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# Encyclopedia of Cancer

Chapter · January 2011

DOI: 10.1007/978-3-642-16483-5\_8007

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# B

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## B Cell

### Definition

A B cell, or B lymphocyte, is one of the two major types of lymphocytes. The antigen receptor on B lymphocytes, usually called the B-cell receptor, is a cell-surface immunoglobulin. On activation by antigen, B cells differentiate into cells producing antibody molecules of the same antigen specificity as this receptor. B Cells play a major role in the body's response to foreign materials (viruses, bacteria, parasites, etc.) by generating antibodies to fight these intruders.

► [Fluoxetine](#)

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## B Symptoms

### Definition

Constitutional symptoms consisting of fever >38°C, night sweats, and/or unintentional weight loss of >10% the body weight over a period of up to 6 months. B symptoms are relevant in non-Hodgkin lymphoma and Hodgkin lymphoma staging and are related to tumor burden and prognosis.

► [Diffuse Large B-Cell Lymphoma](#)  
► [Malignant Lymphoma, Hallmarks and Concepts](#)

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## B-raf-1

► [B-Raf Signaling](#)

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## B-1 Cells

### Definition

B-1 cells, also known as CD5 B cells, are a class of atypical, self-renewing B cells found mainly in the peritoneal and pleural cavities in adults. They have a far less diverse repertoire of receptors than do B-2 cells (also known as conventional B cells), which are generated in the bone marrow throughout life, emerging to populate the blood and lymphoid tissues.

► [Omental Immune Aggregates](#)  
► [Sjögren Syndrome](#)

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## B7 Molecules

### Definition

The major T-cell costimulatory molecules are the B7 molecules, B7.1 (CD80) and B7.2 (CD86). They are closely related members of the immunoglobulin gene superfamily and both bind to the CD28 molecule on

T cells. They are expressed differentially on various antigen-presenting cell types.

► [Sjögren Syndrome](#)

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## BAC

### Definition

Acronym of bacterial artificial chromosomes. A vector system used to clone large DNA fragments.

► [Array CGH](#)

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## BACH1 Helicase

Sharon Cantor and Jenny Xie  
Cancer Biology, UMASS Medical School, Worcester,  
MA, USA

### Synonyms

[BRIP1](#); [FANCJ](#)

### Definition

The ► [BRCA1](#) associated carboxy (C)-terminal helicase (BACH1) ([Fig. 1](#)) gene encodes a 1,249 amino acid nuclear protein that is characteristic of the DEAH family of DNA helicases. BACH1 is an ATP-dependent DNA helicase that catalyzes the destabilization of hydrogen bonds between complementary nucleic acids. The C-terminal region of BACH1, including a phosphorylated serine 990 residue, interacts directly with the C-terminal BRCT repeats of BRCA1. In addition, BACH1 also participates in the ► [Fanconi anemia](#) (FA) ► [DNA Damage Response](#) pathway as it was identified as the FA gene product, FANCJ.

### Characteristics

#### BACH1 and Hereditary Breast Cancer

Breast cancer afflicts over 200,000 individuals each year, and is the leading cause of cancer death among US women with cumulative lifetime risk of one in nine.

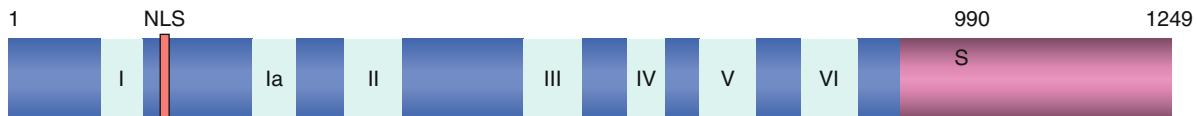
Hereditary breast cancer accounts for approximately 10% of all breast cancers, making it the most commonly inherited cancer in the USA. Germline mutations in the Breast Cancer Associated genes BRCA1 and BRCA2 account for ~30–40% of these cases. Thus, research has focused on understanding how the BRCA gene products function to support normal cell growth and ultimately suppress tumor formation.

#### BACH1 Was Identified by Its Direct Interaction with BRCA1

BRCA1 is a nuclear phosphoprotein with an amino (N)-terminal zinc finger domain and C-terminal BRCT (BR CA1 C-Terminal) repeats. The integrity of the BRCT repeats is critical for BRCA1-mediated DNA damage response. BRCT region missense and deletion mutations disrupt the BRCA1 DNA damage repair function and are also associated with cancer predisposition. Thus, the integrity of the BRCT region is linked to BRCA1 tumor suppression. Consistent with this role, the BRCA1-BRCT repeats are highly conserved and are also present in other proteins involved in the DNA damage response. The BRCT repeats in BRCA1 and other proteins have been shown to bind phosphopeptides. In fact, the BRCA1-BRCT repeats bind directly to BACH1 when a phosphoserine residue (pSer990) at the C-terminal of BACH1 is phosphorylated.

#### BACH1 Is Mutated in Hereditary Breast Cancer Patients

Due to the direct interaction between BRCA1 and BACH1, it was hypothesized that, like BRCA1, BACH1 may also function to suppress breast cancer development. Consistent with this hypothesis, early genetic screens identified two early-onset breast cancer patients with BACH1 germline mutations. Further analysis of these mutations *in vitro* revealed that these sequence changes resulted in defective BACH1 protein disrupting the helicase activity. Recently BACH1 truncating mutations were identified in 9 out of 1,212 breast cancer patients, whereas, similar mutations were only presented in 2 out of 2,018 healthy individuals. Overall, these data suggested that BACH1 mutations conferred a twofold risk in the development of breast cancer.



**BACH1 Helicase.** Fig. 1 Schematic representation of BACH1. BACH1 is composed of 7 helicase domains (*light blue*) which is characteristic of the DEAH helicase, and its interaction with

BRCA1 depends on the ► *phosphorylation* status of a serine residue (S) situated at the carboxy-terminal 990 position

B

### BACH1 Is Important for Mediating the DNA Damage Response

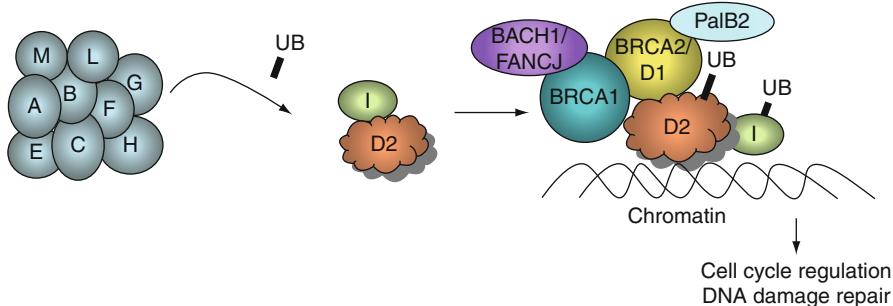
The BRCA1/BACH1 interaction suggests that BACH1 likely contributes to BRCA1 DNA repair functions. BACH1 similar to BRCA1, following DNA damage is modified by phosphorylation, displays a BRCA1-like nuclear foci pattern and colocalizes with ► *gamma* ( $\gamma$ )-H2AX. The BACH1/BRCA1 complex is unaltered by DNA damage, but both proteins contribute to the localization of each other to DNA damage foci. In the absence of BRCA1, BACH1 fails to localize to site of DNA damage. While BACH1 is not required for BRCA1 localization to sites of DNA damage, the intensity of BRCA1 foci is diminished in BACH1-deficient cells. Furthermore, this interaction has been shown to be required for activation of the ionizing radiation (IR)-induced ► *G2/M checkpoint*. Cell cycle analysis revealed a G2/M checkpoint defect in BACH1 siRNA Expressing HeLa cells that was corrected by expression of wild-type BACH1, but not with the S990A version of BACH1 disrupted for BRCA1 binding. BACH1-depleted cells also exhibit an elevated S-phase accumulation compared to control cells after treatment with low dose of aphidicolin, which is known to activate the intra ► *S-phase checkpoint*. BACH1's role in intra S-phase checkpoint was further validated when BACH1-deficient cells showed ► *radioresistant DNA synthesis (RDS)*.

### BACH1 Is Required for DSBR

As part of the DNA damage response, BACH1 is required for the repair of ► *DNA double strand breaks* (DSBs). The contribution of BACH1 to double stranded break repair (DSBR) was first apparent through the observation that BRCA1 clinical mutations that disrupted its interaction with BACH1 also resulted in defective DNA damage repair. In addition, overexpression of a helicase inactive version of BACH1 (K52R) displayed a marked delay in repair, as shown by an increase in the level of unrepaired

breaks detectable after IR. This repair delay was dependent on BACH1 binding to BRCA1 suggesting that the K52R BACH1 functions as a dominant negative to perturb DSBR in a BRCA1-dependent manner.

Moreover, similar to BRCA1, BACH1 promotes the repair of DSBs by promoting ► *Rad51*-dependent ► *homologous recombination* (HR). Specifically, BACH1's role in HR was examined using mammalian cell-based homology directed repair assay, where it was found that suppression of either BRCA1 or BACH1 disrupted HR to a similar degree. In support of a role for BACH1 in HR, BACH1 is able to unwind D-loop recombination intermediates *in vitro*. Since BACH1 does not unwind holiday junction intermediates similar to other DNA repair helicases, such as Bloom syndrome (BLM) and Werner syndrome (WRN), BACH1 most likely has a distinct HR function. The D-loop is the initial structure formed in recombination when the Rad51 Nucleoprotein filament invades the duplex DNA in search of homologous repair template. Thus, Rad51 focal accumulation has been speculated to represent the development of these HR intermediates. Although suppression of either BRCA1 or BACH1 protein results in defective HR, one difference observed between BRCA1 and BACH1 deficient cells was the visualization of Rad51 foci following DNA damage. Rad51 foci formation in BRCA1-suppressed cells is dramatically reduced compared to control cells. However, BACH1-suppressed cells retain robust Rad51 foci. The unaltered presence of Rad51 foci, but reduced HR in BACH1-deficient cells suggests that some form of Rad51-based recombination is active. One possibility is that BRCA1 functions upstream of Rad51 and BACH1 functions downstream of BRCA1. Alternatively, it was proposed that BRCA1 regulates BACH1 helicase activity in a manner that regulates Rad51 foci formation. In this model, it was proposed that in the absence of BRCA1, BACH1 helicase activity would be unregulated and disrupting of Rad51 foci due to perpetual unwinding of recombination intermediates.



**BACH1 Helicase.** Fig. 2 The FA pathway is composed of 12 FA complementation groups, with the central feature being the monoubiquitination of FANCD2 in response to DNA damage. Activated FANCD2 translocates to chromatin-associated foci

where it localizes with BACH1/FANCJ, BRCA1, and BRCA2/FANCD1 to promote cell cycle regulation and DNA damage repair

### BACH1 and Fanconi Anemia

FA is a recessive disorder in which FA patients are characterized by congenital abnormalities, bone marrow failure, and predisposition to leukemia and solid tumors. FA is a multigenic disorder and to date, there are 12 FA complementation groups FA-A, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M (Fig. 2). Germline mutations in the BACH1 gene were identified in patients from the FA-J complementation group. FA-J cells, similar to other FA cells, exhibit chromosome abnormality and hypersensitivity to DNA interstrand cross-linking agents such as mitomycin C and cisplatin. Most likely FA proteins are critical for processing cross-linked DNA. In the absence of processing, ► **interstrand cross links** (ICLs) are extremely deleterious because DNA strands are covalently tethered, which can block DNA replication and compromise cell viability. Consistent with a role for the FA proteins in this process, cellular exposure to ICLs activates the FA core complex (FANCA, -B, -C, -E, -F, -G, -L and -M) and promotes the ► **monoubiquitination** of the effector protein, FANCD2. Activated FANCD2 translocates into chromatin and forms nuclear foci that colocalizes with BRCA1, BRCA2/FANCD1, and BACH1/FANCJ. Activation of the FA pathway through monoubiquitination of FANCD2 and FA protein relocalization are essential for coordinating the ICL repair response. BACH1/FANCJ is considered downstream of the FANCD2, since it is not required for FANCD2 monoubiquitination. The FA proteins, BRCA2/FANCD1, and the newly identified PALB2 proteins are also downstream of FANCD2. While the role of

BACH1/FANCJ in the FA pathway is not yet clear, its helicase activity, but not BRCA1 binding activity appears to be essential to correct the ICL-response in chicken and patient cells.

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### Bacillus Calmette-Guérin

Sven Brandau

Department of Otorhinolaryngology, University  
Duisburg-Essen, Essen, Germany

### Synonyms

BCG; *Mycobacterium bovis* BCG

## Definition

Bacillus Calmette-Guérin (► **BCG**) is a ► **mycobacterium** belonging to the *Mycobacterium tuberculosis* complex. It is a live attenuated organism and generally nonpathogenic for healthy human subjects. Originally developed as a vaccine against tuberculosis, since the mid-1970s it is increasingly used as a successful active immunotherapeutic agent in the treatment of nonmuscle invasive ► **bladder cancer**.

## Characteristics

### Microbiological Background on BCG and Tuberculosis Vaccination

BCG is an attenuated derivative of virulent *Mycobacterium bovis* (*M. bovis*), the causative pathogen of bovine tuberculosis. The name of BCG acknowledges the contributions of two French researchers Albert Calmette, a microbiologist, and Jean-Marie Camille Guérin, a veterinarian. Those two, between 1908 and 1921, passaged a virulent strain of *M. bovis* in an attempt to generate a vaccine against tuberculosis in humans. By using a continuous in vitro passage and a special bile-glycero-potato culture medium, they succeeded in generating a nonvirulent mycobacterial strain that did no longer cause disease in experimental rodent animals. Shortly after these observations the vaccine was first applied to human subjects as a vaccine against tuberculosis. Although controversial since its first use, the vaccine has now been administered to over three billion people worldwide with an excellent safety profile. In spite of BCG's efficacy as a preventive vaccine in general, its highly variable efficacy in preventing pulmonary tuberculosis in adults (with some studies showing no protection at all) remains a major challenge.

The original live BCG vaccine required continuous culture and was spread over various continents for clinical application. By the time lyophilization was available as a method to preserve viable mycobacteria, several substrains of the original strain were created which are collectively known as BCG. Characteristic for BCG is its relatively slow growth in in vitro culture with doubling times between 8 and 36 h depending on the strain and the respective culture conditions. Modern molecular biological analyses are now used to follow the "in vitro evolution" of BCG vaccines.

Although the genetic basis for BCGs attenuation has not yet been fully elucidated, several regions of genetic difference between virulent *M. tuberculosis* and BCG have been identified and characterized.

Today, for use in cancer ► **immunotherapy**, several substrains of BCG mycobacteria are available as freeze-dried lyophilisates with a guaranteed amount of viable bacilli at the end of the shelf life, which usually exceeds 1 year. Easy clinical use is facilitated by the availability of aliquots pretitrated for one single instillation and delivered together with the respective dilution buffer.

### BCG in Cancer Immunotherapy

At the end of the nineteenth century, the American Surgeon W. E. Coley observed occasional remissions of lymphosarcomas following local and/or systemic bacterial infections. Based on these observations, he used bacterial extracts for the adjuvant treatment of cancers of the head and neck which have become known as Coley toxin. Furthermore, it was noted that patients with tuberculosis rarely developed malignant neoplasms. These observations stimulated investigators to further explore the potential use of BCG for various anticancer applications.

In 1976, Morales et al. combined this experience and investigated a new form of application for BCG. They developed a schedule for the effective adjuvant intravesical treatment of nonmuscle invasive bladder tumors following transurethral resection (TUR) with BCG. Since that time, adjuvant BCG immunotherapy for bladder cancer has remained largely unmodified. Additionally, a large number of prospective controlled phase III trials were published confirming the efficacy of BCG and strengthening its role in uro-oncology. Today, an estimated number of one million annual BCG treatments are given to cancer patients.

According to recent metaanalyses of published randomized clinical trials, the following conclusions can be drawn from these trials: (1) patients after TUR in combination with adjuvant BCG will suffer from less recurrences as compared to TUR alone, (2) BCG immunotherapy is superior to intravesical chemotherapy for the reduction of bladder cancer recurrences, and (3) BCG is the only treatment option for preventing and/or delaying bladder cancer progression to muscle invasive disease.

BCG immunotherapy is first applied at 1–3 weeks following complete TUR of the primary tumors.

It consists of a 6-week induction cycle including weekly intravesical instillations of about 81 mg BCG reconstituted in 50 mL saline corresponding to  $1-5 \times 10^8$  ► colony-forming unit (► CFU) viable mycobacteria. Retention of the BCG mycobacteria in the urinary bladder for not less than 2 h is recommended to ensure sufficient immunostimulation. While the efficacy of various BCG strains has never been compared in prospective trials, data from a recent meta-analysis suggest that at least the five most commonly used strains Tice, Pasteur, Connaught, RIVM, and A. Frappier do not differ in terms of preventing tumor progression. Contraindications against the intravesical use of BCG are given in patients with a compromised immune status, active tuberculosis, acute urinary tract infections, lesions in the lower urinary tract, fever of unknown origin, a history of radiotherapy to the bladder and/or pelvis, and during pregnancy or active nursing.

Recent clinical approaches aimed at improving BCG efficacy by addition of a so-called maintenance therapy schedule. BCG maintenance consists of three intravesical instillations given in weekly intervals at 3, 6, 12, 18, 24, 30, and 36 months after initiation of the induction cycle. Initial data suggest that this modified treatment protocol provides additional benefit for preventing tumor recurrence and progression.

In addition to the current success in bladder cancer, historically, BCG has been used as an anticancer agent in a variety of other cancer types including ► melanoma and lung cancer among many others. However, in most cases, these treatment regimens have not become standard clinical practice. With the identification of ► toll-like receptor agonist structures present on the BCG surface, an immunological rationale for the well-known ► adjuvant effect of BCG has now emerged. Currently, a variety of approaches are trying to utilize the immunostimulatory effect of BCG and its subcomponents as additives in autologous tumor vaccines.

### Mode of Action in Antitumor Activity

When BCG was first used in clinical practice more than 30 years ago, its mode of action has been far from understood. Progress in the fields of infection biology, ► innate immunity, and tumor immunology has set the stage for a better understanding of the mechanism of BCG's antitumor activity. Mechanistic and descriptive studies in vitro, in rodent bladder

cancer models, and with patient material have provided a clear picture of the processes leading from instillation of mycobacteria into the bladder lumen to antitumor effects.

Based on in vitro studies initial data have suggested a direct antiproliferative, cytotoxic, or proapoptotic effect of BCG on tumor cells. While this effect can certainly be demonstrated in vitro, mechanistic studies in rodent models now provide convincing evidence that a functional immune system of the tumor-bearing host is absolutely essential for most of BCGs antitumor activity in vivo.

BCG mycobacteria have a characteristic outer cell wall consisting of various complex structural biomolecules. Microbiologists, biochemists, and immunologists have identified and characterized the interaction of a variety of those biomolecules with their respective receptors on target cells of the host. Collectively, immunologically active parts of those molecules are today referred to as ► pathogen-associated molecular patterns (PAMP). Those PAMPs on the mycobacterial surface are biochemically quite diverse and, for example, include peptidoglycan, mycolic acids, lipomannan, and lipoarabinomannan. Although the role of the mycobacterial surface molecules is better defined in infection biology, it can be assumed that initial contact to primary host target cells in physiologic mycobacterial infection and in instillation immunotherapy is mediated by similar mechanisms.

In BCG immunotherapy of bladder cancer the vast majority of the original instillation dose of several hundred millions of mycobacteria will be washed out with the first postoperative micturition. The remaining mycobacteria will adhere to the bladder wall. This contact will lead to an activation of epithelial cells in the bladder, which can respond with the release of immunologically active mediators such as ► Cytokines-like interleukin-(IL)-8, IL-6, and IL-1. Within days after instillation, BCG initiates a complex inflammatory cascade in the bladder wall resulting in the enhanced production of a vast array of cytokines and ► chemokines. Most of these cytokines and chemokines are proinflammatory or promoting a so-called ► T-helper 1 (Th1) response associated with the induction of cell-mediated immunity. Animal experiments have shown that this ► Th1 response is essential for induction of protective antitumor immunity. The local inflammatory response (► inflammation) in the

bladder tissue consists of different phases and changes in the cellular composition of the bladder wall become apparent already a few hours after instillation and can last for up to several months in human patients. A large number of studies have analyzed the cellular immune response (Th1 response) induced by BCG in immunotherapy of bladder cancer. Collectively, these studies suggest the following scenario: In an immediate and early inflammatory phase, the cellular infiltration of the bladder wall is dominated by ► **neutrophil granulocytes** (neutrophils) known to the immunologist as prototypic inflammatory cells. This neutrophilic infiltration is characteristic for the early postinstillation urine and the cellular composition of the urine early after instillation is dominated by more than 90% granulocytes. Polymorphonuclear neutrophil granulocytes (PNG; PMN) then direct in a second phase the subsequent influx of mononuclear cells including macrophages, T cells, and NK cells by mechanisms which are only beginning to emerge. The complex interplay of these immunocompetent cells mediates potent antitumor effects, and mechanistic studies have shown that concurrent activation of different effector cell populations is required for full therapeutic efficacy. In a third chronic inflammatory phase, granuloma-like structures of cellular infiltration can persist for up to several months possibly representing a long-lasting immunostimulatory event.

Inspired by the plethora of immune mediators induced during BCG immunotherapy, researchers have tried to identify a prognostic immunologic marker which could be used to predict a clinical response to this type of immunotherapy. However, until now no definite prognostic immunologic marker has been identified, although certain good candidates (e.g., IL-2) do exist and will be further explored in the future.

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## Bacteriophage

### Definition

A bacteriophage is a virus that infects bacteria.

- **Phage Display**

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## Bacteriophage Display

- **Phage Display**

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## Baculoviral IAP-repeat Containing Protein 5

- **Survivin**

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## Baculovirus

### Definition

Baculovirus is a pathogen that attacks insects and other arthropods. Like some human viruses, they are usually extremely small (less than a thousandth of a millimeter across), and are composed primarily of double-stranded DNA that codes for genes needed for virus establishment and reproduction.

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## BAD

### Definition

BAD is a Bcl-2 antagonist of ► **apoptosis/death**, a pro-apoptotic member of the Bcl-2 family of proteins.

It is a 18 kD protein that binds to Bcl-xL and Bcl-2. It competes out the binding of Bcl-xL and Bcl-2 to Bax, thus promoting apoptosis.

## BAF47

- [hSNF5/INI1/SMARCB1 Tumor Suppressor Gene](#)

## BAK

### Definition

Bcl2-antagonist/killer 1, a member of Bcl-2 protein family. Bak functions in a similar manner as Bax to induce ► [apoptosis](#).

- [PUMA \(p53 Upregulated Modulator of Apoptosis\)](#)

## BAK1

### Synonyms

[BAK-1](#)

### Definition

BAK1 is a Bcl-2 antagonist/killer-1, a pro-apoptotic member of the Bcl-2 family of proteins. It is a 23 kD protein that binds to and antagonizes Bcl-2. It also forms heterodimers with anti-apoptotic Bcl-xL and binds to adenovirus E1b19k.

- [Apoptosis](#)

## BALT

### Definition

The lymphoid cells and organized lymphoid tissue in the respiratory tract are termed the bronchial-associated lymphoid tissues (BALT). These tissues are very

important in the induction of immune responses to inhaled antigens and to respiratory infection.

- [Sjögren Syndrome](#)

## Bannayan-Riley-Ruvalcaba Syndrome

### Definition

Bannayan–Riley–Ruvalcaba syndrome is a rare inherited disorder characterized by excessive growth before and after birth, an abnormally large head (macrocephaly) that is often long and narrow (scaphocephaly), normal intelligence or mild mental retardation, and/or benign tumor-like growths (hamartomas) that, in most cases, occur below the surface of the skin (subcutaneously). The symptoms of this disorder vary greatly from case to case. Additional abnormalities associated with this disorder may include abnormal skin coloration (pigmentation) such as areas of skin that may appear “marbled” (cutis marmorata) and/or the development of freckle-like spots (pigmented macules) on the penis in males or the vulva in females. In some cases, affected individuals may also have skeletal abnormalities and/or abnormalities affecting the muscles (myopathy). Bannayan–Riley–Ruvalcaba syndrome is inherited as an autosomal dominant genetic trait. Bannayan–Riley–Ruvalcaba is the name used to denote the combination of three conditions formerly recognized as separate disorders. These disorders are Bannayan–Zonana syndrome, Riley–Smith syndrome, and Ruvalcaba–Myhre–Smith syndrome.

