic interactions. Because of negative interactions, multiple agents may be given sequentially rather than simultaneously, with more favorable outcome.

COMBINATION CHEMOTHERAPY

With few exceptions, multiagent therapy has proved more effective than single-agent therapy in the treatment of most human cancers. In general, the choice of drugs for combination therapy is made on the basis of mechanistic, pharmacokinetic, and toxicologic considerations. The most important criteria for including drugs in a combination regimen are the following:

1. single-agent activity in the disease to be treated;
2. nonoverlapping toxicities, allowing full doses of each drug to be used;
3. different mechanisms of action; and
4. nonoverlapping mechanisms of resistance.

These are not hard and fast rules. There are numerous examples of clinical regimens that use multiple alkylating agents, multiple natural products (despite their shared cross-resistance), or combinations of myelotoxic drugs. Often, these choices are dictated by impressive single-agent activity in the tumor being treated and the uncertainty of knowledge about clinical mechanisms of drug resistance. For example, individual alkylating agents differ in their mechanisms of activation, transport, and adduct formation. Their DNA adducts are repaired by a variety of enzymatic pathways. It is not clear which of these factors contributes to clinical drug resistance (see Table 9-5). Although these agents belong to the same class of drugs, in the absence of more complete knowledge about resistance, it is possible to construct a reasonable argument for combining different alkylating agents.

The development of targeted drugs has greatly expanded opportunities to address resistance to cytotoxic chemotherapy. Drugs such as bevacizumab and trastuzumab have significantly enhanced the effectiveness of cytotoxic chemotherapy of colon and breast cancers, respectively. Trastuzumab blocks signaling through the MEK-ERK and PI3K pathways that promotes proliferation and survival, whereas bevacizumab normalizes tumor vasculature and improves access of cytotoxics to poorly perfused tumor cells. The further development of targeted drugs in combination may allow successful inactivation of multiple resistance pathways, as exemplified by the joint use of B-RAF and MEK inhibitors in melanoma, a strategy that holds great promise for cancer treatment. To implement this strategy, it is necessary to perform molecular profiling of tumor samples on a routine basis, a capability not widely available in current practice, and one must be able to combine drugs without experiencing excessive toxicity.

SUMMARY

The potential for cancer chemotherapy to cure of metastatic cancer has been fully realized in only a few clinical circumstances, primarily those in which a rapidly proliferating hematologic cancer has been treated aggressively with a multiagent regimen. Solid tumor chemotherapy with curative intent must be given early in the course of disease, carefully interdigitated with irradiation and surgery, and administered in maximal doses. Because of the likelihood of serious side effects and drug–irradiation interactions, such regimens are difficult to design and implement. Thus far, the key drugs for such combinations have been 5-FU and cisplatin or carboplatin. The development of optimal chemoradiotherapy regimens must take into account the advantages and disadvantages of sequential versus concurrent combined-modality approaches. To maximize therapeutic synergy and minimize toxicity, protocol planning must pay careful attention to potential drug–irradiation interactions, drug pharmacokinetics, and drug–drug interactions. The patient population being treated (often, elderly patients with associated conditions such as heart disease or hypertension and other evidence of organ dysfunction) may have limited ability to tolerate the rigors of combined-modality treatment. Advances in cancer biology, particularly in the areas of angiogenesis and metastasis, will lead to newer, more tumor-specific, less-toxic approaches to cancer control. Recent strides in molecular profiling of an individual's tumor may lead to a more rational, tailored design of a patient's treatment, and ultimately beneficial therapy, while sparing the unnecessary toxicity and expense of treating with an ineffective drug.

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