## BAR Domain

### Definition

Banana-shaped domain seen in adapter proteins that bind and tubulate cellular membranes during the dynamic processes of endocytosis, vesicle trafficking, organelle fission, and specialized membrane formation events (e.g., muscle T tubules). BAR domains also bind small GTPases and their regulators as well as phosphatidylinositol lipid–binding proteins.

- [Bin1](#)

## BARD1

Irmgard Irminger-Finger

Molecular Gynecology and Obstetrics Laboratory,  
Department of Gynecology and Obstetrics, Geneva  
University Hospitals, Geneva, Switzerland

### Synonyms

[BRCA1-associated ring domain \(gene/protein\) 1](#)

### Definition

*BARD1* codes for a protein that forms a functional heterodimer with the breast cancer predisposition gene product ► [BRCA1](#). This BRCA1–BARD1 complex has ► [ubiquitin ligase](#) activity, but not the respective monomers. The specific targets of the BRCA1–BARD1 ubiquitin ligase relate to the tumor suppressor functions of BARD1 and BRCA1. BRCA1-independent functions are attributed to BARD1, based on its role as inducer of ► [p53-dependent apoptosis](#).

### Characteristics

The tumor suppressor BARD1 was originally identified as a protein binding to the BRCA1 gene product, BRCA1. BRCA1 depends on binding to BARD1 for most of its tumor suppressor functions mostly through the ubiquitin ligase activity of the BRCA1–BARD1 heterodimer. This is presumably due to the fact that the stability of both proteins depends on their interaction via their N-terminal ► [RING finger](#) domains.

#### BARD1 Structure, Conservation, and Expression

BARD1 is a RING finger protein, like BRCA1, and both have ► [BRCT domains](#) at their C-terminus. *BARD1* genes have been found in several species: mouse, rat, *Xenopus*, *Caenorhabditis elegans*, and a database entry is found for the tropical fish *Takifugu rubripes*. While the N-terminal RING finger and the BRCT domains of BARD1 are evolutionary conserved with at least 90% identity of amino acids, the regions between these structures show only little conservation (Fig. 1). In addition to these two conserved domains, BARD1 possesses ► [ankyrin \(ANK\) repeats](#) that are

found in proteins of diverse functions. Besides similar structure, BARD1 has several features in common with BRCA1: embryonic lethality of knock out in animal models, and genetic instability in cellular model systems of BARD1 or BRCA1 depletion.

BARD1 is composed of 11 exons. First observation of alternate splice variants was BARD1 $\beta$  in preleptotene ► [spermatocytes](#), while the wild-type form of BARD1 was expressed in spermatogonia. An isoform BARD1 $\delta$ , derived from differential splicing, was found in a rat ovarian cancer cell line and in HeLa cells.

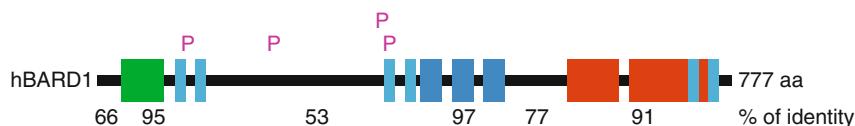
BARD1 mRNA expression was found in most proliferative tissues, mostly parallel with the expression pattern of BRCA1. However, in hormonally regulated tissues BARD1 expression was different from BRCA1. Further evidence for hormonally controlled expression of BARD1 was found in spermatogenesis. BARD1 expression is also induced by hypoxia.

#### Functions of the BARD1–BRCA1 Heterodimer

BARD1 and BRCA1 interact with a number of proteins involved in various functions, most cited are ► [homologous repair](#) and ubiquitin ligase activity. Dissection of repair pathways showed that the BRCA1–BARD1 heterodimer has a role in homologous repair before the branch point of HDR (homology derived repair) and SSA (single strand annealing).

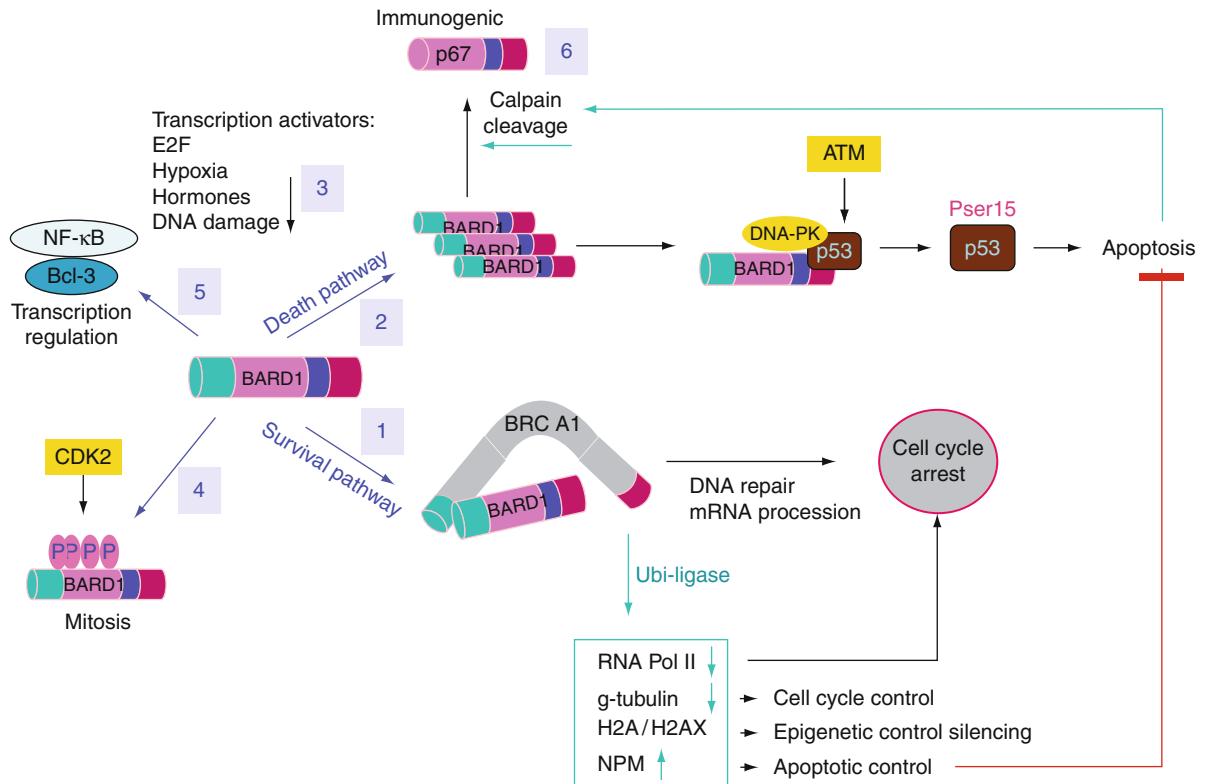
The RING finger domains of BARD1 and BRCA1 are required for ubiquitin ligase functions, and missense mutations in the BRCA1 RING finger that abrogate this ubiquitin E3 ligase function, are found in breast and ovarian cancer, suggesting that the ubiquitin ligase activity is linked to tumor suppressor functions. Specifically, BARD1 protein residues 8–142 could enhance ubiquitin ligase activity of BRCA1. The results of mutagenesis studies indicate that the enhancement of BRCA1 E3 ligase activity by BARD1 depends on direct interaction between BARD1 and BRCA1. Across its ubiquitin ligase activity, the BRCA1–BARD1 heterodimer controls cell cycle progression and exerts tumor suppressor functions. BARD1–BRCA1 might also regulate cell cycle progression by interacting with another potential target for ubiquitinations, PolII, or the nucleolar protein nucleophosmin/B23 (NMP).

A function in regulation of transcription is exerted via BARD1 binding to the ► [Bcl-3 oncoprotein](#), which acts as a bridging factor between ► [NF- \$\kappa\$ B/Rel](#) and nuclear coregulators. The functional interaction of BARD1 with polyadenylation factor CstF-50



**BARD1. Fig. 1** Human BARD1 domain structure. RING (green), ANK (blue), and BRCT (red) domains are indicated and location of potential NLS (light blue) and phosphorylation

sites (P). Evolutionary conservation is indicated as percentage of identical amino acids between human and mouse BARD1 sequences within distinct regions



**BARD1. Fig. 2** Major BARD1 and BRCA1–BARD1 pathways and functions. BARD1 participates in several pathways: (1) survival pathway as BRCA1–BARD1 heterodimer and (2) death pathway in a BRCA1-independent function in apoptosis. BRCA1–BARD1 acts in survival pathway. BRCA1–BARD1 ubiquitin ligase activity leads to RNAPolII degradation and cell cycle arrest, to  $\gamma$ -tubulin degradation and regulation of centromere duplication, to H2A/H2AX ubiquitylation and

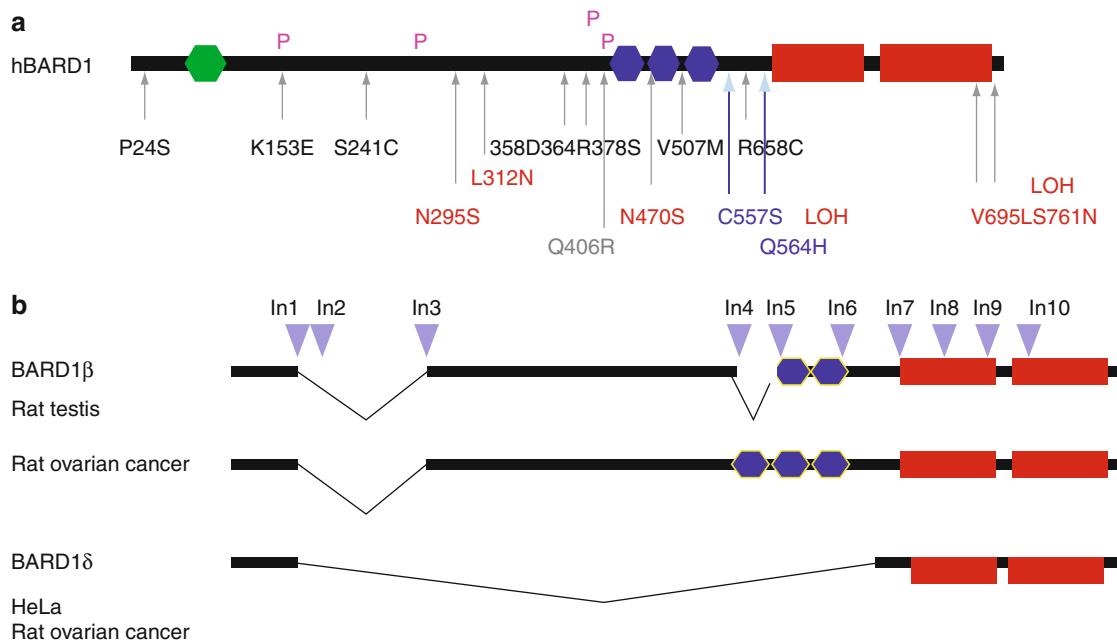
epigenetic control, and to NPB ubiquitylation. Upregulated NPB is a known inhibitor of apoptosis, it causes centromere amplification and genetic instability; hence NPM antagonizes BARD1 functions. (3) BARD1 is transcriptionally upregulated by different conditions, (4) is modified upon apoptosis by phosphorylation, (5) and can modulate NF- $\kappa$ B function. The proteolytic cleavage product p67 is immunogenic and has antitumorigenic properties (6)

provides another possible regulatory mechanism by linking **mRNA 3' end formation** to DNA damage and tumor suppression.

#### BRCA1-Independent Tumor Suppressor Function of BARD1

Independently of BRCA1, BARD1 acts in an **apoptosis** pathway by binding and stabilizing

**p53**. The mechanism of p53-dependent apoptosis, induced by BARD1, is based on BARD1 binding to the **Ku-70** subunit of **DNA-PK** and to p53 thus facilitating or catalyzing the phosphorylation of p53 on serine 15. This phosphorylation is required for stabilization of p53 and for initiation of apoptosis. An excess of BARD1 over BRCA1 can induce apoptosis and binding to BRCA1 inhibits BARD1 apoptotic



**BARD1. Fig. 3** BARD1 mutations and isoforms. **(a)** Schematic drawing of BARD1 protein structure with RING (green), ANK (blue), BRCT (red) motifs indicated. Phosphorylation sites are marked with P. Mutations are marked in red and polymorphisms in black underneath. The Q406R mutation was found recently in

ovarian cancer. **(b)** Splice variants found for *BARD1*: BARD1 $\beta$  in preleptotene spermatocytes, BARD1 $\delta$  in ovarian cancer cell line and in HeLa cells. The N-terminal exons 1–5 were frequently lost in ovarian and ovarian cancer cells cancer, as shown by immunohistochemistry and RT-PCR

function. Upregulation of BARD1 expression can be induced by various types of cellular stress.

BARD1 deletions that lack the RING finger domain are stable, suggesting that the RING finger is targeting BARD1 for degradation, and proteolytic cleavage of the RING finger leads to the apoptosis pathway. During apoptosis BARD1 (97 kDa) translocates to the cytoplasm where it is cleaved to become the more stable p67, and consistently the apoptotic function of BARD1 was mapped to a region within p67, including ANK, the regions between ANK and BRCT, and part of BRCT.

Therefore BARD1 acts as a cellular switch from repair mode with BRCA1 and moderate BARD1 levels to apoptosis without BRCA1 and excessive BARD1 levels (Fig. 2).

#### BARD1 in Mitosis

A role of BARD1 in mitosis was suspected, since BARD1 protein levels are increased in mitosis. Mice deficient for BARD1 are embryonic lethal, and embryos die at day 8 of embryonic development

due to deficient proliferation. A novel function of BARD1–BRCA1 in mitosis implies interaction with the randomized studies indicate that 2-CdA and provides an explanation for the genetic instability and lethality observed in BARD1 repressed cells and embryos.

#### Clinical Relevance

**BARD1 Mutations in Cancer** Interestingly, mutations in *BRCA1* occur all over the protein-coding region, leading to truncated presumably unstable proteins. Missense mutations are mostly found in the RING finger and disrupt the BRCA1–BARD1 interaction, suggesting that the BRCA1 function is linked to the function of the BARD1–BRCA1 heterodimer. On the contrary, mutations in BARD1 are found mostly around the ANK repeats, the BRCT domains and the region in between these domains (Fig. 3). This C-terminal region has been shown to be stable in the absence of BRCA1 and to play a role in apoptosis, which suggests that BARD1 has tumor suppressor functions independently of BRCA1.

Considering the multiple functions of *BARD1* as tumor suppressor, it is expected that its functions might be lost or abrogated in cancer cells. Surprisingly, in human breast and ovarian tumors, elevated expression and mislocalization to the cytoplasm of *BARD1* was found, as compared to the healthy tissue. Most samples showed 5' truncations and the upregulation of *BARD1* isoform expression in cancer was correlated with poor prognosis.

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## Barret Epithelium

### Synonyms

Barret esophagus

## Barrett Esophagus

### Synonyms

Barrett epithelium

### Definition

Barrett esophagus is the metaplastic epithelial lining of the distal esophagus, recognized as a premalignant condition. Metaplasia of the lower esophagus that is characterized by replacement of squamous epithelium

with cells normally found in the intestines (intestinal metaplasia), occurs especially as a result of chronic

► [gastroesophageal reflux disease](#) (GERD), a condition characterized by backflow (regurgitation) of the contents of stomach into the esophagus. The disorder is considered a premalignant condition (► [Colon Cancer Premalignant Lesions](#)), and affected individuals are at an increased risk of developing ► [adenocarcinoma](#) of the esophagus (► [Esophageal Cancer](#)).

### ► Fluorescence Diagnostics

## Barrett High-grade Dysplasia

### Definition

► [Barret esophagus](#).

## Basal Cell Carcinoma

Jorge R. Toro, Sherri Bale and Rasha S. Hamouda  
National Institutes of Health, Bethesda, MD, USA

### Definition

Non-melanoma skin cancers are the most common malignant neoplasms in the United States, representing one third of all cancers diagnosed every year. Basal cell carcinoma (BCC) represents 75% of non-melanoma skin cancers and has an estimated annual incidence of more than 700,000 cases in the United States outnumbering squamous cell carcinoma (SCC) 4–1. Over half a million BCCs are diagnosed in the United States annually, outnumbering ► [squamous cell carcinoma](#) (SCC) 4–1. The US average annual incidence of BCC in whites is currently 191 per 100,000 and is increasing at a rate of 3–7% per year. The peak incidence of BCC occurs in the seventh decade of life and is rare in children.

### Characteristics

Basal cell carcinoma has multiple distinctive clinical forms, and these clinical subtypes can often be

correlated with histologic subtypes. BCC is protean in its manifestations. For this reason, biopsy for histologic confirmation is necessary. The various clinical forms of BCC include nodular, morpheaform and superficial. Nodular BCC is the most frequent form of BCC. It usually presents as a waxy, pearly or translucent papule/nodule with overlying fine telangiectasias, with frequent ulceration or erosion of the surface. BCCs may occasionally be pigmented to varying degrees. Superficial BCCs most commonly arise on the trunk and extremities, but may be seen anywhere on the body. The tumors are characterized by an erythematous macule or patch, which may be variably pigmented. There may also be an overlying fine scale, a superficial erosion or hemorrhagic scale crust. Superficial BCC is the variant most frequently seen in chronic arsenism and as late sequelae of radiation therapy. Individuals may have broad areas of superficial BCC that are multiple and disconnected. Morpheaform or sclerosing has a scar-like appearance. It consists of a dermal plaque with overlying epidermal atrophy in a sun-exposed distribution. As with infiltrative BCC, subclinical extension is often great and treatment failures frequent.

### Risk Factors and Therapy

Location, histologic subtype, clinical characteristics and size are predictive factors for the biologic behavior of BCCs. The typically indolent growth pattern of BCC accounts for the resistance and fusion planes of the central facial zone being a more significant determinant of subclinical extension. Size is also a good predictor of high risk BCCs. Cure rates with [► Mohs micrographic surgery](#) (MMS) decreases as tumor size increases. A cure rate of 99.8% for tumors less than 2 cm in diameter, 98.6% for tumors between 2 and 3 cm and 90.5% for tumors greater than 3 cm has been reported.

Micronodular, infiltrative and [► morpheaform](#) BCCs have a much higher incidence of positive surgical margins after surgical excision (18.6–33.3%) as compared with tumors with a nodular or superficial histologic pattern. Morpheaform BCCs may have significant subclinical extent, with the average subclinical extension being 7.2 mm. Similarly, significant subclinical extension in infiltrative BCC has been noted. BCC with marked squamous differentiation has been determined to be a more virulent tumor (a local recurrence rate of 45.7% and metastatic

incidence 8.6% of 35 such tumors as compared to rates of 24.2%/0.09% for BCC). As with SCC, the perineural space can serve as a conduit for significant subclinical tumor extension.

Although BCC is rarely life threatening its capacity for local tissue destruction can result in significant functional or cosmetic morbidity. Untreated or inadequately treated BCCs have an insidious growth pattern and may result in death. ► [Metastasis](#) from BCC is a rare event, with estimates of metastatic incidence ranging from 0.0028% to 0.1%. Metastasis is associated with the metatypical (basosquamous) BCC and with duration and size of the lesion. The most frequent site of metastasis is the lungs, followed by bone, lymph nodes, and liver. For these reasons, great importance is attached to the early diagnosis and treatment of this malignancy.

BCC is related to chronic ultraviolet radiation ([► UV radiation](#)) exposure. UVR exposure is partly responsible for both BCC and SCC, as evidenced by the preponderance of these lesions on sun-damaged skin after chronic exposure to sunlight. More than 99% of individuals developing BCC are Caucasians, and 85% of these tumors arise on the head and neck. The nose is most common of all sites, accounting for 25–30% of all tumors. Individuals of Scottish, Celtic, or Scandinavian ancestry are at higher risk. Affected persons usually have a history of significant occupational and/or recreational sun exposure. There is evidence that BCC arising before the age of 40 years corresponds with childhood or recreational sun exposure but does not correlate directly with cumulative sun damage. Thus, in areas of the world where the UV radiation is most intense, such as the sun belt in the United States, childhood sun exposure is at a maximum and younger patients are at a higher risk of developing BCC.

It is debatable whether BCC is more aggressive in children. As total incidence rates of BCC continue to rise, childhood cases may become more common. This increase in pediatric BCC may be especially true in areas of high UV radiation exposure. The percentage of sunny days during the year, higher altitude, and location closer to the equator may place children in these areas at increased risk. There exist other significant risk factors for the development of BCC: Prior injury such as trauma, burns, or vaccinations at the tumor site is frequently noted by persons with BCC. Carcinomas arising as a late sequelae of radiation therapy most frequently takes the form of BCC on the

head, neck, and trunk, and SCC on the hands. Prior exposure to inorganic arsenic can also lead to the formation of BCC. In this setting, tumors are often multiple, truncal, and superficial lesions. Immunosuppressed individuals are also prone to the development of BCC, although their risk is greater for SCC than for BCC.

Basal cell ► **nevus** syndrome (BCNS), ► **xeroderma pigmentosum** (XP), Baze syndrome and albinism represent inherited genetic disorders that predispose those affects to BCC and SCC. Patients with basal cell nevus syndrome are found to have a germline mutation in PTCH, a ► **tumor suppressor gene** located on 9q22.3. PTCH is the human homolog of Drosophila patched. Approximately 1/3 of cases result from a new germline mutation. Approximately 80% of PTCH mutations result in premature truncation of the patched protein. Inactivation of this gene was found in tumor tissue in 68% of BCCs examined and did not correlate directly with sun exposure or age. Typically, multiple BCCs develop at a young age in ► BCNS. Multiple BCCs, odontogenic keratocysts and palmo-plantar pits constitute the primary features of BCNS. Approximately 5% of infants with BCNS develop ► **medulloblastoma**. ► **Radiation therapy** for the medulloblastoma can result in a crop of BCCs in the radiation port.

Xeroderma pigmentosum (XP) is due to a genetic defect in the biochemical pathway to eliminate the carcinogenic potential caused by the damage of ultraviolet light B (UVB) to DNA. Several genes, those for XP groups B, D, and G and ► **Cockayne syndrome** groups B code for components of transcription factors, the protein complexes that bind the promoter regions and control gene transcription. BCCs and SCCs occur at a much higher rate and a much earlier age. Keratoacanthomas, fibrosarcomas and melanomas are also common in patients with XP.

BCC can be treated with multiple modalities providing 90% cure rates for primary disease in most cases. Cure rates for ablative surgery and excisional surgery vary with a number of factors including the clinical size of the tumor, the location, the histological subtype and whether or not it is recurrent. Cure rates for ablative surgery are less than 90% for BCC exceeding 0.5 cm in diameter on the face and over 2.0 cm in diameter on the trunk and extremities. In these instances, consideration should be given for excisional surgery with adequate margin control. BCCs exceeding 0.5 mm in diameter of the central

facial zone and aggressive growth pattern tumors with sclerosing stromas are best treated with Mohs micrographic surgery. The histologic subtype or growth pattern is a good predictor of cure rate. These tumors do not respond well to superficial or ablative surgery. Nodular and superficial BCC respond well to curettage and electrodesiccation, cryotherapy or shave excision can result in less morbidity than full-thickness excisional surgery. For adequate cure rates morphaform or sclerosing, micronodular or infiltrative variants of BCC require excisional surgery with histologic margin control.

### Squamous Cell Carcinoma

SCC is the second most common skin cancer, representing 20% of cutaneous malignancies. Over 100,000 cases of SCC are diagnosed annually in the United States, accounting for an incidence of 41.4 per 100,000. SCC of the skin is the fifth most common cancer among men and the sixth most common cancer among women in Sweden. SCC in situ or ► **Bowen disease** is the most common benign/precancerous tumor among men, while among women, it is second only to in situ cervical cancer. It most commonly affects individuals in mid to late life. SCC usually cause local tissue destruction and in advanced cases it may cause cosmetic and functional morbidity.

Clinically, typical SCC is a hyperkeratotic papule, nodule or plaque with variable ► **erythema**. Associated pain may suggest perineural extension. The central part of the face is the area at highest risk for recurrence. Tumors in this region tend to grow down or extend at various resistance planes such as the perichondrium of auricular and nasal cartilages. The tarsal plates of the eyelids or embryonic fusion planes at the junction of the nasal and nasolabial folds, and along the nasal columella or in the periauricular region. The size of the tumor also affects risk for recurrence. Tumors less than 1 cm have a 99.5% cure rate by Mohs micrographic surgery, compared with 82.3% for tumors 2–3 cm and 58.9% for tumors greater than 3 cm. Tumors under 2 cm of diameter have a local recurrence rate of 7.4% in contrast to a 15.2% recurrence rate for tumors greater than 2 cm. Therefore, margins of excision are adjusted according to size, with a 4 mm margin recommended for tumors less than 2 cm and 6 mm for tumors of 2 cm or greater.

Deeply invasive tumors have a greater tendency for local recurrence and metastases. Tumors with less than

4 mm in depth have a local recurrence rate of 5.3% compared with a rate of 17.2% for tumors 4 mm or greater. SCCs that penetrate through the dermis to the subcutaneous tissue have a recurrence rate of 19.8%. Tumors greater than 1 cm in diameter or a histologic grade 2 or higher are more likely to extend to subcutaneous tissue. The degree of histologic differentiation has a propensity for aggressive disease. SCC Broder grades 2 or higher usually require larger resections and has greater risk of local recurrence. Well differentiated SCC have a 13.6% recurrence rate in contrast to a 28.6% recurrence rate for poorly differentiated SCC. SCC with neurotropic growth pattern, which invade the perineural space, have a greater risk for local recurrence.

SCC usually have a low metastasis rates ranging from 0.3% to 3.7%. SCC arising in the lip, ear, penis, scrotum and anus have a higher risk for metastases. There is a greater risk of metastases for SCC more than 2 cm in size, with depth of invasion to at least 4 mm, ► Broader histologic classification of 2 or greater and perineural extension. SCC usually metastasis to the regional lymph nodes. The 5 year survival rates for patients with regional lymph node metastases is 26%, and 23% in patients with distant metastases.

### Risk Factors and Therapy

The risk factors for SCC include exposure to UV light and ► arsenic compounds, immunosuppression and underlying genetic predisposition. Cellular atypia is often equally high among those with *in situ* SCC or invasive SCC, and it is difficult to use cytological criteria to define *in situ* SCC as a benign lesion, in spite of an intact basement membrane in histological specimens. Even when using molecular markers such as the expression of p53 gene, *in situ* and invasive SCC are indistinguishable. The incidence of invasive SCC is two times higher for men, whereas *in situ* SCC is more common among women. It is possible that there are close etiological links between *in situ* and invasive SCC, and *in situ* appears to be as important a marker for subsequent cancer risk as invasive SCC.

SCC can also be treated satisfactorily with different modalities. Histologic growth pattern is less important in SCC than clinical size and depth of invasion, with the exception of rare histologic subtypes such as adenosquamous cell carcinoma. SCC exceeding 1 cm in diameter and tumors that invade into the mid-dermis

or deeper, particularly those involving cartilage and bone, are high-risk tumors. SCC on the lip, ear, temple, genitalia and those associated with preexistent conditions such as radiation or burn scars are all higher risk tumors. In these instances excisional surgery with careful margin control should be the treatment of choice. Postoperative radiation therapy may also be considered for these aggressive high-risk tumors on a case-by-case basis. Superficial or ablative procedures such as curettage and electrodesiccation, cryotherapy and shave excision should be reserved for SCC *in situ* (► Bowen disease) or SCC that invades only the superficial dermis. The depth of the invasion can be measured with an adequate preoperative biopsy. Indurated tumors with an undermining infiltrative border are often deeply invasive and should be treated with excisional surgery.

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## Basal Cell Nevus Syndrome

### Synonyms

### BCNS

### Definition

Is a heritable autosomal dominant tumor syndrome, characterized by a multitude of developmental abnormalities including basal cell carcinomas of the skin, keratocysts of the jaw, palmar and plantar pits, fibromas of the ovaries and heart, medulloblastomas, and less commonly polydactyly, syndactyly, and spina bifida. Approximately, 2% of patients with BCNS

develop medulloblastomas. The disease results from germline mutations in the *PTCH1* gene in chromosome 9q22.3.

- ▶ Gorlin Syndrome
- ▶ Medulloblastoma
- ▶ Naevoid Basal Cell Carcinoma Syndrome
- ▶ NBCCS

## Basal Phenotype Breast Cancer

- ▶ Basal-like Breast Cancer

## Basalioma

### Definition

Refers to a skin cancer with extended growth, but no metastasis; develops in skin areas exposed to sun, such as face, nose, or ears.

## Basal-like Breast Cancer

Vincent L. Cryns<sup>1</sup>, Mervi Jumppanen<sup>2</sup> and Jorma Isola<sup>3</sup>

<sup>1</sup>Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

<sup>2</sup>Department of Pathology, Central Hospital of Seinäjoki, Seinäjoki, Finland

<sup>3</sup>Institute of Medical Technology, University of Tampere, Tampere, Finland

### Synonyms

Basal phenotype breast cancer; Basal-subtype breast cancer; Basal-type breast cancer

### Definition

Basal-like ▶ breast cancer is an aggressive type of breast cancer that expresses genes characteristic of

epithelial cells in the outer or basal layer of the normal breast, including the basal ▶ cytokeratins. These tumors are usually hormone receptor negative and have a poor prognosis.

## Characteristics

The normal adult breast is a glandular tissue organized into ducts and lobules that are made up of an inner layer of ▶ luminal epithelial cells, which surround the lumen of these structures and produce milk, and an outer or basal layer of ▶ myoepithelial cells (basal epithelial cells), which are surrounded by a basement membrane, separating them from the adjacent connective tissue. Luminal and basal epithelial cells can be distinguished by their expression patterns of the cytokeratins (CK), a family of cytoskeletal proteins. To understand the basis for nomenclature, it should be noted that the name “basal cytokeratin” was originally given to those (namely, CK5/14/17) expressed in the basal cells of the stratified epithelium of the skin. Cytokeratins 8, 18, and 19 were named as “luminal” according to their strong expression in luminal epithelial cells. The basal or suprabasal layer of the breast also contains a population of multipotent CK5-positive ▶ progenitor or adult stem cells (▶ breast stem cells) that are capable of generating both epithelial cell types and likely play an important role in regenerating the gland after pregnancy and lactation.

Intriguingly, human breast tumors have distinctive ▶ gene expression profiles or “molecular portraits” that likely reflect the contribution of luminal epithelial cells, myoepithelial cells, and/or breast stem cells. These gene signatures have recently been used to classify breast cancer into several molecular types, including ▶ estrogen receptor (ER)-positive luminal groups, which expresses genes characteristic of luminal epithelial cells (ER, ▶ progesterone receptor (PR), and the luminal cytokeratins 8 and 18), and two major ER-negative groups, the ▶ HER2 group, characterized by ▶ amplification and overexpression of the proto-oncogene HER2, and the basal-like group, which express genes characteristic of myoepithelial cells (basal cytokeratins 5 and 17 and HER1/EGFR). The vast majority of basal-like breast tumors are negative for ER and PR and lack amplification of HER2.

Hence, basal-like tumors are sometimes referred to as ► **triple negative breast cancer**, although not all basal-like tumors are triple negative and vice versa. Other genes expressed in basal-like breast cancer have been implicated in ► **apoptosis**-resistance (e.g.,  $\alpha$ 6 and  $\beta$ 4 integrins, the molecular ► **chaperone**  $\alpha$ B-crystallin, and ► **pleiotrophin**) and multiple molecular events in metastasis, including ► **epithelial-to-mesenchymal transition** (EMT), cell ► **migration**, and ► **invasion** (e.g., TGF $\beta$ 2, the ► **chemokine** CXCL1, and the ► **matrix metalloproteinase** MMP14). These genes may contribute to the aggressive nature of basal-like breast cancer, which has a poor prognosis due in large part to its tendency to metastasize to distant organs. Basal-like tumors also express genes characteristic of ► **breast cancer stem cells**, such as ► **KIT**, vimentin, and  $\alpha$ 6 integrin, suggesting that these tumors may have their origin in primitive breast stem/progenitor cells rather than in differentiated myoepithelial cells.

Basal-like breast tumors have also been identified by their expression of basal cytokeratins. In general, all or nearly all sporadic breast carcinomas show strong expression of luminal cytokeratins CK8, CK18, and CK19. The tumors expressing only luminal CK8, CK18, and CK19 are called “luminal” or “non-basal.” A small fraction (~10%) of breast cancers also express CK5 together with its major partners CK14 and CK17, which are normally found in the basal layer of the breast, leading to their designation as “basal” or “basal phenotype” tumors. Tumor classification into the basal-like subtype by cytokeratin immunohistochemistry and gene expression microarrays is very similar, but not completely identical. Some breast tumors lacking immunohistochemically detectable CK5 and CK14 may display a typical basal-like gene expression profile, and vice versa.

Basal cytokeratin expression can be seen in the tumor either uniformly in almost every carcinoma cell or strikingly heterogeneously in a checker-board manner. This feature can be used to further classify basal phenotype tumors as “basal” and “basoluminal” subtypes. All tumors in both subtypes show strong expression for luminal cytokeratins. Thus, in heterogeneously CK5/14-positive tumors, a large number of tumor cells express luminal cytokeratins only. Based on this observation, these tumors were called “basoluminal,” in contrast to the uniformly CK5/14-positive “basal” tumors. Basoluminal tumors are

also characterized by higher rates of HER2 amplification and a poorer prognosis than “basal” tumors.

### Molecular Etiology

Despite the distinctive ► **gene expression profile** of basal-like breast cancer, which has been validated in multiple studies, the specific role of individual basal-like genes in the etiology of these tumors is poorly understood at the present time. Nevertheless, the gene signature of basal-like tumors has provided invaluable insights into potential pathogenic mechanisms. The observation that the gene expression profiles of breast tumors in women who have inherited a mutation in the ► **BRCA1 tumor suppressor gene** are largely basal-like strongly suggests that defects in BRCA1 function may play an important role in the etiology of non-hereditary basal-like breast cancer. BRCA1 normally functions to protect the genome through its actions in ► **DNA damage repair** and ► **cell cycle checkpoint** activation. Although mutations in *BRCA1* do not occur in non-hereditary breast cancer, basal-like tumors often have diminished expression of BRCA1 mRNA and protein due to ► **promoter** ► **methylation** and other mechanisms. Similar to basal-like tumors, hereditary *BRCA1*-related breast tumors are typically triple negative. Moreover, mutations in the ► **TP53** tumor suppressor gene and overexpression of the ► **cell cycle** regulator cyclin E occur commonly in both hereditary *BRCA1*-related breast tumors and non-hereditary basal-like tumors and may contribute to the ► **genomic instability** and the rapid proliferation rates of these tumors.

Defective X chromosome inactivation is also a hallmark of both tumor types (and BRCA1 dysfunction), although the role of this abnormality in their pathogenesis is unclear. Taken together, these findings suggest that BRCA1 dysfunction and resultant defects in DNA damage repair may play a key role in the etiology of basal-like breast cancer and provide a molecular target for therapy.

### Diagnosis

The highly reproducible gene expression profile of basal-like breast cancer remains the definitive method for diagnosing basal-like tumors. However, ► **gene expression profiling** is not routinely available in the clinic. Consequently, basal-like tumors can be identified by basal cytokeratin expression by ► **immunohistochemistry** or by double negative (ER and

► HER2 negative) status combined with positive immunostaining for either CK 5/6 or HER1/EGFR. These methods identify similar, but not identical, subsets of breast tumors given the likely heterogeneity of the basal-like group, an important caveat for clinical studies. In the near future, it may become possible to diagnose basal-like tumors using gene signatures of a subset of basal-like genes (20–50 genes) determined by ► reverse transcription-polymerase chain reaction (RT-PCR) methods on clinical specimens.

### Clinical Features

The unfavorable prognosis of basal cytokeratin expressing breast cancer was first described in 1987. Thereafter, it has been shown in many immunohistochemical studies that basal cytokeratin expressing tumors associate with poor survival and early relapse. More recently, the molecular classification of breast cancer by ► gene expression profiling has been shown to provide important prognostic information for patients. Patients with basal-like and ► HER2 tumors have the shortest overall and relapse-free survival of all the molecular types. When basal-like tumors are further subclassified into basoluminal and basal subgroups, the basoluminal tumors show shorter survival estimates than the basal tumors. This difference is not due to more frequent ► amplification of HER-2 in the basoluminal subgroup.

Consistent with their aggressive behavior, basal-like tumors are poorly differentiated, highly proliferative with high mitotic counts, and are characterized by genomic or chromosomal instability, resulting in numerous chromosome abnormalities, including gains and losses. In addition to these properties, basal-like tumors often show aggressive morphological features like pushing borders, lymphocyte infiltration, tumor necrosis, central scarring, and the presence of spindle cells. Many of these features are typical for medullary and/or metaplastic carcinomas, rare histologic types of breast cancer, which express basal cytokeratins and have a basal-like gene expression profile. Moreover, basal-like breast cancer tends to metastasize via the blood stream ("hematogenous" route) to distant organs such as the brain and lung, rather than spreading through the lymphatic system to adjacent lymph nodes. These metastases are particularly prevalent during the first 5 years after diagnosis and are largely responsible for the poor survival of patients with basal-like breast cancer. Intriguingly, hereditary

► BRCA1-related breast tumors are also characterized by early metastases to distant organs that account for their poor prognosis. Approximately 10–20% of all breast cancer cases are basal-like. However, basal-like tumors appear to be more prevalent in younger women, particularly African American women. Hence, the clinical impact of these poor prognosis tumors is compounded by their tendency to strike younger women.

### Treatment

Unlike the other molecular types of breast cancer, there are no currently available, ► targeted therapies for basal-like breast cancer. For instance, most basal-like breast tumors do not respond to ► endocrine therapy such as ► tamoxifen or ► aromatase inhibitors because they are ► estrogen receptor (ER)-negative. Similarly, ► trastuzumab (► Herceptin), a therapeutic antibody targeting ► HER2, is of little benefit against most basal-like tumors because they lack ► amplification of HER2. Although our understanding of the molecular etiology of basal-like tumors is limited, several candidate drug targets have been identified by gene profiling, including the ► receptor tyrosine kinases HER1/ ► EGFR and ► KIT, the MEK-ERK ► MAP kinase pathway (activated by HER1/EGFR and c-Kit), and the cell cycle regulator cyclin E. Drugs targeting several of these molecules have already been developed and may prove to be useful in treating basal-like tumors. Another potential therapeutic target is the likely BRCA1 dysfunction in basal-like tumors. Breast cancer cells expressing mutant BRCA1 are highly sensitive to chemotherapy drugs which cause DNA double-strand breaks (e.g., topoisomerase II inhibitors) or cross-link DNA (e.g., ► cisplatin), consistent with the established role of BRCA1 in ► DNA damage repair. Hence, these agents may be effective against basal-like tumors, particularly those with reduced BRCA1 expression. Moreover, inhibition of BRCA1 expression by ► RNA interference sensitizes cancer cells to ► apoptosis by inhibitors of the DNA repair enzyme ► poly(ADP-ribose) polymerase (PARP), which catalyzes poly(ADP-ribosylation) of target proteins. These findings suggest that PARP inhibitors may be of benefit in basal-like tumors. Given the aggressive nature of basal-like tumors and the lack of targeted therapies at present, there is a clearly a pressing need for carefully designed clinical trials to evaluate these and other agents in basal-like breast cancer.

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## Basal-subtype Breast Cancer

- [Basal-like Breast Cancer](#)

## Basal-type Breast Cancer

- [Basal-like Breast Cancer](#)

## Base Excision Repair

### Definition

Is a DNA repair pathway that repairs mainly non-bulky adducts that cause only minor disturbances in the helical structure of DNA, such as oxidized, alkylated, or even absent bases.

- [Mutagen Sensitivity](#)
- [Nucleotide Excision Repair](#)

## Basedow Disease

### Synonyms

[Graves' disease](#)

## Basement Membrane

### Definition

The basement membrane (BM) is a thin mat of extracellular matrix that separates epithelial sheets and many types of cells, such as muscle cells and fat cells, from connective tissue. The characteristic components of BMs are laminin, collagen type IV, and heparan sulfate proteoglycan.

- [Adhesion](#)
- [Basal-like Breast Cancer](#)
- [Extracellular Matrix Remodeling](#)
- [Heparanase](#)
- [Metastatic Colonization](#)

## Basic FGF

### Definition

- [Fibroblast Growth Factor 2](#)

## Basic Fibroblast Growth Factor

Is a ► [cytokine](#) that belongs to a homologous family of at least 18 proteins. Basic FGF binds FGF receptors 1 and 2, acts as a potent mitogen for ► [endothelial cells](#), and is expressed in many benign and malignant tumors.

- [Antiangiogenesis](#)
- [Fibroblast Growth Factors](#)

## Basic Helix-Loop-Helix Domain

### Definition

A phylogenetically conserved domain that defines a class of transcription factors. The basic region, rich in positively charged amino acids, mediates interactions

with DNA while two amphipathic alpha helices separated by a flexible, unstructured loop mediate hetero- or homodimeric interactions with other bHLH-containing transcription factors.

- E2A-PBX1
- Myc Oncogene

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## Basolateral Surface

### Definition

As applied to the gastrointestinal tract wall, it refers to the wall surface on the outside (the side exposed to the body's blood flow) of the GI tract or in Caco-2 cell culture the cell surface that mimics the outside of the GI tract. In Caco-2 cultures this is the cell surface that grows on the semi-permeable support membrane.

- ADMET Screen

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## Basophil

### Definition

A white blood cell that contributes to inflammatory reactions. Along with mast cells, basophils are responsible for the symptoms of allergy.

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## BAX

### Definition

Bax is a Bcl-2-associated X protein of 192 aa and 21 kDa that is membrane-bound and expressed widely in different tissues. It has proapoptotic activity, by binding and antagonizing the antiapoptotic Bcl-2, thereby accelerating apoptosis. The gene maps to 19q13.

- Apoptosis

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## Bbc3

- PUMA

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## BBI

- Bowman-Birk Inhibitor

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## BBS

- Bombesin

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## BCC

### Definition

- Basal Cell Carcinoma
- Photodynamic Therapy

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## BCDF

- Interleukin-6

---

## B-cell CLL/lymphoma 2

- BCL2

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## B-cell Differentiation Factor

- Interleukin-6

---

## B-cell Interferon

- Interferon- $\alpha$

## B-cell Leukemia/Lymphoma-2 Gene

► [BCL2](#)

## B-cell Leukemias

► [B-cell Tumors](#)

## B-cell Lymphoid Neoplasm

► [B-cell Tumors](#)

## B-cell Lymphoma

### Definition

B-cell lymphoma is a tumor of B-cells; ► [B-cell Tumors](#).

► [B-cell Tumors](#)  
► [Diffuse Large B-cell Lymphoma](#)  
► [Photodynamic Therapy](#)

## B-cell Lymphoma Protein 2

► [BCL2](#)

## B-cell lymphoproliferative Disorders/Diseases

► [B-cell Tumors](#)

## B-cell Malignancy

► [B-cell Tumors](#)

## B-Cell Response

### Definition

Response of B-1 and B-2 cells to antigen stimulation. B-1 cells bear high levels of surface IgM, lower levels of surface IgD, and most express the cell surface antigen CD5, they frequently secrete high levels of polyspecific antibody with relatively low affinity. The majority of B-cells are B-2 cells which express low levels of surface IgM, do not express CD5, and secrete highly specific antibody. While B-1 cells are CD43+ and CD23-, B-2 cells are CD43- and CD23+.

► [Autoantibodies](#)

## B-cell Stimulating Factor-2

► [Interleukin-6](#)

## B-Cell Tumors

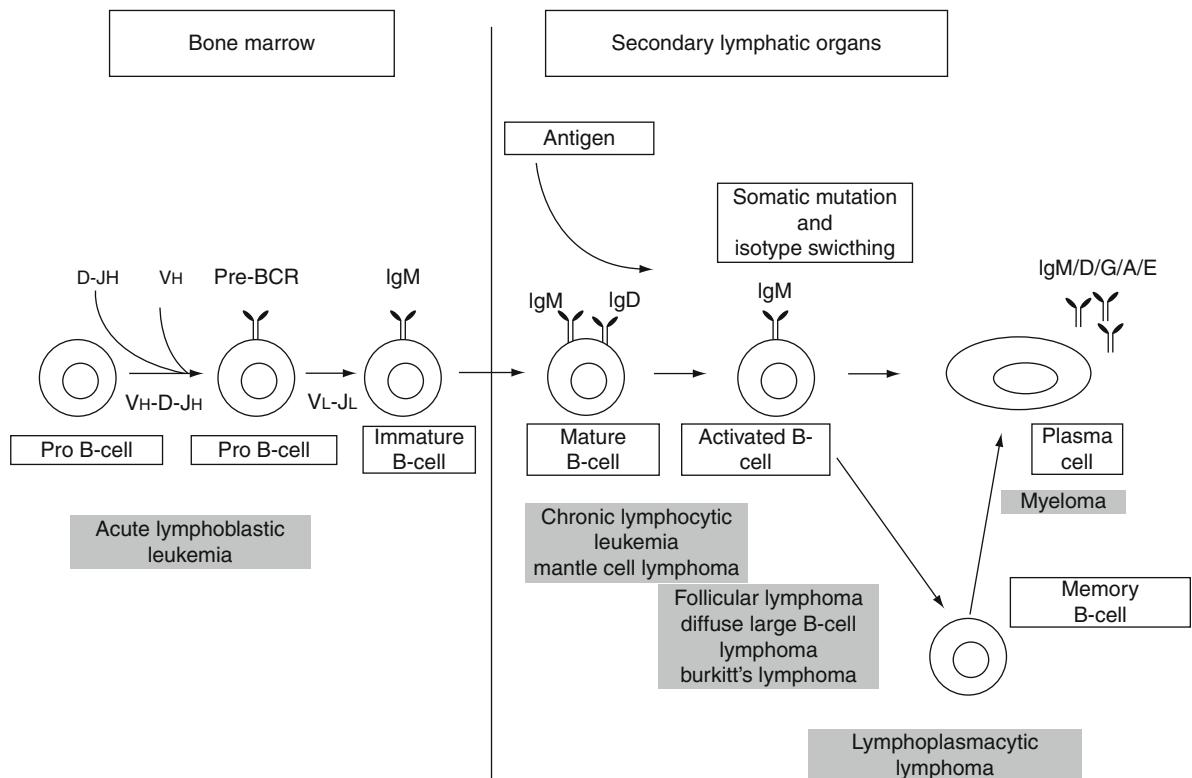
Christian Ottensmeier and Freda Stevenson  
CRC Wessex Oncology Unit, Southampton General Hospital and Tenovous Laboratory, Southampton University Hospital Trust, Southampton, UK

### Synonyms

B-cell leukemias; B-cell lymphoid neoplasm; B-cell lymphomas; B-cell lymphoproliferative disorders/diseases; B-cell malignancy; Cancer of B-lymphocytes; Hodgkin and non-Hodgkin lymphomas

### Definition

B-cell lymphomas are malignant tumors of B-lymphocytes. They arise at all stages of B-cell differentiation, from immature B-lymphocytes in the bone marrow through to terminally differentiated plasma cells (Fig. 1). It is now possible to use immunogenetic



**B-Cell Tumors. Fig. 1** In the bone marrow, first the D-JH then VH-D-JH recombination takes place. This heavy chain is expressed on the cell surface with the surrogate light chain to form the pre-B-cell receptor (pre-BCR). Next the light chain genes are rearranged. The B-cell now expresses surface Ig and leaves the bone marrow. Mature B-cells encounter antigen, and

are stimulated to somatically mutate their V genes. Additionally class switching is initiated. Some B-cells then leave the germinal center to become plasma cells; some become memory cells. The gray blocks illustrate at which stage of B-cell differentiation some B-cell tumors are thought to originate

analyses to define more clearly the cell origin and clonal history of B-cell tumors.

## Characteristics

### What Is a B-Lymphocyte?

B-lymphocytes are cells of the immune system that are destined to express immunoglobulins (antibody molecules). These immunoglobulins (Igs) play a central role in the recognition of foreign antigens like infectious organisms, which could threaten the integrity of the individual.

Igs are glycoproteins that can exist either as membrane-bound molecules on the cell surface or as secreted molecules in the serum. There are five classes of Ig with different size, structure, and function: IgM, IgD, IgG, IgA, and IgE. Each basic Ig molecule contains two identical heavy chains ( $\mu$ ,  $\delta$ ,  $\gamma$ ,  $\alpha$ , or  $\epsilon$ ) and

two identical light chains ( $\kappa$  or  $\lambda$ ). A mature B-cell carries about 105–106 identical Igs on its cell surface. Both light chains and heavy chains can be subdivided into distinct regions. The N-terminal variable (V-) regions mediate antigen contact, and their amino acid sequence is specific to each B-cell. The C-terminal constant regions are common to all antibodies of the same class.

The sequence variability, which is necessary to recognize the vast number of different antigens present in the environment, is created by two processes. The first is ► **immunoglobulin gene** rearrangement and the second is somatic mutation. ► **Class switching** changes Ig effector function.

### Immunoglobulin Gene Rearrangement

During this remarkable process, double-stranded DNA breaks are created and repaired in a tightly controlled fashion. Rearrangement brings together one

representative from different gene families: variable region (VH) genes, diversity region (D) genes, and joining region (JH) genes for the Ig heavy chain, VL and JL for the Ig light chain (Fig. 1). The process of VH-D-JH and VL-JL joining is imprecise; non-templated nucleotides (N-additions) can be inserted and the ends of the joined segments can be trimmed back. Thus the final products of rearrangement, the VH-D-JH and VL-JL, will have a unique nucleotide sequence V(D)J Recombination.

The heavy chain variable (VH) region is about 120 amino acids (aa) long and can be subdivided into discrete structural sections. Three complementarity determining regions form the classical antigen binding site: CDR1, CDR2, and CDR3. While CDR1 and CDR2 are encoded in the germline, CDR3 is created de novo in each B-cell by VH-D-JH rearrangement. In the antibody molecule, this sequence corresponds to the central part of the antigen recognition site. The CDRs alternate with four framework regions (FR1–4). Light chain variable (VL) regions are about ten aa shorter, but contain similar structural motifs.

In the bone marrow, the Ig heavy chain genes are rearranged first followed by the light chain genes (Fig. 1). Ultimately, a B-cell that successfully completes this process will have a unique immunoglobulin heavy and light chain gene sequence for antigen recognition. The nucleotide sequence in CDR3 can be viewed as its molecular marker or “fingerprint.”

### Somatic Mutation and Class Switching

Following antigen encounter, further variability is introduced into the rearranged variable region genes by somatic mutation that occurs in secondary lymphatic organs. Somatic mutation can change the amino acid sequence of variable region genes and may therefore impact on the antigen binding of the resulting protein.

The process of class switching changes the effector function of the antibody molecule (complement activation, binding to Fc receptors, or uptake by phagocytic cells). During class switching, the DNA segment of one constant region (e.g., IgM) is deleted and the variable region of the heavy chain brought into the vicinity of another constant region gene (e.g., IgG or IgA). This process conserves the unique variable region.

### What Is a B-Cell Tumor?

In the broadest sense, ► **B-cell tumors** are malignancies in which tumor cells have undergone rearrangements of their ► **immunoglobulin genes**. Analysis of the status of these genes provides information that defines origin and clonal history of the tumor cell. Figure 1 shows key steps in normal B-cell development, and gives examples of B-cell malignancies that may arise at a particular stage. Within each cancer, the tumor cells are clonally related, as revealed by the common CDR3 sequence in the tumor cell population.

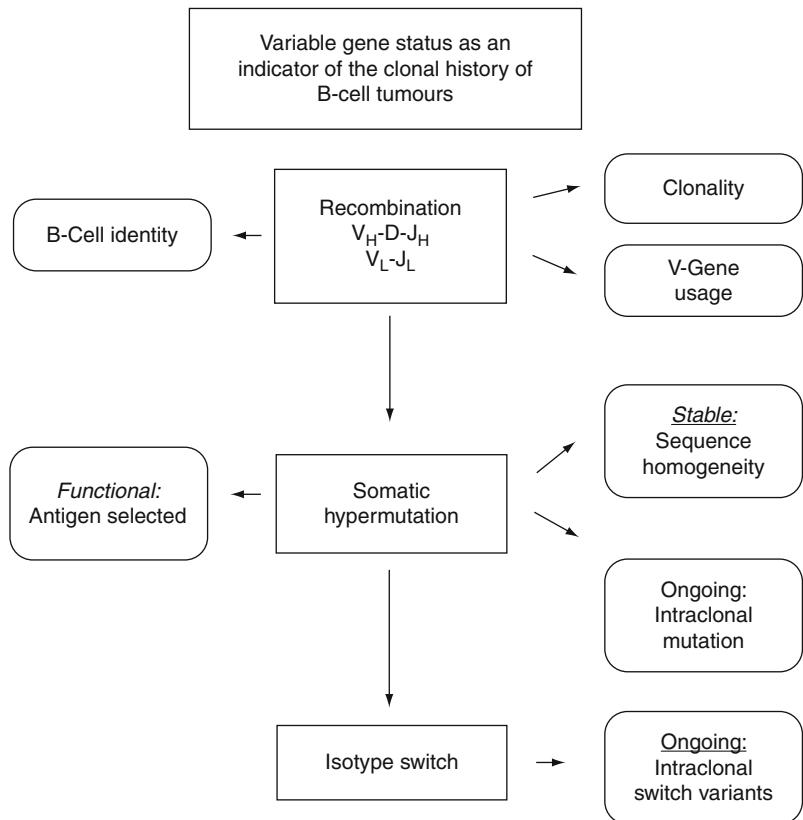
In some lymphomas the tumor clone has non-functionally rearranged VH genes, as appears to be the case in Hodgkin lymphoma (► **Hodgkin disease**). This sets these lymphomas apart from normal B-cells, which can only survive if they express immunoglobulins. The antigenic determinants, derived from the variable regions of the immunoglobulin molecule, provide us with a unique tumor antigen called ► **idiotype**. This tumor antigen is now being exploited in new immunotherapeutic strategies.

### Characteristics of B-Cell Tumors

B-cell tumors account for about 3% of all cancers. For unknown reasons, their incidence is rising steadily at about 6% per year worldwide. B-cell tumors are the most common malignancies in childhood. In adults, the frequency of B-cell tumors increases steadily with age, with a median of 50–60 years. They occur more frequently in men than women.

The presentation of B-cell tumors at the clinical and morphological level can vary widely. Aggressive malignancies are at the one end of the spectrum, which if untreated will cause death in weeks but are frequently curable with combination chemotherapy. Indolent malignancies are at the other end of the spectrum, which are usually incurable but can remain untreated for decades. The diagnosis of B-cell malignancies relies on the clinical picture, histological analysis, and immunophenotype of the tumor. Increasingly, hallmark genetic abnormalities are being defined in individual entities. They frequently involve translocations into the immunoglobulin loci of the heavy chain genes on chromosome 14 or the  $\kappa$  or  $\lambda$  light chain loci on chromosomes 2 and 22.

Different ways of grouping lymphoid malignancies in a logical fashion have been applied. They were based on the need of clinicians to determine a suitable course of treatment as well as the desire of pathologists

**B-Cell Tumors. Fig. 2**

to distinguish morphological similarities. Although these classifications were used in parallel, they are difficult to compare since similar entities were often attributed to different categories. In 1994, an attempt was made to divide lymphoid malignancies taking into account the combined available information from clinical patterns, morphology, immunophenotype, and genetic characteristics. Also, as far as possible, the normal counterparts were attributed to each malignancy. This led to the “Revised European-American Classification of Lymphoid Neoplasms” (REAL). This REAL classification provides the first truly international view of lymphomas and has been developed further in the form of the recently proposed WHO classification.

### Immunogenetics of B-Cell Lymphomas

Genetic analysis of B-cell lymphomas has aided our understanding of malignant lymphoma. Specific chromosomal rearrangements in many of the lymphoma entities indicate that a particular type of genetic

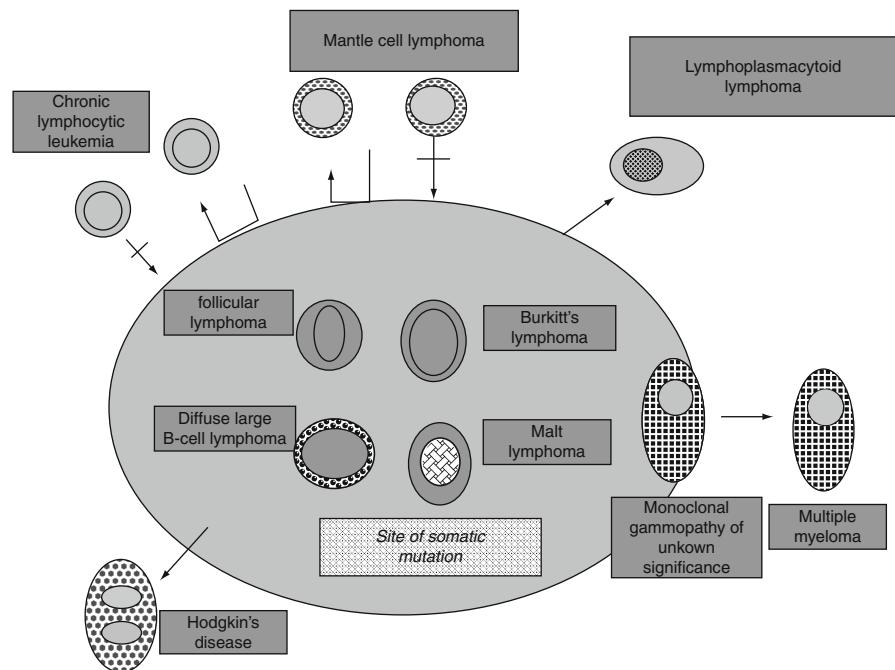
damage in the precursor cell is important for the development of the lymphoma. For example, t(14;18) translocation is characteristic of ► **follicular lymphoma**. The isolation of the same translocation in cells from healthy individuals suggests that this genetic change may be a necessary but insufficient condition for the development of follicular lymphoma.

More recently, the analysis of the status of the immunoglobulin genes in B-cell tumors has shed new light on the events which shape the malignant cell. **Figure 2** summarizes the information that V-gene analysis of B-cell tumors can reveal. The presence of rearranged immunoglobulin genes defines the cells under investigation as being of B-cell origin (**Fig. 2**). In this way, it could finally be established that in the majority of cases Hodgkin lymphoma is a B-cell tumor.

Sometimes, it can be very difficult to assess if an abnormal population of B-cells represents a true malignancy. Examples include low grade ► **MALT lymphomas** in the stomach or lymphoproliferations after organ

**B-Cell Tumors.**

**Fig. 3** Origin and development of B-cell tumors in relation to the site of somatic mutation in the germinal center (GC). V-gene mutational patterns can be used to classify tumors as follows: (i) not entering the GC (*blocked arrows*); (ii) passing through the GC (*arrows out*); (iii) remaining in the GC (*no arrows*). Monoclonal gammopathy of unknown significance may in some cases remain in the GC. CLL has subgroups, with different patterns of mutations, which thus possibly arise from different stages of B-cell development



transplants. Here the analysis of the Ig genes can help to separate a poly- or oligo-clonal and pre-malignant lesion from a truly clonal and cancerous one (Fig. 2).

In some B-cell lymphomas (follicular lymphoma, diffuse large B-cell lymphomas), the observed VH gene usage is similar to that of normal B-cells. In other tumor types, however, a marked over- or under-representation of certain VH genes has been detected. For example, a member of the VH4 gene family, called V4–34, is used by about 6% of normal cells. In contrast, all known cases of ► **Waldenstrom macroglobulinemia** with cold agglutinins of anti-I activity use the V4–34 gene. This suggests that B-cell superantigens may play a role in the pathogenesis of cancer in these B-cell lymphomas.

Since gene rearrangement, somatic mutation and class switching all leave their traces in the Ig-genotype of a B-cell, Ig analysis can provide important information about the clonal history of the malignant B-cell. V-gene analysis allows us to determine which processes the B-cell has been exposed to and also suggests which “normal” counterpart the tumor cell may be related to.

The majority of B-cells in the periphery will have been exposed to somatic mutation in the germinal center of the secondary lymphatic organs. Figure 3

relates the origin and development of B-cell tumors relative to the germinal center. The analysis of the tumor related VH-D-JH genes in tumors can reveal evidence that the tumor cell clone has entered this site, if somatic mutations are found in the VH-D-JH gene. Within the same tumor type some cases may show somatic mutation, while others do not. ► **Chronic lymphocytic leukemia** (CLL) segregates into two categories; patients with unmutated (pre-germinal center) CLL have a significantly worse prognosis than those with mutated VH-D-JH genes.

Evidence for ongoing mutation can be identified by detecting micro-heterogeneity in clonally related sequences from the tumor. While the clonal fingerprint of the tumor is shared between all cells, some cells have acquired additional mutations that are not shared by other cells. This type of pattern is found in follicular lymphoma, ► **Burkitt lymphoma**, and diffuse large B-cell lymphomas (DLBCL) (Fig. 3). The tumor cells of DLBCL and hairy cell leukemias are also able to produce transcripts for more than one Ig isotype. This provides additional evidence that the malignant tumor cells are less frozen in their development than previously thought.

Malignancies like multiple myeloma (MM) have mutated VH-D-JH genes, but all sequences are

identical (they are “stable”). This suggests that MM has undergone somatic mutation but that the tumor cells have then left the site of somatic mutation (post germinal center tumors). ► **Monoclonal gammopathy of undetermined significance** (MGUS) can show or lack intraclonal heterogeneity.

The available data now allow us a detailed description of human B-cell tumors. Immunogenetics has contributed to the classification by providing information that is independent of the morphology and clarifies the developmental stage at which the final transforming event occurred.

It is likely that in the future new methods like the gene ► **microarray technology** will help us understand which disease entities should be further subdivided. Additionally, we are likely to predict better within lymphoma entities and tailor treatment according to more accurate prognostic factors.

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## BCG

### Definition

Attenuated form of *Mycobacterium bovis*, ► **Bacillus Calmette-Guérin**. Generated by multiple in vitro passages on a special culture medium. Named after two French microbiologists who worked at the Pasteur Institute in the 1920s.

► **Bacillus Calmette-Guérin**

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## Bcl2

Marco Ruggiero

Department of Experimental Pathology and Oncology,  
University of Firenze, Firenze, Italy

### Synonyms

Apoptosis regulator Bcl2; B-cell CLL/lymphoma 2;  
B-cell leukemia/lymphoma-2 gene (*Bcl-2*); B-cell  
lymphoma protein 2; Bcl-2

### Definition

The gene defined in the title of the article as Bcl2 could be found written in different ways, with or without a line or a space between *Bcl* and 2. Just like any other gene, technically it should be *italicized*. In this entry, however, I decided to pay attention to specify whether I was referring to the gene or to the protein.

The Bcl2 family of proteins belong to a peculiar class of proteins regulating ► **apoptosis**, ► **cell cycle**, ► **differentiation**, and ► **autophagy**; in oncology, the genes coding for these proteins could not be defined either as dominant transforming ► **oncogenes** (such as ► *myc*), or as ► **tumor suppressor genes** (such as ► *p53*). They could be best defined as apoptosis-related genes, a definition that stresses the importance of apoptosis (and of its dysregulation) in the genesis and development of cancer in humans and other species. Dysregulation of apoptosis is involved also in the development of diseases other than cancer, such as ► **autoimmune diseases**, AIDS, and various degenerative pathologies. *Bcl2*, a gene coding for inhibitors of apoptosis, is the prototype of this family of genes even though other members of the family show proapoptotic properties (► **BAX**, ► **BAD**, ► **BAK**, and Bok among others). There are about 25 genes in the *Bcl2* family known to date. *Bcl2* derives its name from B-cell leukemia/lymphoma 2, as it was the second member of a range of genes initially described as a ► **reciprocal translocation** involving chromosomes 14 and 18 in ► **follicular lymphoma**. *Bcl2* ► **orthologs** have been identified in numerous mammals for which complete genome data are available.

### Characteristics and Molecular Anatomy

The Bcl2 family encompasses several members divided into antiapoptotic and proapoptotic (genes and proteins); among the antiapoptotic proteins are Bcl2 and Bcl-XL, whereas among the proapoptotic are Bax, Bak [BAK1], Bid, and Bad. Looking at protein structure, it is worth noting that these proteins contain conserved Bcl2 homology (BH) domains (termed BH1, BH2, BH3, and BH4), together with a transmembrane domain, all being identified as crucial for regulation of apoptosis. Thus, deletion of these domains via molecular cloning affects survival/apoptosis rates. In addition, based on functional studies and the conservation of BH domains, the Bcl2 family of proteins can be further divided into three subgroups. The Bcl2 subgroup includes all antiapoptotic proteins, such as Bcl2 and Bcl-XL that conserve all four BH domains. The Bax subgroup consists of proapoptotic members, such as Bax, Bak, and Bad. Both groups contain more than one BH domain. The third subgroup contains BH3-only proteins, such as Bid and Bim, which can interact with either antiapoptotic proteins or proapoptotic members. The observation that inhibitors and inducers of cell death interact with each other by forming homodimers or heterodimers suggests that apoptosis is regulated, at least in part, by protein-protein interaction. By means of two alternative transcripts (a and b), Bcl2 codes for a protein of 205 amino acids (Bcl2b), or 239 amino acids (Bcl2a); both proteins contain BH domains for homo/heterodimerization with members of the Bcl2 family of proteins. The BH4 domain is required for antiapoptotic activity and also for interaction with the serine/threonine kinase encoded by the proto-oncogene Raf-1, a gene coding for a protein homologous to protein kinase C, which is the target of several tumor promoters including phorbol esters. The hydrophobic carboxyl terminus of the protein determines association with cellular membranes; also this tail seems necessary for the antiapoptotic function. In fact, the Bcl2 family of proteins shows a general structure that consists of long hydrophobic helices surrounded by short amphipathic helices. Many members of the family have transmembrane domains. Genes and proteins of the Bcl2 gene family are evolutionarily conserved from the sponges to man.

### Biological Functions: Apoptosis, Cell Survival, Differentiation, and Autophagy

The main biological function of Bcl2 protein is to inhibit apoptosis or, conversely, to promote cell survival. Other related biological functions concern the control of cell cycle. In fact, Bcl2, as well as the antiapoptotic members of this family of proteins, is anti-proliferative by facilitating G0, thus suggesting that cell survival is maintained at the expense of proliferation. In hematopoietic cell lines, these functions are crucial for differentiation, and Bcl2 might also have a direct role in cell fate decision beyond strict cell survival. In addition, Bcl2 family members are involved in the control of autophagy. As far as cell survival is concerned, it appears that the cell fate is dependent on the amount of intracellular Bcl2 protein; overexpression of *Bcl2* is associated with prolonged survival and apoptotic protection, whereas decrease of Bcl2 protein level is associated with apoptosis or enhanced sensitivity to apoptosis-inducing agents. In the development of cancer, *Bcl2* overexpression inhibits the apoptosis of cancer cells bearing mutations, thus being a key determinant of neoplastic cell expansion and resistance to anticancer treatments. As a consequence, cancer cell death is delayed, and cancer cell accumulation occurs. Conversely, HIV-specific CD8+ T cells show a significantly reduced expression of *Bcl2*, potentially priming them to apoptosis. The relationship between HIV and Bcl2 family of proteins, however, is complex and presents wide-ranging implications in cancer. In fact, there exists a HIV accessory protein termed viral protein R (Vpr) that plays a key role in virus replication and also induces cell cycle arrest and apoptosis in various cell types including T cells, neuronal, and tumor cells. Vpr-induced apoptosis is mediated by inhibition of downstream antiapoptotic *Bcl2*. Thus, as odd as it may seem, a protein produced by HIV could be exploited as a beneficial anti-tumor agent in cancers overexpressing *Bcl2*. In recent years, several investigators have studied the potential use of Vpr as an anti-tumor therapeutic. In vitro studies have indicated that Vpr is cytotoxic against a large number of different tumor cell types and it is presumable that those cancers overexpressing *Bcl2* are the most sensitive to the proapoptotic effect of Vpr. The anti-tumor properties of certain HIV proteins might even have been responsible for establishing a symbiotic relationship in

humans, considering that it is estimated that HIV has been in humans for more than 100 years, thus establishing a delicate survival balance. Conversely, in the development of cancer, *Bcl2* overexpression inhibits the apoptosis of cancer cells bearing mutations, thus being a key determinant of neoplastic cell expansion and resistance to anticancer treatments. As a consequence, cancer cell death is delayed, and cancer cell accumulation occurs. At the molecular level, inhibition of apoptosis as well as control of cell cycle, differentiation, and autophagy occur through a complex process of protein–protein interaction. In the inhibition of apoptosis this process involves heterodimerization, especially with the proapoptotic member of the Bcl2 family. In addition to homo/heterodimerization within the Bcl2 family members, the antiapoptotic members of the Bcl2 family also interact with other proteins regulating apoptosis, such as ► Caspases and ► APAF1. Formation of complexes with these proteins involved in the actuation of apoptosis prevents them to initiate the protease cascade, eventually leading to cell death.

The multiple independent functions of Bcl2 proteins are mediated by the BH domains and the hydrophobic helices. These functions can be grouped in two main categories: (1) a function as membrane channels for ions and proteins and (2) a function as membrane adaptor/docking proteins. The first hint about Bcl2 function came from studies on the three-dimensional structure of the Bcl2 analog, the antiapoptotic Bcl-XL. It showed a surprising similarity to the pore-forming domains of some bacterial toxins that cause the formation of channels for ions, proteins, or both. It was observed that Bcl2 protein and its homologues are localized to intracellular membranes, in particular, the outer mitochondrial membrane, the endoplasmic reticulum, and the intracellular membrane of the nuclear envelope. In these areas, they have a membrane transport function for calcium ions and proteins. The channels created by Bcl2 insertion into membranes resemble the pores formed by certain bacterial toxins. Thus, the two long hydrophobic helices of the protein core insert deeply through the phospholipid bilayer, perpendicular to the membrane surface, and the rest of the protein undergoes conformational changes resembling the opening of an umbrella with the five surrounding amphipathic helices resting on the top of the membrane. The ability to form channels, by insertion of the two hydrophobic helices, is essential

for Bcl2 antiapoptotic function. However, by analogy with other channel-forming proteins, the Bcl2 channels are formed by two or more proteins of the Bcl2 family. Thus, there is the possibility that anti- and proapoptotic members of the Bcl2 family form homo- or heterodimers. In fact, the proapoptotic members of the family also have channel-forming activity, although the channels formed by these proteins might have different transport selectivity or subcellular localization. Heterodimerization of anti- and proapoptotic Bcl2 family proteins might lead to the formation of different channels or, alternatively, the heterodimers might be unable to form channels at all. Schematically, the channels formed by Bcl2 and the other antiapoptotic members prevent apoptosis, possibly transporting back, and thus antagonizing, the proapoptotic factors that outflow through the channels formed by the proapoptotic members of the Bcl2 family. For example, Fas-ligand, a well characterized inducer of apoptosis, activates a member of the caspase family (caspase 8) that cleaves proapoptotic Bid. Once truncated, Bid translocates to mitochondria where it might function as a channel protein to release cytochrome *c*, thus activating cytosolic caspases which are the terminal effectors of apoptosis. Bcl2 inhibits the release of cytochrome *c* either by plugging the channels opened by Bid, or by transporting cytochrome *c* back to the mitochondria. Also in this case, the level of gene expression and the ratio between antiapoptotic and proapoptotic Bcl2 family proteins is critical in deciding cell death or survival.

In addition to the channel-forming properties, Bcl2 family proteins interact with a number of signal transducing proteins involved in apoptosis and other crucial cellular processes. These include the protein kinase C homologue Raf-1, the ► G-proteins H-Ras and R-Ras, the p53-binding protein p53-BP2, the proapoptotic protein CED-4, (homologue to APAF1), and the protein phosphatase calcineurin. These interactions are mediated by specific BH domains; for example, the BH4 domain has been reported to bind with calcineurin, Raf-1, and CED-4. The association between Bcl2 and these proteins might be responsible for their translocation to intracellular membranes where Bcl2 is anchored. This may lead to changes of their activity, such that they might be sequestered and inactivated, or targeted for interaction with other membrane-associated proteins. For example, Raf-1 is

a serine/threonine kinase which transduces mitogenic signals from membrane receptors to the nucleus. Association between Raf-1 and Bcl2 causes translocation of the protein kinase to the mitochondrial membrane where Bcl2 is located. Once there, Raf-1 phosphorylates and inactivates Bad, one of the proapoptotic members of the Bcl2 family. Phosphorylated Bad is sequestered in the cytosol, engaged by an adaptor protein termed 14-3-3, and thus unable to induce apoptosis. In the absence of growth/survival factors (such as in IL-3 deprivation of IL-3-dependent hematopoietic cell lines), Raf-1 is not activated and the unphosphorylated Bad is able to induce apoptosis. Protein–protein interaction is also responsible for Bcl2 biological functions other than control of apoptosis. In fact, interaction between the catalytic domain of Raf-1 and the BH4 domain of Bcl2 in multipotent hematopoietic progenitor cells is critical in determining the erythroid/myeloid fate of differentiating cells. Another protein originally isolated as a Bcl2-interacting protein is Beclin 1, the first identified mammalian autophagy gene product. Bcl2 negatively regulates Beclin 1-dependent autophagy and Beclin 1-dependent autophagic cell death, thus raising the possibility that proteins of the Bcl2 family might also regulate autophagy.

### Regulation of Gene Expression

The first association between Bcl2 and human cancer was observed in follicular lymphoma bearing the t(14;18) chromosomal translocation by which the gene was cloned. This translocation brings the Bcl2 gene to chromosomal location 18q21 into juxtaposition with the immunoglobulin heavy-chain locus at 14q32, resulting in transcriptional deregulation of the Bcl2 gene. This event does not involve alterations of the coding regions of the gene. Subsequently, Bcl2 overexpression was recognized as a general feature of various types of hematological and solid malignancies. Thus, many members of the Bcl2 family have been found to be differentially expressed in various malignancies, and some are useful prognostic cancer biomarkers (Biomarkers in Prognosis and Prediction). Whether through its function as a channel protein or as an adaptor/docking protein, the final result on cell fate, however, depends upon the level of expression of Bcl2. Therefore, the control of Bcl2 expression has been the object of numerous studies of transcriptional, translational, and posttranslational regulation.

Overexpression of Bcl2 has been associated with hypomethylation in the promoter region and resulted in increased cell survival. It should be noticed, however, that regulation of Bcl2 gene expression is likely to be more complex than previously imagined and might encompass interaction between different proteins, each regulating Bcl2 expression. HIV-infected monocytes represent a good example of such a complexity. HIV-Tat protein upregulates Bcl2 expression thus increasing survival of infected cells, whereas HIV-Vpr synthetic peptide downregulates Bcl2 expression thus inducing monocyte apoptosis. As mentioned above, however, the net result depends upon the balance between these opposite effects, and it appears that in HIV infection upregulation prevails over downregulation with the final result of increased survival of monocytes/macrophages during HIV infection. This role of Bcl2 in monocytes/macrophages survival is of utmost importance in cancer since macrophages are the targets of Gc-Macrophage Activating Factor (GcMAF), a stimulator of the immune system and an anticancer agent tested with success in advanced cancers [9]. Although the effects of GcMAF on Bcl2 expression have not been studied as yet, it would not be surprising to discover that at least some of the effects of GcMAF are mediated through Bcl2.

### Regulation of Protein Function

In normal cells, once apoptosis is initiated, Bcl2 protein is proteolytically cleaved by caspases. The cleaved protein, lacking the BH4 domain, has proapoptotic activity, and causes the release of cytochrome c into the cytosol thus promoting further caspase activity. Bcl2 family proteins are also regulated by phosphorylation that affects their activity and conformation. The structural analysis of antiapoptotic members of Bcl2 family led to the discovery of an unstructured “loop region” near the N-terminus exposed to the cytoplasm. The antiapoptotic members of Bcl2 family such as Bcl2 and Bcl-XL are phosphorylated on specific serine/threonine residues within this unstructured loop in response to diverse stimuli including treatment with chemotherapeutic or chemopreventive agents. In most instances, such phosphorylation has been associated with the loss of their biological (antiapoptotic) function. The chemoresistant tumors often overexpress Bcl2/Bcl-XL. In these instances, the apoptosis yielding effect due to phosphorylation of antiapoptotic Bcl2 family

members is quite interesting because phosphorylation–dephosphorylation pathway of these antiapoptotic proteins could be an ideal molecular target for therapy of subpopulation of cancer in which these cell death repressors are essential prognostic markers. Thus, further gaining the knowledge on the mechanism of inactivation of Bcl2/Bcl-XL by phosphorylation might be of significant importance to therapy for human malignancies in which overexpression of these antiapoptotic proteins is recognized. It should be noticed, however, that, as odd as it may seem, in some instances Bcl2 can be considered a favorable prognostic marker. In fact, a recent meta-analysis demonstrated the prognostic role of assessing Bcl2 protein by immunohistochemistry in breast cancer [7]. This effect was found independent of lymph node status, tumor size, and tumor grade. According to this meta-analysis, Bcl2 almost paradoxically exerted a tumor suppressor effect, and its expression was associated with favorable prognostic features such as low grade, estrogen receptor positivity, and good outcome. The mechanisms through which Bcl2 might exert such a protective effect in solid epithelial tumors including breast cancer are still unclear and might even open new perspectives on the multifaceted role of Bcl2 in cancer. In fact, it was demonstrated that in vitro Bcl2 interferes with the cell cycle slowing G1 progression and G1-S transition by prolonging G0, thus inhibiting cell proliferation. It is conceivable that in some instances these effects of Bcl2 on the cell cycle might prevail over the antiapoptotic effects with the net, paradoxical, result that Bcl2 could perform a tumor suppressor role in solid epithelial tumors such as breast cancer.

## Bioactivity

Oncogenes and tumor suppressor genes modulate *Bcl2* expression with profound results on death or survival of cancer cells. The tumor suppressor gene [[TP53]] can induce apoptotic cell death by downregulation of *Bcl2* and upregulation of [[*Bax*]]. The p53-dependent negative response element on *Bcl2* has the features of a ► transcription silencer, mediating inhibition of transcription in an orientation-dependent manner. In a variety of tumors, *p53* expression is associated with ► apoptosis and with sensitivity to ► DNA damaging agents (anticancer drugs and ► ionizing radiations), by enhancing the transcription of a gene that favors apoptosis (*Bax*), at the same time blocking the transcription of a gene that would protect cancer cells from

apoptosis (i.e., *Bcl2*). *Bcl2* overexpression is able to hinder p53-induced apoptosis, but it is ineffective against p53-dependent growth arrest. However, when *Bcl2* is expressed together with the ► *MYC* gene, both p53-induced growth arrest and apoptosis are counteracted. In recent years, however, the role of mutations of single genes in the genesis of cancer has been questioned, and it was proposed instead that cancer is a chromosomal disease. According to this hypothesis, carcinogens initiate chromosomal evolutions via unspecific aneuploidies. By unbalancing thousands of genes, ► aneuploidy corrupts teams of proteins that segregate, synthesize, and repair chromosomes. Aneuploidy is thus considered a steady source of karyotypic–phenotypic variations from which selection of further cancer-specific aneuploidies encourages the evolution and subsequent malignant progression of cancer cells. The rates of these variations are proportional to the degrees of aneuploidy, and can exceed conventional mutation by 4–7 orders of magnitude. In this scenario, the role of antiapoptotic genes, such as *Bcl2*, is even more paramount as they provide the opportunity for cancer cells to survive despite gross aneuploidy and to accumulate complex, malignant phenotypes.

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## Bcl-2 Binding Component 3

► [PUMA](#)

## Bcl-2 Family Proteins

### Definition

These molecules can regulate apoptosis positively or negatively at the site of mitochondria. The prototype is ► [BCL-2](#), which is originally isolated at the t(14, 18) breakpoint from the follicular lymphoma. All members share the Bcl-2 homology (BH) domain. Three subgroups have been defined. The anti-death members include Bcl-2, Bcl-x<sub>L</sub>, Mcl-1, and Ced-9. They possess the BH1, BH2, BH3, and BH4 domains. The multi-domain pro-death members include ► [Bax](#), ► [Bak](#), and Bok, which have the BH1, BH2, and BH3 domains. The BH3-only pro-death members consist of Bad, Bik/Nbk/Blk, Bid, Bim, Bmf, Hrk/DP5, EGL-1, Noxa, and ► [PUMA](#), which all contain the BH3 domain only.

- [Apoptosis](#)
- [Bid](#)

## BCL3

Katja Brocke-Heidrich

Institute of Clinical Immunology and Transfusion Medicine, University Hospital of Leipzig, Leipzig, Germany

### Definition

BCL3 stands for B-cell leukemia/► [lymphoma](#) 3. The BCL3 gene is a proto-oncogene mapping to chromosomal band 19q13. It encodes a phosphoprotein of 446 amino acids exhibiting an apparent molecular weight between 47 and 60 kDa. BCL3 is an ► [IkB](#)-like protein that primarily functions as a transcriptional cofactor, especially in cooperation with ► [NF-κB](#) (nuclear factor κB).

## Characteristics

### Structure and Molecular Function

As its main structural feature, BCL3 protein exhibits seven so-called ankyrin repeat elements in its central domain. This structure is characteristic of the IkB family of proteins. Ankyrin repeats are tandemly arranged modules of about 33 amino acids. Through these motifs, IkB proteins interact with, and modulate the activity of NF-κB transcription factors. The NF-κB family consists of five members called RelA, RelB, c-Rel, p50, and p52. These subunits form various homo- and heterodimers that regulate the transcription of target genes by binding to specific (κB) sites present in promoter or enhancer elements. Unlike the other NF-κB subunits, p50 and p52 contain a DNA-binding domain but lack a transactivation domain. Thus, DNA-bound p50 and p52 homodimers inhibit gene transcription.

BCL3 differs from classical IkB family members by acting as a transcriptional cofactor. Hence, in many cells BCL3 is primarily located in the nucleus. Its proline-rich amino terminus and proline-/serine-rich carboxyl terminus appear to function as transactivation domains. BCL3 preferentially binds to NF-κB p50 and p52 homodimers. These complexes can either activate or repress transcription of target genes. Two mechanisms of transcriptional activation by BCL3 have been described. It can either directly activate transcription by providing its transactivation activity to p50 and p52 homodimers, or cause de-repression by removing these inhibitory subunits from κB sites. Alternatively, BCL3 can also enhance binding of p50 and p52 to DNA, thereby inducing transcriptional repression. The circumstances leading to either effect are not well understood. The dual role of BCL3 in transcriptional regulation is reflected by its interaction with the basal transcription machinery and coactivators such as ► [p300/CBP](#), [SRC-1](#), and the ► [histone acetyltransferase](#) Tip60 and with corepressors such as ► [histone deacetylases](#) (HDACs). In addition to its role in NF-κB-dependent transcription, BCL3 has been described to function as a coactivator in complex with transcription factors ► [AP-1](#) and ► [retinoid X receptor](#) by potentiating their activities.

Additionally, apart from its role in transcriptional regulation, BCL3 seems to exert a function in intracellular signaling. This conclusion results from the observation of BCL3 expression in thrombin-activated platelets. These cells are anuclear and incapable of

gene transcription. Here, BCL3 has been found to associate with the ► [Src](#)-related protein kinase Fyn. The molecular relevance of this interaction is not known.

### Regulation

BCL3 protein is modified by phosphorylation and polyubiquitination (► [Ubiquitination](#)). Phosphorylation occurs extensively and constitutively, predominantly at the serine-rich C-terminal domain. BCL3 exhibits several protein forms differing in their phosphorylation state. A major protein kinase shown to act on BCL3 is ► [glycogen synthase kinase-3](#) (GSK3) that constitutively phosphorylates BCL3 at serines 394 and 398. This modification is followed by polyubiquitin linkage on N-terminal lysine residues of BCL3 and its subsequent degradation through the proteasome pathway. Therefore, this mechanism regulates BCL3 turnover. In addition, GSK3-mediated phosphorylation also influences the transcriptional function of BCL3 by modulating its interaction with HDAC transcriptional repressors, and attenuates its oncogenicity. Independent of these findings, the extent of BCL3 phosphorylation has been shown to affect its interaction with both NF-κB p50 and p52. Further information on signaling pathways leading to BCL3 phosphorylation is missing.

In addition, polyubiquitination has also been shown to regulate BCL3 entry into the nucleus. In B and T cells, BCL3 exhibits a predominantly nuclear localization, while in several other cell types (e.g., erythroblasts, hepatocytes, keratinocytes) BCL3 resides in the cytoplasm and needs activation prior to nuclear translocation. Current data reveal that BCL3 requires a lysine 63-linked polyubiquitin chain in order to enter the nucleus and regulate gene transcription. This polyubiquitin modification acts as a “molecular ticket,” probably by facilitating the interaction with nuclear transport receptors (called importins) and mediating transport through the nuclear core complex. Nuclear translocation of BCL3 is prevented by the de-ubiquitinating enzyme ► [CYLD](#), which was identified as a tumor suppressor. Loss of CYLD results in de-ubiquitylation of BCL3, which in turn facilitates nuclear accumulation of BCL3 and transcription of target genes which are able to promote cellular transformation.

### Expression

The BCL3 gene is composed of nine exons, spanning 11.5 kb. Its transcript shows a broad expression pattern

in multiple cell types. It is highly expressed in spleen and liver, with no apparent expression in brain. Transcription of BCL3 is regulated through several signaling pathways. In addition to regulatory elements in the promoter, two enhancer regions have been identified within the second intron.

BCL3 itself is an NF-κB target gene whose expression is initiated by a number of classic NF-κB-inducing stimuli (e.g., TNF- $\alpha$ , interleukin-1) but also upon activation of the T-cell receptor. The corresponding κB sites have been found in the promoter and first intronic enhancer. BCL3 transcription is further induced by the Jak/Stat pathway (► [Signal transducers and activators of transcription in oncogenesis](#)). Stat3-activating cytokines (e.g., ► [interleukin-6, -9 and -10](#)) initiate BCL3 transcription primarily via Stat binding sites in the second enhancer. In mice, an AP1-dependent mechanism of BCL3 gene expression was found in T cells upon ► [interleukin-4](#) stimulation. Moreover, BCL3 autoregulates its own transcription in a repressive manner. The negative feedback is mediated via the κB motifs.

In platelets, which lack nuclei and cannot synthesize mRNA, BCL3 expression is regulated on the translational level. In resting platelets, a preformed BCL3 mRNA pool exists whose translation is constitutively repressed. Upon activation, an ► [mTOR](#)-dependent rapid increase of BCL3 protein synthesis takes place. This specialized translational control pathway is mediated by a cascade also involving PI3K (► [PI3K signaling](#)) and PDK1 protein kinases and culminates in phosphorylation of the translation repressor 4EBP-1, causing its dissociation from eukaryotic translation initiation factor 4E and allowing translation to proceed.

### Physiological Function

Knockout mouse studies provide some information on the physiological function of BCL3. Although BCL3 is widely expressed, it seems to play its primary role in the immune system. BCL3 knockout mice appear developmentally normal, but are susceptible to certain kinds of pathogens. They are severely impaired in producing antigen-specific T and B cell responses. The altered microarchitecture in the spleen and lymph nodes, including the lack of ► [germinal center](#) formation, is thought to underlie the immunological defects.

In accordance with its observed role in immune responses, BCL3 functions have been found in immunologically relevant cells. BCL3 is selectively

up-regulated in mature dendritic cells, and its absence results in failure of normal follicular dendritic cell differentiation. This finding might be the main reason for the observed defects in the microarchitecture of secondary ► lymphoid organs and T-cell responses in BCL3-deficient mice. BCL3 was further shown to be required for the survival of activated T cells as well as for the attenuation of the pro-inflammatory (► inflammation) action of activated macrophages.

Moreover, BCL3 has been found to be transiently up-regulated by DNA damage and to suppress p53 activation (see below). The data suggest a physiological role of BCL3 in B cell development. According to this hypothesis, BCL3 expression allows germinal center B cells to tolerate the DNA damage required for immunoglobulin ► class switch recombination and ► somatic hypermutation without mounting an apoptotic (► apoptosis) response.

BCL3 expression is highly up-regulated in thrombin-activated platelets. In these activated platelets, BCL3 is required for retraction of fibrin clots, which is an important step in wound healing.

### Oncological Relevance

The BCL3 gene was initially identified through its involvement in a t(14;19)(q32;q13) chromosomal translocation found in some patients with chronic lymphocytic leukemia (B-CLL) or other B-cell neoplasms. This translocation leads to juxtaposition of the BCL3 locus at chromosome 19q13 to the enhancer of the immunoglobulin heavy chain gene on chromosome 14q32, resulting in high-level expression of the BCL3 transcript. Recent studies have shown that elevated BCL3 expression is not limited to the rare cases of CLL or lymphomas with this translocation. High BCL3 expression has also been reported in subsets of ► diffuse large B-cell lymphomas, T-cell lymphomas (especially ► anaplastic large cell lymphoma), and ► Hodgkin disease. Furthermore, increased nuclear levels of BCL3 have been demonstrated in a growing number of non-lymphoid tumors such as breast cancer and nasopharyngeal carcinomas. Oncogenically activating mutations within the coding region of BCL3 have not been found so far. Consequently, elevated expression of BCL3 is hypothesized to contribute to oncogenesis by dysregulating target genes involved in cell proliferation, apoptosis, and differentiation.

Consistent with a direct oncogenic function, BCL3 overexpression has been shown to lead to

transformation of murine fibroblasts and induction of tumor growth in vivo. In contrast, transgenic mice expressing BCL3 in both B and T cells develop a lymphoproliferative disorder but no lymphoid neoplasms, indicating that BCL3 overexpression alone is not sufficient for the direct transformation of lymphoid cells.

A few target genes potentially involved in the oncogenic potential of BCL3 have been identified so far. Transcription of the cyclin D1 (► cyclin D) gene, whose product acts as a key factor in driving cell cycle progression, is activated by BCL3 through its cooperation with p52 homodimers bound to an NF- $\kappa$  B motif in the cyclin D1 promoter. Concerted elevation of BCL3, p52 and cyclin D1 levels have been found in breast cancer cells. In these cells, in vitro studies also suggested a BCL3-mediated activation of the anti-apoptotic ► BCL-2 gene.

BCL3 can suppress the activation of tumor suppressor protein p53 (► p53 protein, Biological and Clinical Aspects), which is a crucial guardian of genomic integrity. Normally, p53 is kept at low levels mainly by its interaction with the Mdm2 protein, which mediates the proteasomal degradation of p53. When cells are exposed to genotoxic stress, this interaction is disrupted, and p53 accumulation results in either cell-cycle arrest or apoptosis. One proposed mechanism in this regulatory circuit is the ability of BCL3 to induce the expression of the p53 inhibitor Mdm2 via its recruitment to  $\kappa$ B sites in the promoter occupied by p50 or p52. A more complete understanding of the role of BCL3 in human cancers is still lacking.

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### BCL-6

#### Definition

B-cell CLL/lymphoma 6, also known as BCL-6, BCL5, LAZ3, and zinc finger protein 51 (ZNF51) is

a zinc finger protein of 706 amino acids and 78 kD. The human BCL-6 locus maps to 3q27 and the mouse BCL-6 gene locus to chromosome 16 (13.90 cM). BCL-6 is a transcriptional regulator that probably plays an important role in lymphomagenesis. It is involved in a form of B-cell non-Hodgkin lymphoma characterized by chromosomal translocation t(3;14)(q27;q32) and t(3;22)(q27;q11) that involves BCL-6 and immunoglobulin gene regions, and also in a t(3;4)(q27;p11) chromosomal translocation with Arhh (Ttf).

#### ► BCL-6 Translocations in B-Cell Tumor

## BCL6 Translocations in B-Cell Tumors

Hitoshi Ohno

Department of Internal Medicine, Faculty of Medicine,  
Kyoto University, Kyoto, Japan

### Synonyms

BCL6 translocations in [non-Hodgkin's lymphoma](#) of B-cell type (B-NHL) are chromosomal translocations involving the 3q27 chromosome band

### Definition

B-NHLs are often associated with chromosomal translocations that lead to the juxtaposition of cellular oncogenes with the [► immunoglobulin gene](#) (IG) loci. The 3q27 translocation is unique, fusing the BCL6 gene on 3q27 to either one of the three IGs but also another non-IG partner. Cytogenetic and molecular analyses have demonstrated that alteration of 3q27 and/or BCL6 is one of the most common genetic abnormalities in B-NHLs.

### Characteristics

#### The BCL6 Gene and Gene Product

The BCL6 gene spans 24-kb and contains 10 exons. The ATG signal for the initiation of protein synthesis is within exon 3 and is followed by an open reading frame ([Fig. 1](#)). The Bcl-6 protein, consisting of

706 amino acids with a calculated molecular weight of 79 kD, is a sequence-specific transcription factor that can repress transcription from promoters containing its DNA-binding site [\[1\]](#). The C-terminal region comprises six Cys2-His2 ► [zinc finger](#) motifs, each separated by a conserved stretch of seven amino acids. Hence, the Bcl-6 protein was classified as belonging to the Krüppel-like subfamily of ► [zinc finger proteins](#) ([Fig. 1](#)).

The BTB/POZ domain at the N-terminus is a conserved 120-amino acid motif, which is found in 5–10% of ► [zinc finger proteins](#) ([Fig. 1](#)). The primary function of the BTB/POZ domain appears to be the mediation of protein–protein interactions. The repressive effect of Bcl-6 on the target gene is exerted via the recruitment of SMRT, NCoR, and BCoR corepressors [\[2, 3\]](#). Crystallographic analysis of the BTB/POZ domain revealed that it forms a butterfly-shaped homodimer to generate a “lateral groove” motif that interfaces with a 17-residue sequence (BBB motif) of SMRT [\[2, 3\]](#).

The central portion of Bcl-6 contains a second domain required for the repressive transcriptional activity. The KKYK motif within the PEST sequence is targeted by p300-mediated acetylation, and this posttranslational modification disrupts the ability of Bcl-6 to recruit histone deacetylase (HDAC), thereby hindering its capacity to repress transcription. Interaction with MTA3 corepressor is sensitive to Bcl-6 acetylation status.

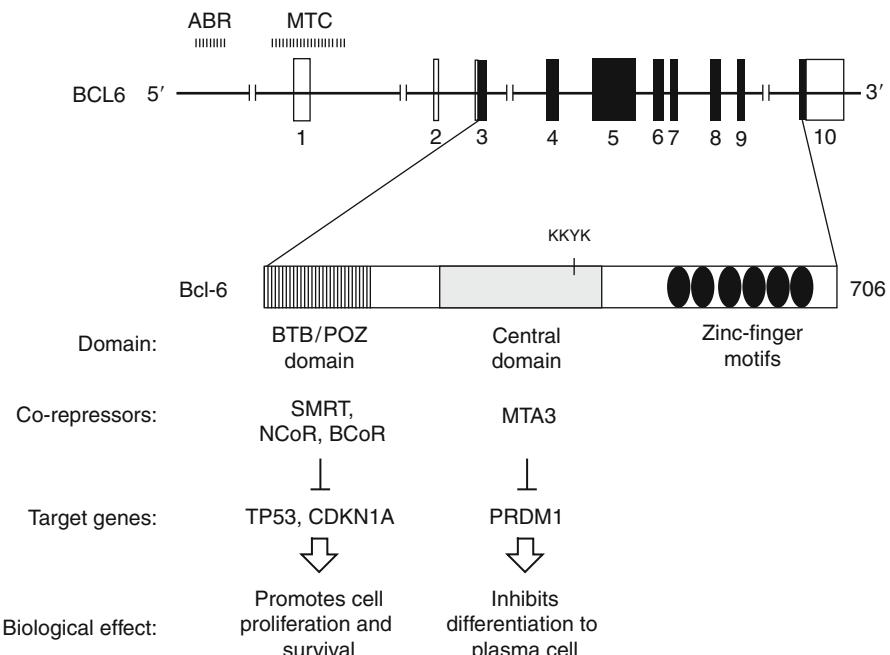
Within the B-cell lineage, BCL6 is expressed exclusively in ► [germinal center](#) (GC) B-cells. Targeted inactivation of BCL6 in the mouse germline prevents GC formation in the lymphoid tissues and alters Th2-mediated immune responses. A prominent target gene of Bcl-6 is PRDM1 (Blimp-1), which plays a key role in the differentiation of B-cells into plasma cells by turning off the entire mature B-cell gene expression program [\[4\]](#). On the other hand, repression of other Bcl-6 target genes, including TP53 and CDKN1A, promotes cell proliferation and survival ([Fig. 1](#)). It is therefore presumed that BCL6 is the master gene for the generation by B-cells of a GC.

#### BCL6 Translocation Affecting the IG and Non-IG Loci

Chromosomal translocation involving the 3q27 chromosomal band occurs within the Major Translocation Cluster (MTC) of BCL6, which spans the promoter,

### BCL6 Translocations in B-Cell Tumors.

**Fig. 1** Schematic presentation of the BCL6 gene and its protein product. Repressor function of Bcl-6 is segregated into two domains [13]. The POZ/BTB domain recruits SMART, NCoR and BCoR corepressors, and target genes involved in B-cell proliferation and survival, whereas the central domain recruits another set of corepressors (MTA3) and controls genes in B-cell differentiation. ABR, alternative breakpoint region; MTC, major breakpoint cluster; KKYK, where K = lysine and Y = tyrosine



the non-coding exon 1 and the 5' region of intron 1 (Fig. 1) [5]. In the majority of cases, breakpoints are localized immediately 3' of exon 1. The translocation, therefore, does not interrupt the protein-coding region of BCL6. The most common type of BCL6 translocation is t(3;14)(q27;q32), involving the IGH heavy chain gene (IGH) on 14q32 as the partner. On the der(3)(3;14)(q27;q32), the IGH upstream sequences are juxtaposed to the BCL6 in the same transcriptional orientation, whereas the 5'-BCL6 sequences are fused to downstream sequences of IGH on the reciprocal der(14)t(3;14)(q27;q32). As the result of t(3;14)(q27;q32), BCL6 expression is initiated from the IGH germline transcript promoters, which are followed by the BCL6 coding sequences. Two "variant" translocations, t(3;22)(q27;q11) involving the  $\lambda$ -light chain gene (IGL $\lambda$ ) on 22q11 and t(2;3)(p12;q27) involving the  $\kappa$ -light chain gene (IGL $\kappa$ ) on 2p12, lead to juxtaposition of the 3' sequences of IGL $\lambda$  or IGL $\kappa$  to BCL6 in divergent orientation [6].

Non-IG partner genes and their chromosomal sites are listed in Table 1. The partners are not random but instead have been recurrently identified. These include the genes for a transcription factor, serine/threonine-protein kinase, cytokine receptor, Ras small GTPase, heat shock proteins, and so on. In spite of this marked diversity of protein products, there are common

features in the molecular anatomy of non-IG/BCL6 translocations. First, the gene fusion occurs in the same transcriptional orientation; second, the breakpoint on the partner gene is located in close proximity to the promoter sequence; and third, the complete sequence of the promoter is fused upstream of the coding region of BCL6 on the der(3) chromosome. As the result of non-IG/BCL6 translocation, many types of regulatory sequences of each partner gene substitute for the 5' untranslated region of BCL6, and the rearranged BCL6 comes under the control of the replaced promoter activity (promoter substitution) (Fig. 2) [6].

### The 5' Non-coding Region of BCL6 Undergoes Somatic Hypermutation

Somatic mutations within the 5' non-coding region of BCL6 have been described in a significant proportion of ► GC/post-GC type B-cell tumors [7]. The majority of the mutations cluster around the 3' of exon 1, which has been referred to as the Major Mutation Cluster (MMC), apparently overlapping the MTC. These mutations are often multiple, are frequently biallelic, and are independent of BCL6 translocation or linkage to IGs. Somatic mutations within the MMC were also observed in a large proportion of memory B-cells isolated from normal individuals as well as GC B-cells from a reactive tonsil. The presence of cis-acting

**BCL6 Translocations in B-Cell Tumors. Table 1** Non-IG partner genes of *BCL6* translocation

Gene symbol (Alias)	Gene product	Chromosomal locus
<i>MBNL1</i> ( <i>KIAA0428</i> )	Muscleblind-like protein (Triplet-expansion RNA-binding protein)	3q25/3q25.1
<i>TFRC</i>	Transferrin receptor (p90, CD71)	q26.2-qter/3q29
<i>ST6GAL1</i> ( <i>CD75</i> )	Sialyltransferase 1 (beta-galactoside alpha-2,6-sialyltransferase)	3q27-q28/3q27.3
<i>EIF4A2</i>	Eukaryotic translation initiation factor 4A, isoform 2	3q28/3q27.3
<i>RHOH</i> ( <i>RhoH, TTF</i> )	Rho-related GTP-binding protein RhoH (GTP-binding protein TTF)	4p13/4p14
<i>H4</i>	H4 histone	6p21.3
<i>HSPCB</i> ( <i>HSP90<math>\beta</math></i> )	Heat shock 90 kDa protein 1, beta	6p12/6p21.1
<i>PIM1</i>	Pim-1 oncogene product	6p21.2
<i>SFRS3</i> ( <i>SRp20</i> )	Splicing factor, arginine/serine-rich 3 (Pre-mRNA splicing factor SRP20)	6p21/6p21.31
<i>HIST1H4I</i> ( <i>H4/m</i> )	H4 histone family, member M	6p21.33
<i>U50HG</i>	Small nucleolar RNA	6q15
<i>ZNFN1AI</i> ( <i>IKAROS</i> )	Ikaros (zinc finger protein)	7p13-p11.1
<i>GRHPR</i> ( <i>GLXR</i> )	Glyoxylate reductase/hydroxypyruvate reductase	9q12/9p13.2
<i>POU2AF1</i> ( <i>BOB1, OBF-1</i> )	POU domain class 2, associating factor 1 (B-cell-specific coactivator OBF-1) (OCT binding factor 1) (BOB-1) (OCA-B)	11q23.1
<i>LRMP</i> ( <i>JAW1</i> )	Lymphoid-restricted membrane protein	12p12.1
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	12p13.31
<i>NACA</i>	Nascent-polypeptide-associated complex alpha polypeptide	12q23-q24.1/12q13.3
<i>LCP1</i>	L-plastin (Lymphocyte cytosolic protein 1) (LCP-1) (LC64P)	13q14.3/ 13q14.13
<i>HSPCA</i> ( <i>HSP90<math>\alpha</math></i> )	Heat shock 90 kDa protein 1, alpha	14q32.33/14q32.31
<i>IL21R</i>	Interleukin-21 receptor	16p11/16p12.1
<i>CIITA</i>	MHC class II transactivator	16p13/16p13.13

elements in BCL6, which are shared with IG and essential for targeting the mutation, has been suggested. On the other hand, PIM1 and RHOH (► **Rho family proteins**), both of which are non-IG partners (Table 1), are mutated in B-cell tumors and the regions involved in the mutation match those in the translocation [8]. These observations suggest that somatic mutations and translocations involving BCL6 are mediated by common molecular mechanisms.

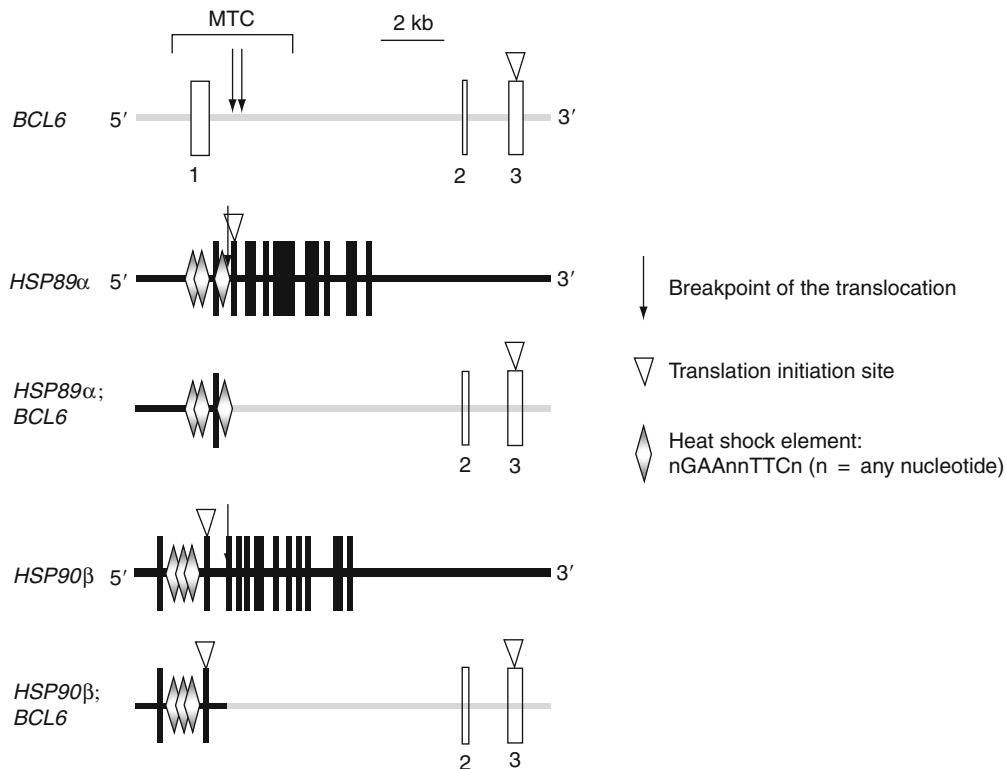
### Mouse Model of BCL6 Translocation to Develop Lymphoma

To investigate the role of BCL6 translocation in the development of B-NHL, mouse models that carried a recombinant gene mimicking t(3;14)(q27;q32) translocation were established [9]. As expected, BCL6 was constitutively expressed in mature B-cell and GC formation markedly increased in response to antigen stimulation. After 13 months of age, the mice developed lymphoma showing the features of human B-NHL [9]. This experiment provided the evidence that BCL6 can act as an oncogene.

### Clinical Relevance

BCL6 translocations are detected by conventional cytogenetic analysis and Southern blotting with an MTC probe. More conveniently, fluorescence in situ hybridization (FISH) using a dual-color, break-apart probe for the MTC is applied to metaphase/interphase nuclei. BCL6 translocations involving IG and non-IG partners occur in about equal frequency [10].

Although an initial study indicated a specific correlation of BCL6 translocation with diffuse large B-cell lymphoma (► **DLBCL**), later studies of panels of many B-NHL types invariably showed that a significant number of cases with ► **follicular lymphoma** (FL) carried such translocations. The range of BCL6 translocations in B-NHL subtypes are 5–15% in FL, 20–40% in DLBCL and its variants, and 20% in ► **acquired immunodeficiency syndrome** (AIDS)-associated DLBCL. BCL6 translocation can occur within the alternative breakpoint region (ABR) that is located 245–285-kb 5' to BCL6 (Fig. 1). Translocation at the ABR is reported to be frequently associated with grade 3B FL.



**BCL6 Translocations in B-Cell Tumors.** **Fig. 2** Non-IG/BCL6 translocations involving HSP89 $\alpha$  heat shock protein gene, and HSP90 $\beta$  gene. Open (*BCL6*) and closed (partner genes) boxes indicate the exons. The breakpoints on the *BCL6* gene were within the MTC region, while those on the HSP genes

were either 5' or 3' of the translation initiation sites. Transcriptional control of HSP genes is mediated by three tandem copies of heat shock element (HSE). As the result of translocation, the complete set of the HSEs is fused upstream of *BCL6*

*BCL6* translocations sometimes coexist with other IG translocations associated with B-cell tumors, i.e., t(8;14)(q24;q32) and t(14;18)(q32;q21) and their variants. In some cases, alteration of the *BCL6* locus was not a primary genetic abnormality but may have occurred at the time of transformation from low- to high-grade disease [11]. A cDNA microarray analysis revealed that DLBCL patients with the GC B-cell-like (GCB) pattern of gene expression have a significantly better survival than those with the activated B-cell-like expression profile. *BCL6* is a representative gene of the GCB-type signature, and high-level expression of *BCL6* at both the mRNA and protein levels has been shown to be a predictor of a favorable treatment outcome in cases of DLBCL. In contrast, *BCL6* translocation are observed with a higher frequency in non-GCB DLBCL subtype and studies on the influence of *BCL6* translocation on treatment outcome yielded conflicting results. One study showed that *BCL6*

translocation was significantly associated with an unfavorable impact on survival of DLBCL patients who were treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone [12]. In another series, however, *BCL6* translocation showed no association with overall survival in DLBCL as a single entity or in subtype analysis [10].

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that protects cells exposed to diverse cytotoxic conditions by promoting cell survival. It is a member of an evolutionarily conserved “stress” pathway, which is triggered by developmental cues and diverse intracellular stresses that activate caspase-9 on a scaffold formed by Apaf-1 in response to cytochrome c released from damaged mitochondria. This pathway, also termed “mitochondrial” or “intrinsic,” is primarily regulated by the Bcl-2 family.

► [NUP98-HOXA9 Fusion](#)

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## BCNS

### Synonyms

[Gorlin syndrome](#); [Nevoid basal cell carcinoma syndrome \(NBCCS\)](#)

### Definition

- [Basal Cell Nevus Syndrome](#)  
► [Bcl-X<sub>L</sub>](#)

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## BCR-ABL1

Christine M. Morris and Suzanne M. Benjes  
Cancer Genetics Research Group, University of Otago at Christchurch, Christchurch, New Zealand

### Definition

*BCR-ABL1* is a hybrid (fusion or chimeric) gene that arises when genomic ► [DNA](#) of the *BCR* gene on chromosome 22 and of the *ABL1* gene on chromosome 9 breaks and recombines. The *BCR-ABL1* hybrid gene is transcribed to produce a hybrid mRNA that is subsequently translated into a functional BCR-ABL1 protein. The *BCR-ABL1* mutation causes and is diagnostic of human ► [Chronic Myeloid Leukemia](#) (CML) and some acute leukemias including particularly ► [acute lymphoblastic leukemia](#) (ALL).

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## BCL-x

### Definition

Bcl-x belongs to the ► [BCL-2](#) family of proteins and is associated with cell survival.

- [Signal Transducers and Activators of Transcription in Oncogenesis](#)

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## Bcl-X<sub>L</sub>

### Definition

Is a member of the pro-survival subfamily (► [Bcl-2](#), Bcl-xL, Bcl-w, Mcl-1, A1, and also Bcl-B in humans)

## Characteristics

### A Somatic Mutation of Bone Marrow Progenitor Cells

The *BCR-ABL1* mutation is somatically acquired. ► **Recombination** between the *BCR* and *ABL1* genes occurs in an early progenitor or stem cell of the bone marrow, and usually results in the microscopically visible ► **Chromosome Translocation** t(9;22)(q34; q11) (Fig. 1).

One product of this translocation is the well known Philadelphia (Ph) chromosome (Fig. 2) a shortened chromosome 22 identifiable in leukemic metaphase cells of ~90% of patients with CML. The Ph and associated *BCR-ABL1* hybrid gene are also found recurrently in ALL, although with higher frequency in adult (15–20%) compared with childhood ALL (5%). The discovery of the Ph chromosome in 1960 was a milestone for cancer research, providing the first clear indication that specific cancer types were characterized by recurrent genetic changes with potential proliferative advantage.

### Molecular Features of BCR-ABL1 Recombination

Recombination between the *BCR* and *ABL1* genes usually generates two products: a 5'BL1-3'BCR hybrid gene on the derivative 9q + chromosome that is in some cases transcribed but not apparently translated, and a 5'BCR-3'ABL1 product on the derivative 22q- or Ph chromosome that is both transcribed and translated. The leukemia-causing properties of the 5'BCR-3'ABL1 protein have been proven in a variety of animal models, and it is to this product that the BCR-ABL1 acronym usually refers.

Both *BCR* and *ABL1* are large genes, at ~138 and 174 kb, respectively (Fig. 3). Several viable in-frame *BCR-ABL1* fusions have been reported or predicted. However, depending on the location of the breakpoint site within *BCR*, those associated with leukemia generally differ according to the number of *BCR* exons that link with the constant *ABL1* exons 2–11 (Fig. 4).

- P210 BCR-ABL1: Breaks occur within the 5-kb major breakpoint cluster region (M-Bcr) of *BCR* in most cases of CML, and in about 50% of BCR-ABL1 rearrangement-positive cases of ► **acute lymphoblastic leukemia** (ALL). In these cases, the *BCR-ABL1* fusion gene is transcribed as a large chimeric mRNA that is spliced into an 8-kb

mRNA with *BCR* exon 13:ABL1 exon 2 (e13a2) and/or *BCR* exon 14:ABL1 exon 2 (e14a2) junctions. This hybrid mRNA is in turn translated to form a 210-kD BCR-ABL1 fusion protein.

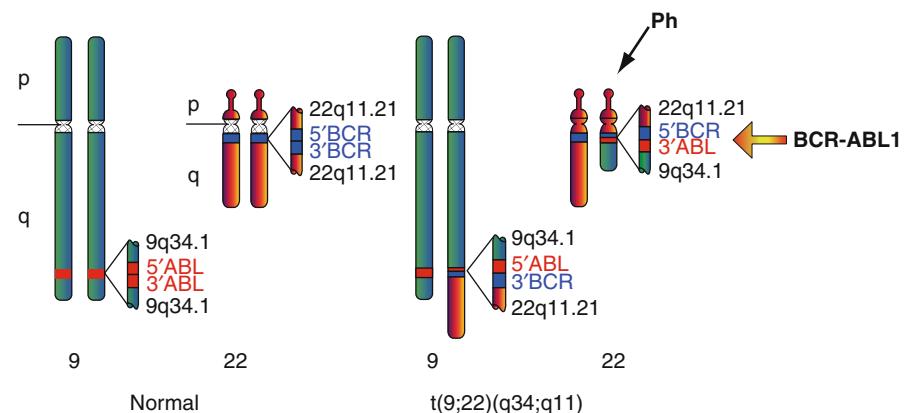
- P230 BCR-ABL1: A larger 230-kD protein identifies a subgroup of patients with neutrophilic CML (CML-N) that present with a lower white cell count than usual and for whom progression to blast crisis is slow. In these cases, a breakpoint occurs in a region more 3' in *BCR* (m-Bcr) to form a *BCR* exon 19:ABL1 exon 2 (e19a2) mRNA transcript in which almost the entire *BCR* gene is joined with *ABL1*.
- P190 BCR-ABL1: For the remaining 50% of BCR-ABL1 positive ALL cases, breakpoints usually occur at different sites across a wider ~35-kb region designated m-Bcr (minor breakpoint cluster region), which maps ~46-kb upstream of M-Bcr. A *BCR* exon 1:ABL1 exon 2 (e1a2) transcript is expressed in these cases, which is translated into a smaller 185-kD BCR-ABL1 protein. The e1a2 transcript is occasionally found in CML patients when it may be associated with a more aggressive clinical course.
- For all BCR-ABL1 leukemias, although sites of preferential breakage in the *ABL1* gene have been identified, breakpoint locations overall are more variable than those in *BCR*, and may occur at different sites within a >200-kb region extending from a point 9-kb 5' of the entire gene to exon 2.

### Complex BCR-ABL1 Rearrangements

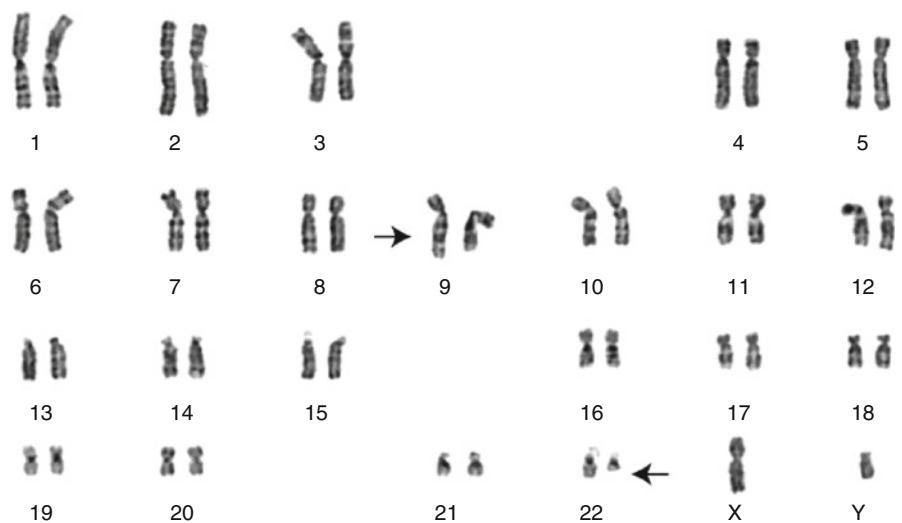
About 10% of CML cases show more complex *BCR-ABL1* rearrangements that involve other chromosomal sites, and which may be camouflaged by a normal karyotype. In all of these cases, the 5' part of *BCR* is fused with the 3' part of *ABL1* to form the characteristic *BCR-ABL1* fusion gene essential for the development of CML. However, the 3' part of *BCR*, which unites with the 5' *ABL1* remnant in the standard t(9;22)(q34;q11), usually recombines with one of the additional chromosomes in the complex translocations or with a part of chromosome 9 outside of the *ABL1* gene. Although patients present with clinical features typical of BCR-ABL1 leukemia, the biological and pathological consequences of complex recombination variants to treatment response and disease course is still unresolved.

**BCR-ABL1.**

**Fig. 1** Ideogrammatic representation of chromosomes 9 and 22 before (left) and after (right) recombination between the *BCR* and *ABL1* genes to form the leukemia-initiating hybrid *BCR-ABL1* gene



**BCR-ABL1. Fig. 2** Karyotype of a leukemic metaphase cell showing the standard Ph translocation, 46,XY,t(9;22) (q34;q11)

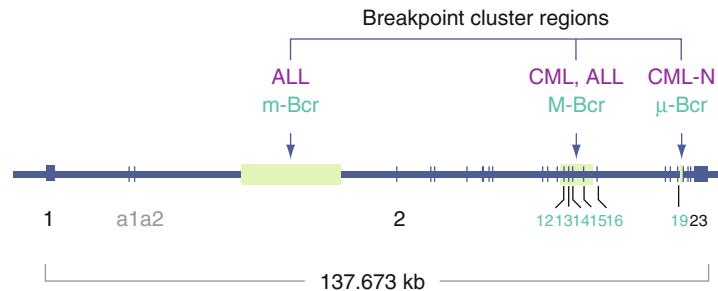
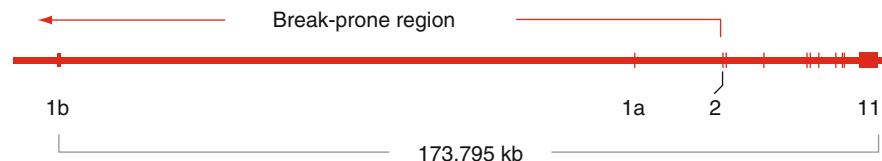
**Translocation-Associated Genomic Deletions**

Another level of complexity in *BCR-ABL1* rearrangement is found in the form of translocation-associated deletions. These genomic deletions, either proximal to the 5' *ABL1* breakpoint or distal to the 3' *BCR* breakpoint are associated with the derivative 9q + of the standard t(9;22) or with sites of recombination on additional partner chromosomes in complex variant *BCR-ABL1* rearrangements. The deletions, which were initially identified fortuitously after development of new fluorescent in situ hybridization (► FISH) probe systems for detecting ► minimal residual disease in interphase cells of CML patients, are found in ~10–15% of all CML patients, with an increased frequency reportedly associated with complex *BCR-ABL1* rearrangements. The deletions can be large, with variable proximal and distal breakpoints

located up to 8 Mb from *ABL1* and 4 Mb from *BCR* on the derivative 9q + derivative additional partner chromosome. They can occur simultaneously during the *BCR-ABL1* recombination translocation-forming process or occasionally as a subsequent step after the initial translocation. Patients having translocation-associated deletions tend to have a considerably worse prognosis and survival than patients without deletions. The biological basis for the survival disadvantage associated with positive deletion status is presently not known, but may possibly be due to loss of tumor suppressor genes within the deleted region.

**Detection of BCR-ABL1**

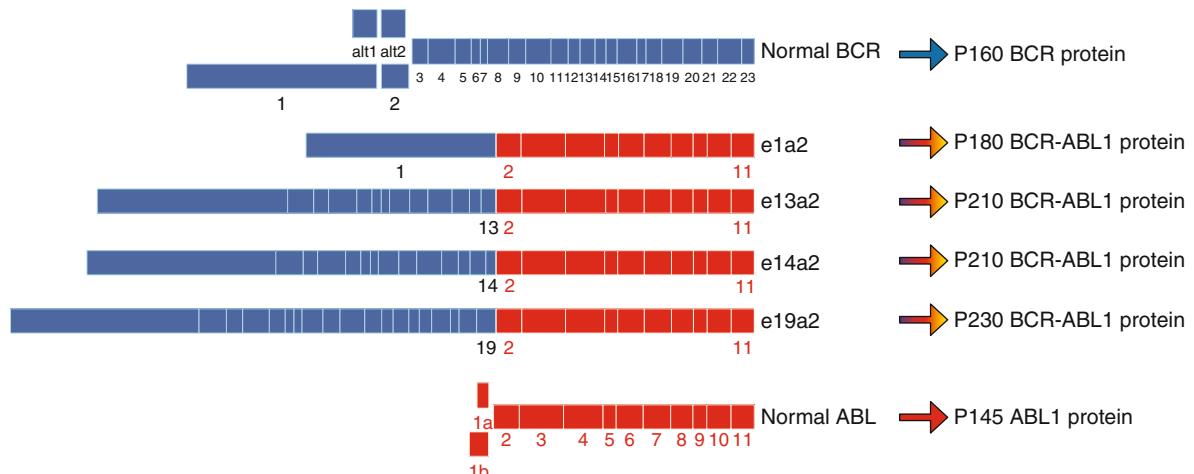
The *BCR-ABL1* fusion gene may be detected in leukemic cells by one or more of the following molecular procedures:

**a** Genomic structure of BCR**b** Genomic structure of ABL1

**BCR-ABL1. Fig. 3** Genomic structure and features of the human *BCR* and *ABL1* genes. (a) Exons 1–23 of *BCR* and alternatives (a) are indicated as blue boxes; The minor breakpoint cluster region (m-Bcr), major ▶ **breakpoint cluster region** (M-Bcr) and micro-breakpoint cluster region ( $\mu$ -Bcr) are shaded in green. Disease

subtypes associated with the different regions are shown as ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; and CML-N, neutrophilic chronic myeloid leukemia; (b) Genomic structure of the human *ABL1* gene. Exons 1–11 and alternatives (a) are indicated as red boxed regions

## BCR-ABL1 Transcripts



**BCR-ABL1. Fig. 4** Normal BCR and *ABL1* transcripts and the most frequently detected variant BCR-ABL1 fusion transcripts. Corresponding protein products are shown to the right.

Alternative (alt) exons are marked above the normal transcript for BCR and as 1a or 1b for *ABL1*

- Amplification of predicted e1a2, e13a2, e14a2, e19a2, or variant splice junctions using polymerase chain reaction (▶ PCR) after reverse transcription of leukemic cell mRNA (RT-PCR)
- FISH on single cells (metaphase or interphase) using a combination of large insert *BCR* and *ABL1* probes
- Southern blotting using leukemic DNA digested with appropriate restriction enzymes and one or

- more probes from within M-Bcr or other relevant regions of *BCR*
- By immunoprecipitation and Western blot analysis of the chimeric protein

### What Causes BCR-ABL1?

The mechanism that underlies *BCR-ABL1* gene rearrangement in most leukemias is unknown, but new and relevant clues are gradually emerging. For example, there is a clear association, both epidemiologically and in the laboratory, between exposure to ionizing radiation and the development of BCR-ABL1 leukemia. This increased risk is reflected in the increased incidence of CML in atomic bomb survivors compared to the general population, and in the increased occurrence of *BCR-ABL1* mutations in cultured cells subjected to high-dose gamma-irradiation and X-irradiation. Recent findings suggest that ionizing radiation can influence the generation of leukemia-specific fusion genes by juxtaposing genes normally distanced in the interphase cell nucleus, and that certain cell types with a lineage-specific 3-D chromatin distribution may be more or less susceptible to a particular fusion gene rearrangement than others.

### BCR-ABL1 in Healthy Individuals

*BCR-ABL1* transcripts have been identified, using RT-PCR, at very low levels in circulating peripheral blood granulocytes of more than two thirds of healthy adults. The identification of other leukemia-associated fusion transcripts in different studies provides good evidence that aberrant recombination occurs ubiquitously at a baseline level in somatic cells of normal individuals. These findings also suggest that additional selective processes, such as immunological tolerance or cell type origin and stage of differentiation, are required to provide *BCR-ABL1* cells with a proliferative advantage and produce the leukemic phenotype.

### Why Breakpoint Cluster Regions?

The molecular factors that determine preferential breakage sites in *BCR*, and precipitate *BCR-ABL1* recombination are presently unknown, but the ► **Alu element** is a strong candidate to facilitate this process. Sequence analysis of M-Bcr has identified a single Alu element central within an ~3-kb region where more than 70% of the breakpoints occur. In addition,

analysis of reciprocal *BCR-ABL1* and *ABL1-BCR* breakpoint junctions from several cases of CML and ALL has identified sequence homology to Alu elements at, or close to, the sites of recombination. M-Bcr also recombines preferentially with Alu elements at chromosomal sites outside of *ABL1* in complex *BCR-ABL1* rearrangements, and in these cases an association with gene coding domains and ► **translin**-specific binding motifs was also suggested. Further research is needed to clarify the significance of these findings.

### Molecular Consequences of BCR-ABL1

The leukemia-causing properties of the BCR-ABL1 protein have been demonstrated in a range of in vivo and in vitro laboratory models, including mice made transgenic for different forms of the hybrid oncogene or transplanted with BCR-ABL1 transfected stem cells. BCR-ABL1 cells are more proliferatively active, differentiate abnormally, show an increased resistance to ► **apoptosis** and have altered adhesion properties compared with their normal counterparts. Much recent work has sought to understand the mechanisms that precipitate these features and precisely how the BCR-ABL1 mutation activates cell transformation in vivo.

Normal BCR proteins are found in the cytoplasm and have at least two enzymatic activities, serine/threonine kinase at the N-terminal, and GAP activity at the C-terminal. The normal ABL1 protein is a nonreceptor protein tyrosine kinase that is localized to the cytoplasm, where it is weakly associated with actin filaments, and the nucleus, where it is associated with chromatin. In BCR-ABL1 hybrid proteins, the fused BCR sequences block nuclear translocation and activate the actin binding function that is required for BCR-ABL1 to efficiently transform cells. Because of its heightened ► **tyrosine kinase** activity, the *BCR-ABL1* protein can phosphorylate a range of different substrates, so activating multiple different cytoplasmic and nuclear signal-transduction pathways relevant to hematopoietic cell growth and differentiation. Examples of signaling cascades activated by BCR-ABL1 include the ► **Ras** pathway, the Jun-kinase pathway, the phosphatidylinositol-3 kinase (► **PI3K Signaling**) pathway, a variety of CRKL-linked signaling processes, the Jak-STAT (► **Signal Transducers and Activators of Transcription in Oncogenesis**) pathway, and the Src pathway.

### Clinical Relevance

- Chronic myeloid leukemia (CML) is a myeloproliferative disorder that develops after the BCR-ABL1 mutation occurs in a pluripotent bone marrow stem cell. The affected stem cell gains a proliferative advantage and a malignant leukemic clone becomes established. CML, characterized by overproduction of granulocytes in the bone marrow and peripheral blood, accounts for about 25% of all human leukemias, with an incidence of ~1 in 100,000 per year. CML affects both sexes and all age groups, but occurs most commonly at 40–50 years. Patients typically present with symptoms of fatigue, bleeding, moderate weight loss, an enlarged palpable spleen, and a high white blood cell count.
- **Blast crisis** CML: CML is a biphasic disease, and without effective treatment usually progresses within 3–5 years of diagnosis to an aggressive and terminal acute phase or blast crisis. The precise molecular events that determine blast crisis are still unknown, although there is much evidence from cytogenetic and molecular studies that nonrandom and lineage-specific accumulation of gene mutations may be important.
- BCR-ABL1 is also found in leukemic cells of patients with adult (10–20%) or childhood (5%) acute lymphoblastic leukemia (ALL L1 or L2), and in rare cases (~3%) of acute myeloid leukemia (AML, mostly M1 or M2).

### Anti-BCR-ABL1 Therapies

Bone marrow transplantation and/or alpha-interferon therapy have been the treatments of choice for BCR-ABL1 leukemias. But the introduction of ► **imatinib mesylate**, a synthetic tyrosine kinase inhibitor designed to specifically impede BCR-ABL1 fusion protein activity, has significantly improved the overall outlook and prognosis for the majority of CML patients and this drug is now considered standard therapy for CML. New anti-BCR-ABL1 therapies are additionally being developed to improve outlook for the proportion of patients who do not respond or to help overcome leukemic cell resistance developed in some cases to imatinib.

### References

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### BCSG1

#### Definition

Breast cancer specific gene 1. Encodes the protein ► **synuclein  $\gamma$** .

► **Synuclein**

### BD

► **Behcet Disease**

### BDNF

#### Definition

Brain-Derived Neurotrophic Factor

### Beckwith-Wiedemann Syndrome

#### Definition

BWS is a rare, congenital overgrowth disorder in which babies are large at birth and may develop low blood sugar. Other common symptoms include a large tongue, large internal organs, and defects of the abdominal wall near the navel. Beckwith-Wiedemann

syndrome increases the risk of developing certain cancers, especially ► [Wilms tumor](#).

- [Bacillus Calmette–Guerin](#)
- [Beckwith–Wiedemann Syndrome–Associated Childhood Tumors](#)

## Beckwith–Wiedemann Syndrome Associated Childhood Tumors

Marcel Mannens

Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

### Definition

Beckwith–Wiedemann syndrome (BWS) is a complex overgrowth disorder caused by a number of genes that are subject to genomic imprinting. A high incidence of solid childhood tumors is seen in patients that present with BWS.

### Characteristics

#### Diagnostic Criteria

Beckwith–Wiedemann syndrome is a disorder first described by Beckwith in 1963 at the 11th annual meeting of the Western Society for Pediatric Research. Later, Wiedemann and Beckwith described the syndrome in more detail [2]. BWS is characterized by a great variety of clinical features, among which are abdominal wall defects, macroglossia, pre- and postnatal gigantism, earlobe pits or creases, facial nevus flammeus, hypoglycemia, renal abnormalities, and hemihypertrophy. BWS patients have a 7.5% risk of developing (mostly intra-abdominal) childhood tumors. Tumors most frequently found are ► [Wilms tumor](#) (WT), adrenocortical carcinoma (ACC), rhabdomyosarcoma (RMS), and hepatoblastoma (HB). Patients can be classified as having BWS according to the clinical criteria proposed by Elliot or DeBaun, although cases of BWS are known that do not comply with either set of criteria.

#### (Epi)genetics

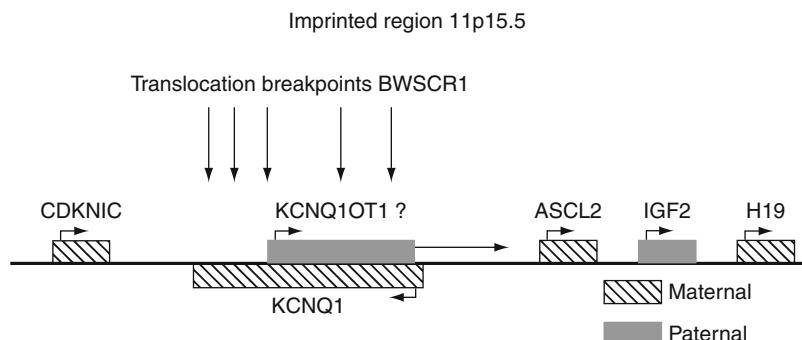
The syndrome occurs with an estimated incidence of 1:13,700 and most cases are sporadic (85%). The genetic predisposition for BWS lies on chromosome 11p15 (linkage analysis, chromosome abnormalities, loss of imprinting (LOI), gene mutations). The syndrome is subject to genomic imprinting since maternal transmission seems to be predominant. In addition chromosomal translocations are of maternal origin, duplications and uniparental disomies (UPD) of paternal origin. All hitherto known causative genes are imprinted. The translocation breakpoints on chromosome 11 map to three distinct regions within 11p15.3pter: Beckwith–Wiedemann syndrome chromosome region 1 (BWSCR1) near INS/IGF2, BWSCR2 5-Mb proximal to BWSCR1, and BWSCR3 2-Mb even more proximal. This already points to genetic heterogeneity, but also at the clinical level there seems to be heterogeneity. Chromosomal translocations in BWSCR1 and BWSCR3 are associated with the classical BWS phenotype, and BWSCR2 with minor BWS features but pronounced hemihypertrophy. BWSCR 1 and BWSCR2 have been cloned, and genes isolated from this region were shown to be involved in the development of this disorder. All genes involved are subject to genomic ► [imprinting](#).

#### BWSCR1

This region consists of a number of imprinted genes ([Fig. 1](#)). All known translocation breakpoints disrupt KCNQ1, a gene coding for a potassium channel involved in the Romano–Ward and Jervell–Lange–Nielsen cardiac arrhythmias syndromes. However, this imprinted gene is most likely not directly involved in BWS, but a gene transcribed in the antisense orientation of KCNQ1 clearly is. This gene, KCNQ1OT1, shows aberrant methylation in 50–80% of BWS cases. It does not code for a protein and functions through its RNA. CDKN1C is an inhibitor of cyclin-dependent kinases. Heterozygous mutations have been identified in about 20% of BWS patients in two studies. Others, however, have not been able to confirm this mutation frequency. The gene is not a major cause of BWS. It is, however, possible that in certain countries the mutation frequency is elevated (e.g., Asia). In addition, it has been reported that this gene is more frequently involved in familial cases of BWS. CDKN1C mouse models revealed some of the clinical BWS features

### **Beckwith-Wiedemann Syndrome Associated Childhood Tumors.**

**Fig. 1** Imprinted genes on 11p15 involved in BWS. The parental expression (imprinting) of these genes is indicated



such as omphalocele and renal adrenal cortex anomalies. In humans, *CDKN1C* also seems to be more frequently associated with abdominal wall defects. Another strong candidate for involvement in the etiology of BWS is the embryonic growth factor *IGF2*. Mouse models overexpressing *IGF2* displayed a phenotype overlapping with the BWS phenotype. Loss of *IGF2* imprinting is often seen in BWS patients. *H19*, another noncoding gene, lies downstream of *IGF2* and the expression of *IGF2* and *H19* seems to be linked. *H19* is important for the maintenance of the imprinting status of *IGF2*. Mouse studies underline the link between *IGF2* and *H19* expression and overgrowth phenotypes were found. *H19* loss of imprinting (silencing of the gene) is frequently seen in BWS cases, although not always in combination with *IGF2* loss of imprinting (LOI). Interestingly, overexpression of *H19* seems to lead to the Silver Russell Syndrome (SRS), characterized by intrauterine growth retardation, poor postnatal growth, asymmetry a classic facial phenotype and no increased risk for childhood tumors. Finally a gene called *ASCL2* is localized to the 11p15-imprinted region. Although no direct involvement in the BWS etiology is known, this gene might account for the fact that most, if not all, BWS cases with uniparental disomy (UPD) present in a mosaic form. The mouse homologue codes for a transcription factor, which is expressed during early mouse development and is essential for the development of the placenta. Therefore, also in humans, complete lack of expression might be lethal.

### **BWSCR2**

Two patients with balanced chromosomal translocations define this second chromosomal region, one of which developed a Wilms tumor. Both translocations

in 11p15.4 disrupt a paternally imprinted zinc-binding finger gene *ZNF215*. Parts of the 3' end of this gene are transcribed from the antisense strand of a second zinc-finger gene, *ZNF214*. Although putative mutations in these genes in other sporadic BWS cases were found, their involvement in BWS needs to be further elucidated by functional studies.

### **Diagnostics**

BWS can be diagnosed in the laboratory with cytogenetics (<5%) or DNA-diagnosis. The current major test involves methylation assays or LOI studies at the RNA level. The majority of cases (50–80%) exhibit aberrant methylation of *KCNQ1OT1* with or without aberrant methylation of *IGF2/H19*. These former cases often show UPD for 11p15 (in a mosaic form) which explains this aberrant methylation for multiple genes. However, the majority of cases with *KCNQ1OT1* defects and some cases with *H19/IGF2* defects have no UPD 11p15. Therefore, an imprinting switch can be assumed, involving an imprinting center analogous to the Prader-Willi and Angelman syndromes. The current data are most compatible with two distinct imprinting centers for either *KCNQ1OT1* or *IGF2/H19*. *CDKN1C* mutation analyses might be considered, especially in familial cases of BWS. The increased tumor risk for BWS patients seems to be associated with UPD in general and *H19* methylation defects in particular. *KCNQOT1* methylation defects only seem to be a reliable prognostic factor, since tumors are seldom associated with this group of patients. Recurrence risks for a second pregnancy can be assessed with UPD studies. In cases of a UPD in a mosaic form, there is no increased recurrence risk for BWS in a second pregnancy since the genetic defect occurred post-fertilization.

### BWS-Associated Tumors

Although childhood solid tumors associated with BWS share some common genetic features, the spectrum of genetic changes found in these tumors is diverse and complicated with many genetic alterations seen.

#### Wilms Tumor

The tumor most often found to be associated with BWS is Wilms tumor (WT) or nephroblastoma (59% of the tumors found in BWS patients). Overall it occurs with a frequency of 1 in 10,000 children, mostly in children under the age of 5 years. In patients suffering from BWS the incidence is 800–1,000 times increased. A high percentage (38%) shows loss of heterozygosity (LOH) of chromosome 11p. This region can be subdivided roughly into two parts: LOH of markers on 11p13 and LOH of markers on 11p15. The region on 11p13 has been shown to be deleted in patients affected by WAGR. WAGR stands for the combined occurrence of sporadic aniridia, WT, genitourinary abnormalities, and mental retardation. A gene in the candidate region (WT1) has been cloned. Mutations of this gene occur in only 10–15% of sporadic Wilms tumors, suggesting the existence of additional genes involved in the development of this tumor. The Denys-Drash syndrome, another syndrome associated with Wilms tumor, shows constitutional mutations of the WT1. The region on 11p15 showing LOH in WTs can be subdivided into two regions: An 800 kb region containing the WT2 locus near IGF2 and an additional locus of 336 kb proximal to WT2. WT can also be found in association with other syndromes, like the trisomy 18 syndrome, the Perlman syndrome and the Simpson-Golabi-Behmel, the Sotos syndrome, and the Klippel-Trenaunay syndrome. The ► [Li-Fraumeni syndrome](#) is a rare familial tumor syndrome and patients suffering from this disease contain germline point mutations in the ► [p53](#) tumor suppressor gene. The tumors that develop in these patients show a deletion of the wild type p53 allele. Although WT is not considered to be part of the Li-Fraumeni syndrome there have been few reports of the occurrence of WT in families affected by this syndrome. Mutations in the tumor suppressor gene p53 have been found in sporadic WTs and seem to be associated with a histological subtype. In a series of 140 WTs, mutations were restricted to tumors of the anaplastic subtype, showing aberrations in 8/11 samples.

This subtype is linked to a poor prognosis. In 10–25% of the Wilms tumors, LOH of 16q markers is found. It has been suggested that LOH of 16q is associated with an adverse prognosis. Another genetic abnormality, which seems to confer an adverse outcome, is LOH of 1p. This abnormality was found in 12% and 18% of the cases, respectively. Chromosome 7 also seems to be involved in Wilms tumor. According to the literature in 23% of the cases chromosome 7 is rearranged. Another region found to be frequently involved in LOH (14%) is on chromosome 22q. In a study which quantified chromosome 12 allelic imbalance in a series of 28 Wilms tumors, duplications were detected in 18%. An inventory of all quantitative chromosome aberrations occurring in a series of 46 WTs was made using comparative genomic hybridization analysis (CGH). Chromosome regions showing loss of DNA in three or more samples included 1p (11%), 11p (9%), 16q (13%), and 17p (7%). Regions showing gain of DNA in three or more samples included 1q (20%), 7q (9%), 8 (7%), 12q (17%), 17q (7%), and 18 (7%). In 2007, it became clear that a somatic deletion of an X-linked gene (WTX) is found in 1/3 Wilms tumors.

As expected, imprinting seems to play a major role in WT development since 11p15 LOH is always of maternal origin. This resulted in the hypothesis that a paternally imprinted tumor suppressor gene is involved in Wilms tumorigenesis. Alternatively, a maternally imprinted gene involved in stimulation of cell growth could be involved in the cases showing paternal UPD of (part of) chromosome 11. At present there are three candidate genes on 11p15 that show parent-of-origin-dependent monoallelic expression and belong to one of these two categories: the tumor suppressor genes H19 and CDKN1C which are maternally expressed and the paternally expressed growth-promoting gene IGF2. Evidence for the involvement of these genes has been found, i.e., loss of imprinting, or increased expression of IGF2, or reduced expression of CDKN1C or H19.

#### Adrenocortical Carcinoma

The second most common tumor found in BWS-patients is adrenocortical carcinoma (ACC). It is found in 15% of patients that develop a tumor. In the general population ACC is found to be an extremely rare tumor with an incidence of 1.7 new cases per

1,000,000 per year. As in BWS, IGF2 seems to be involved in sporadic ACC-tumorigenesis. A considerable proportion of the malignant tumors (~60%) display LOH of the 11p15.5 region, presumably all representing uniparental disomies. This is seen in both adult and childhood ACCs. In these cases a good correlation was found with overexpression of the IGF2 gene. These phenomena were found in a much smaller percentage in the benign adenomas. It has been hypothesized that adrenocortical tumorigenesis is a multistep process with sequential progression from the normal to the adenomatous and then to the malignant cell. If this is the case then IGF2 could be involved in the transition from adenoma to carcinoma. ACC is also found in association with other syndromes. One of these is the Li-Fraumeni syndrome, which is associated with mutations of the p53 gene on chromosome 17p. In one study, in which sporadic ACCs were analyzed for the presence of LOH at three different chromosome regions, chromosome 17p (containing the p53 gene) had become homozygous in all informative samples. LOH of 17p was not found in adrenocortical adenoma, the benign counterpart of ACC. Again, if the hypothesis that adrenocortical tumors develop from normal tissue to adenomas to carcinomas is correct, this would mean that LOH of 17p could be a late event in ACC tumorigenesis. Two other groups identified mutations in the p53 gene in ~30% of sporadic ACCs. In addition, CGH analysis showed loss of 17p in 50% of the (sporadic) cases. Another hereditary tumor syndrome associated with adrenocortical tumors is ► **multiple endocrine neoplasia type 1 (MEN1)**. In most cases associated with MEN1 adrenocortical adenomas are found. The disease is caused by mutation of the men in tumor suppressor gene (MEN1), located at 11q13.

Other regions found to be lost in ACCs include chromosome 13q, which was shown to have lost heterozygosity in 50% of informative patients, and chromosome 2. Genetic aberrations that were found in 38% of the tumors in this study were gains of chromosomes 12, 15q, 16q, and 19p and losses of chromosomes 3p, 6q, 8p, 9p, 11p, 17q, 18q, and 22q. There are numerous differences between the genetic aberrations found in adrenocortical adenomas and adrenocortical carcinomas. These differences may reflect various stages along the carcinogenic pathway.

Evidence for an involvement of imprinting in ACC again comes from LOH 11p15 studies (maternal loss) and LOI and expression studies for IGF2 and H19. It should be noted that LOI of IGF2 was associated with the malignant phenotype, since it was not detected in the adenomas but only in the carcinomas.

#### Rhabdomyosarcoma

Although rare, rhabdomyosarcoma (RMS) represents the most common soft-tissue sarcoma in children under the age of 15 years. It occurs with a frequency of 1.3–4.5 cases per million children per year. Based on their histology, rhabdomyosarcomas can be subdivided into three major subtypes: embryonal (E-RMS), alveolar (A-RMS), and pleomorphic (P-RMS) rhabdomyosarcoma, of which E-RMS is the subtype associated with BWS. Of all newly diagnosed cases 60% are E-RMS and 20% are A-RMS. Patients with E-RMS have a better prognosis than patients with A-RMS. LOH of chromosome 11p is an abnormality found frequently in RMS. In one study it was found in 72% of primary E-RMS and 20% of primary A-RMS. A gene located in this region, GOK (gene on chromosome 11) or STIM1 (stromal interaction molecule 1) was postulated to be a candidate tumor suppressor gene in RMS. No expression was found in seven RMS cell lines, and transfection of the gene into the RMS cell line RD was followed by growth arrest of the cells. LOH of 16q was also found in both types (in 55% of E-RMS and 40% of A-RMS). In total, LOH of 6p was found in 28% and LOH of 18p in 32% of the cases. Studies of A-RMS have shown that they often (~90%) contain a specific translocation. In most of these cases (68%) a t(2;13)(q35;q14) is found. In a smaller subset of A-RMS (14%) a variant translocation of t(1;13) (p36;q14) has been detected. Both these translocations cause the formation of a chimeric protein. In the case of the t(2;13) a PAX3-FKHR fusion product is expressed and in tumors with the t(1;13) a PAX7-► **FOXO1A** product is detected. PAX3 and PAX7 are both transcription factors involved in embryonal myogenesis. In the chimeric proteins the DNA binding domains of the PAX genes are retained and fused to the C-terminal region of the FKHR gene containing a strong transactivation domain. It has therefore been proposed that both fusion proteins function as transcription factors that aberrantly regulate transcription of genes, controlled by PAX3 or PAX7 binding

sites. The PAX3-FOXO1A fusion protein has been shown to be a strong transcriptional activator. In addition both PAX3-FOXO1A and PAX7-FOXO1A are overexpressed in A-RMS either by increased transcription (PAX3-FOXO1A) or by gene amplification (PAX7-FOXO1A). Although the presence of either translocation is considered to be a characteristic of A-RMS, some cases with the t(1;13) show mixed histology of both the embryonal and the alveolar type, and a case of E-RMS containing the t(2;13) has been described. In addition the age at diagnosis in patients with the t(1;13) is more consistent with E-RMS. Cytogenetic analysis of RMS showed a high incidence of trisomy 2 (in 9/9 E-RMS samples) and a high incidence of structural rearrangements of chromosomes 1 and 3 (both in 4/5 RMS samples). The alterations on chromosome 3 seem to cluster within 3p14–21. The presence of a der(16)t(1;16)(q21;q13) is also noted in both RMS types and has been categorized as a secondary structural abnormality. RMS was one of the first tumors found to be associated with the Li-Fraumeni syndrome. DNA amplifications have been identified for regions on chromosome 2p and 12q. Both A-RMS and E-RMS have been studied by CGH and the results showed clear differences between the two RMS subtypes. Aberrations found in E-RMS concerned gains and losses of whole chromosomes or large parts of chromosomes: Gains were most frequently found for chromosomes 2, 8, 12, 13 (in 6/10 cases), chromosome 7 (in 5/10 cases), and chromosomes 17, 18, and 19 (in 4/10 cases). Losses were identified most often for chromosome 16 (in 4/10 cases), chromosome 10 (in 3/10 cases) and chromosomes 14 and 15 (in 2/10 cases). One tumor showed an amplification of 12q13–q15. In the A-RMS samples whole (or part of) chromosome gains and losses were found to a much smaller extent. In ten tumors and four cell lines gain of chromosome 17q was found in four cases. However, in a high percentage amplifications were present. Chromosome regions most often involved were 12q13–q15 (in seven cases) and 2p25 (in five cases). The latter region contains the N-MYC gene which is known to be amplified in A-RMS. The regions containing the PAX7 and FKHR genes on 1p36 and 13q14 were found to be amplified in two cases.

As for Wilms tumor, abnormal genomic imprinting of chromosome region 11p15 appears to play a role in the development of RMS (paternal LOH, LOI of IGF2). Increased expression of IGF2 in tumors with

monoallelic expression of the gene confirms the important role postulated for IGF2 in the development of this tumor. The imprinting status of H19 has also been examined in RMS and was found to be normal in both subtypes. However, the expression was reduced significantly in 13/15 E-RMS and 2/11 A-RMS. This phenomenon was associated with either loss of the maternal (expressed) allele or LOI of IGF2. In contrast to the situation for Wilms tumor, reduced expression of H19 was not seen in all cases with LOI of IGF2.

### Hepatoblastoma

Hepatoblastoma (HB) is a rare malignant epithelial tumor of the liver with an incidence of one case per million children. However, it is the most common malignant hepatic neoplasm of childhood, and occurs with a predominance in males. Although most cases are sporadic, some HBs are associated with either BWS or familial adenomatous polyposis coli (FAP; ► [APC gene in Familial Adenomatous Polyposis](#)). Since FAP patients carry mutations in the adenomatous polyposis coli (APC) gene, sporadic HBs have also been analyzed for the presence of mutations in this gene. Indeed, alterations of the APC gene were found in 69% of the sporadic cases. When FAP occurs in combination with extracolonic symptoms it is commonly referred to as Gardner syndrome. Patients suffering from this disease also have an increased risk for the development of HB. The trisomy 18 syndrome can also be associated with HB, as has been found in four patients. One of the phenotypic features of trisomy 18 syndrome is the presence of an omphalocele (also found in BWS patients). It has been suggested that this feature may be one of the factors important in the development of HB in cases in which part of the liver has herniated into the omphalocele. As was found for the other BWS-associated tumors, LOH of 11p15 has also been found independently by several researchers for HB (up to 33%). An LOH study of chromosome 1 showed frequent loss of alleles in HBs. In 32 cases 34% had lost heterozygosity for (a part of) chromosome 1, of which 22% were homozygous for markers on the (distal) short arm. There has been a report of the occurrence of HB in the Li-Fraumeni syndrome, and in addition one study showed mutation of the p53 gene in 1/3 sporadic HB samples. Cytogenetic analysis of HB revealed certain consistent chromosome anomalies. Extra copies of chromosomes 2q and 20 are most frequently found. There has also been a report about

a recurring translocation: t(1;4)(q12;q34) that results in partial trisomy of most of chromosome arm 1q and partial monosomy of distal 4q. CGH analysis identified mostly gain of DNA. Chromosomes affected in more than 30% of the cases included 1, 2, 7, 8, and 17. When determining the parental origin of 11p alleles lost in HBs it became clear that in this BWS-associated tumor LOH of 11p15.5 was exclusively of maternal origin. When looking directly at the imprinting status of the IGF2 and H19 genes biallelic expression was detected. Two studies showed LOI of IGF2 with normal imprinting of H19 in 1/3 HBs and in 1/5 HBs. A third study showed LOI of both genes in 1/5 cases.

### Common Genetic Pathways

When reviewing all genetic and epigenetic data it becomes clear that the most evident abnormality found in all BWS-associated tumors affects chromosome region 11p15. This is the region to which the syndrome has been linked. All four tumor types show LOH of markers in this region. To date, data has been published for all except ACC showing LOH affecting the maternal allele, with retention of the paternal allele (one ACC with paternal UPD has been described). This suggests the involvement of genomic imprinting. Indeed, abnormal imprinting was found for these tumors, as it was for BWS: They display LOI of the maternally imprinted IGF2 gene. Therefore, this growth factor may play a central role in the development of the overgrowth syndrome and its associated tumors. Increased expression has been noted for WT, ACC and E-RMS, and LOI of IGF2 has been associated with decreased expression of the supposed tumor suppressor gene H19. There is an additional genetic abnormality common between all four types of neoplasms. They all show mutations in the p53 gene. However, this is found in a large proportion of all cancers and, therefore, is considered not to be specific for the development of tumors associated with the BWS.

Besides genetic evidence there are also pathological data indicating an association between these tumors. Both WT and HB may contain rhabdomyomatous tissue, whereas primary tumors of the liver have been shown to consist of ACC and RMS.

There are also several chromosome aberrations found in a subset of these tumors. When considering abnormalities found in three of the four tumor-types there seems to be a strong connection between WT,

E-RMS, and HB. They share seven common genetic abnormalities. Besides the abnormalities already mentioned above, they all might contain extra copies of chromosomes 7q, 8, and 17q. Therefore, these chromosome regions may contain genes that play a role in the normal embryonic development of the affected tissues. Since these affected regions are large it would be very difficult to identify the genes involved. More interesting, therefore, is the abnormality of chromosome 1p that was found in these tumors. This presented either as LOH or structural abnormality of the short arm of chromosome 1. Since these aberrations affect small(er) regions of the chromosome they may be very helpful in the identification of genes. This applies especially to the analysis of translocation breakpoint regions, as has been shown for the regions involved in BWS. Extra copies of chromosome 12 have been identified in the subset consisting of WT, ACC, and E-RMS. These tumors are also characterized by increased expression of IGF2.

When analyzing the published data, it becomes clear that WT and E-RMS share most genetic aberrations, with a total of 12. Therefore, the genetic relationship is most evident between these two tumor-types. In addition to the abnormalities already mentioned, they have both been shown to contain extra copies of chromosome 18, and in both tumor-types, decreased expression of H19 has been found. Further elucidation of the common genetic pathways involved in the etiology of the BWS-associated tumors awaits identification of the genes involved.

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## Beclin 1

### Definition

Mammalian homologue of the yeast autophagy protein Atg6/Vps30. Found in an inhibitory complex with BCL-2. After release of ► **BCL-2**, beclin 1 can associate with class III PI3K and stimulate ► **autophagy**. Beclin 1 is monoallelically deleted in some human cancers.

## Becquerel

### Definition

A Becquerel (Bq) is a measure for the disintegration per second. A disintegration of 1 nucleus per second equals 1 Bq.

► [Radon](#)

## Behcet Disease

### Synonyms

BD

### Definition

Behcet's syndrome was named in 1937 after the Turkish dermatologist Hulusi Behçet, who first described the triple-symptom complex of recurrent oral aphthous ulcers, genital ulcers, and uveitis. This complex, multisystemic disease includes involvement of the mucocutaneous, ocular, cardiovascular, renal, gastrointestinal, pulmonary, urologic, and central nervous systems and the joints, blood vessels, and lungs. It is characterized by oral aphthae and by at least two of the following: (1) genital aphthae, (2) synovitis, (3) posterior uveitis, (4) cutaneous pustular vasculitis, (5) meningoencephalitis, (6) recurrent genital ulcers, and (7) uveitis in the absence of inflammatory bowel disease or collagen vascular disease. The cause of BD is not

known; however, immunogenetics, immune regulation, vascular abnormalities, or bacterial and viral infection may have a role in its development.

## Benign Prostate Hyperplasia

### Definition

(BPH) or “enlarged prostate”; is a condition that can cause many of the same symptoms as prostate cancer. BPH is a non-cancerous increase in the size and number of cells that make up the prostate. BPH is almost always found in older men. Since women do not have a prostate, they cannot get BPH. Young men almost never experience symptoms of an enlarged prostate either. The prostate enlarges over the course of many years of exposure to male hormones, and young men typically have not had enough years of exposure for symptoms to show up. During puberty, the prostate goes through a phase of very rapid enlargement, but this levels off once puberty is completed. Starting in mid-life, the prostate begins growing again, but very slowly this time. It is thought that these periods of growth result from increased levels of male hormones such as ► [testosterone](#). Testosterone is produced throughout a man's life and, subsequently, the prostate grows throughout a man's life. Due to the slow progression of this growth, most men do not notice any symptoms of BPH until they are older and the prostate has grown to such a size that it impinges on the outflow of urine from the bladder.

### Symptoms

Due to the location of the prostate, BPH causes a number of urinary symptoms. The prostate is located just below where the bladder empties into the urethra (which is a thin tube that carries urine from the bladder, through the penis, to outside the body). As the prostate enlarges, it impinges the flow of urine through the urethra. The most common symptoms are:

1. Frequency – urinating much more often than normal.
2. Urgency – having a sensation that you need to urinate immediately.
3. Nocturia – getting up to urinate multiple times during the night.
4. Hesitancy – difficulty starting the urine stream.

These symptoms can be identical to those experienced by men with ► prostate cancer. There is no way to tell if your symptoms are due to BPH or prostate cancer, so it is essential to visit your physician if you develop any of these symptoms. To diagnose BPH, prostate cancer must first be ruled out. To rule out prostate cancer, you need to undergo a digital rectal examination (DRE) and a ► prostate-specific antigen (PSA) blood test at the minimum. These tests are used to diagnose prostate cancer and, if both are negative, then your chances of having prostate cancer are very low.

<http://prostatecancer.about.com/od/prostatecancer101/a/bphbasics.htm>.

## Benign Tumor

### Definition

A tumor that remains confined to its site of origin and neither invades the surrounding tissue nor spreads to other organ sites.

## Benzene

► Benzene and Leukemia

## Benzene and Leukemia

Valentina Bollati<sup>1</sup> and Alessandra Forni<sup>2</sup>

<sup>1</sup>Department of Preventive Medicine,  
IRCCS Maggiore Hospital, Mangiagalli and  
Regina Elena Foundation, Milan, Italy

<sup>2</sup>Department of Occupational and Environmental  
Health “Clinica del Lavoro L. Devoto”, University of  
Milan, Milan, Italy

### Definition

Benzene and Leukemia address the leukemogenic effect of benzene, representing a complex model of chemical carcinogenesis in humans.

## Characteristics

The relationship between benzene, the smallest and most stable ► aromatic hydrocarbon, and leukemia has been reported in the past for workers with high exposures, when benzene in the commercial form (► Benzol) was used largely as a solvent, especially in the shoe industry and in rotogravure printing. Today, occupational exposures are controlled by law and are at most reserved to workers in the petrochemical industry, workers exposed to automobile emissions such as urban officers or gas station attendants, firefighters, and vehicle mechanics. Currently, most European countries and the USA have fixed the threshold of acceptable occupational exposure at 1.63–3.25 mg/m<sup>3</sup> (0.5–1 ► ppm). Benzene, even at much lower concentrations, is also a pollutant of the general environment. Among major sources of benzene for the general population (usually below 50 µg/m<sup>3</sup>, 15 ► ppb) are traffic exhaust fumes, since benzene is still a typical component of gasoline (1%), and cigarette smoking, which remains a significant source of exposure in both occupationally and nonoccupationally exposed individuals.

### Benzene Toxicity and Carcinogenicity

Since the nineteenth century, benzene has been recognized as the cause of hematotoxicity of various degrees, up to aplastic anemia, in workers chronically exposed to high concentrations. However, high-dose benzene leukemogenicity was first reported only in 1928 by Delore and Borgomano in a subject showing benzene intoxication. Subsequent studies confirmed an increased risk of leukemia in different occupational settings characterized by high exposure. In Italy, outbreaks of severe benzene poisoning and leukemia have been observed from the thirties to the early sixties, when benzene as a solvent was prohibited by law. Similar findings were reported in the seventies in Turkey and more recently in China. In Italy, most cases of fatal aplastic anemia and leukemia occurred in shoe manufacturing and in rotogravure printing where commercial benzene was used as a solvent of glues and inks respectively. The estimated or measured exposures were in the order of hundreds ppm. Less severe cases of benzene toxicity were observed in subjects exposed to several tens ppm. The acceptable threshold in the 1960s (25 ppm) was later reduced in many countries after the confirmation of benzene leukemogenic activity at lower exposures, claimed on

the basis of some epidemiologic studies in chemical and rubber workers, in USA.

Benzene was recognized as a group A carcinogen ("a known carcinogen") by ► EPA in 1979 and as a group 1 human carcinogen ("known to be carcinogenic to humans") by ► IARC in 1982. Based on the general assumption that no threshold might exist for carcinogenic substances and the fact that benzene exposures in certain industries cannot be avoided, the threshold has been lowered to less than 1 ppm, anyway the lowest technically possible threshold.

Conflicting results, however, have been reported in low-level exposure populations such as drivers, police traffic officers, and gasoline station attendants. Concerns about health effects of benzene at very low doses have been raised recently by results of a study showing a reduction of white blood cells and platelets also in subjects chronically exposed to less than 1 ppm in air, but the values reported were anyway within normal ranges.

The bone marrow depression of chronic benzene poisoning, resulting in hyporegenerative anemia, leukopenia, and thrombocytopenia of varying degree, may slowly recover after removal from exposure, but sometimes persists and evolves into fatal aplastic anemia or into ► acute myeloid leukemia (AML). AML may be preceded by a myelodysplastic syndrome (or preleukemic syndrome), consisting in abnormalities of bone marrow cells surviving ► apoptosis and of blood precursor cells differentiation.

The majority of benzene AMLs are myeloblastic, but other rarer subtypes (e.g., erythroleukemia) have been reported. Many cases of benzene leukemia have low white cell counts, or show only a moderate leukocytosis with a small percentage of immature cells, except in the terminal stage.

Aplastic anemia may occur in subjects while they are still exposed to high concentration of benzene. Leukemia may occur at the same time, or more or less shortly after cessation of exposure. In a few cases, a long latency period between the end of work with benzene and occurrence of leukemia has been reported.

### Benzene Metabolism and Toxicity

Benzene is not toxic and carcinogenic per se, but rather its toxicity is through its metabolites. Experimental evidences indicate that reactive intermediates are necessary for benzene carcinogenicity and toxicity, but the metabolite(s) responsible are still not fully identified.

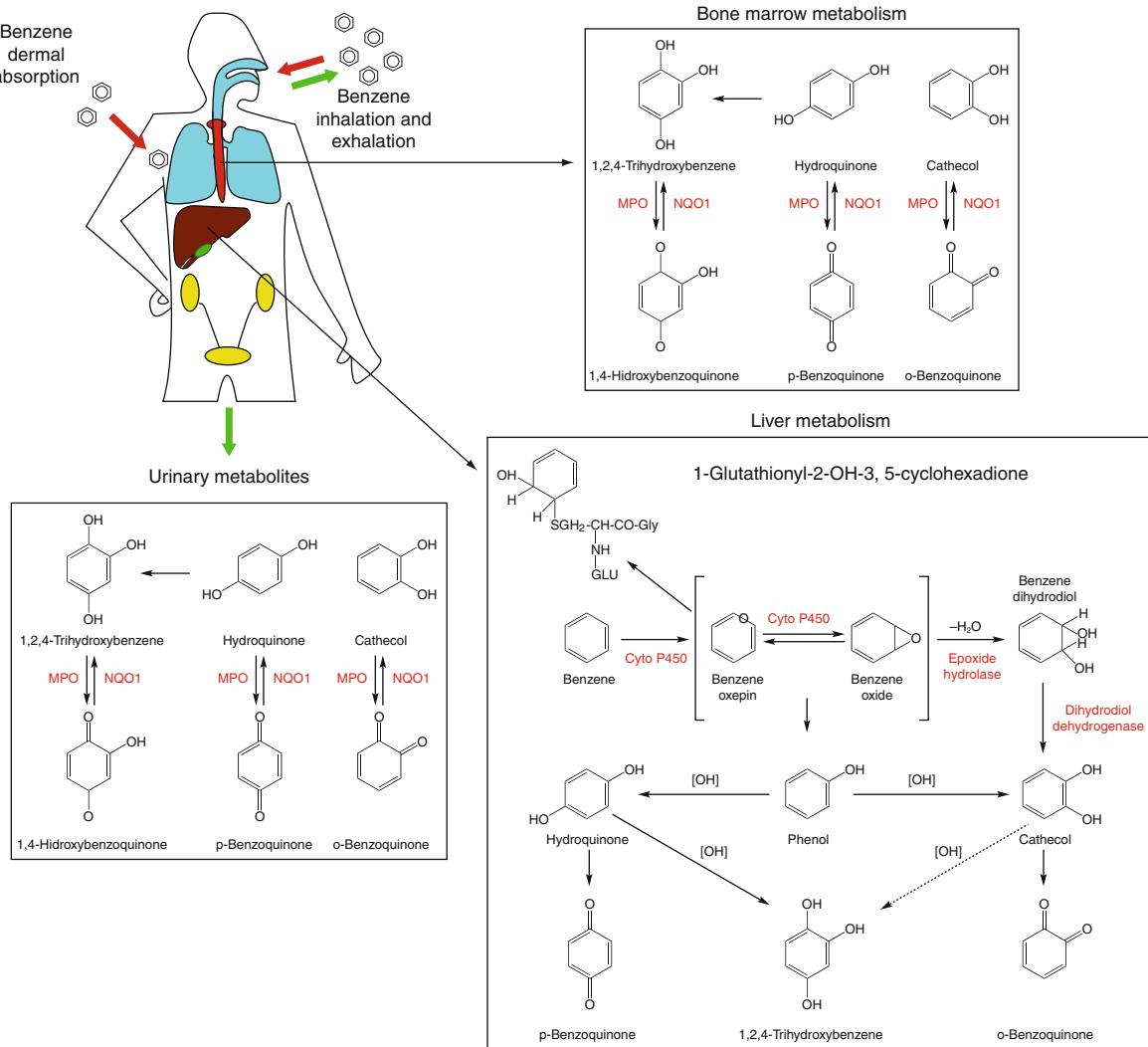
Inhaled benzene is partly eliminated in the exhaled air. The remaining is rapidly distributed, crosses blood-brain, placental, and gonadal barriers, and is found in several organs including the bone marrow. Benzene is transformed in the liver to benzene oxide, phenol, catechol, hydroquinone, and 1,2,4-trihydroxybenzene by the microsomal ► cytochrome P-450 monooxygenase system (► CYP2E1). Catechol and hydroquinone oxidation results into the reactive intermediates *ortho*-benzoquinone and *para*-hydroquinone. Hydroquinones may also be produced from benzene derived ► quinones via ► NAD(P)H:quinone oxidoreductase (► NQO1) (Fig. 1). Benzene metabolites such as hydroquinone and catechol, reach the bone marrow and can be further activated by ► myeloperoxidase (► MPO), present at high levels in stromal ► macrophages, resulting in the production of quinones and ► reactive oxygen species, which bind covalently to biological macromolecules. Unmetabolized benzene and several metabolites are eliminated through the kidney and some of them can be measured to assess benzene exposure.

In order to explain the different susceptibility to benzene poisoning in workers with similar levels of exposure, some ► metabolic polymorphisms have been examined in benzene-exposed subjects. A rapid CYP2E1 activity and a loss of NQO1 function polymorphism were found to be associated with increased benzene toxicity in workers exposed to high levels of benzene (>10 ppm) in Shanghai (China). More recent results highlighted the role of MPO and NQO1 polymorphisms even at exposures lower than 1 ppm. CYP2E1 and NQO1 are polymorphically distributed in human populations: in Caucasians, the estimated frequency of CYP2E1 rapid metabolizers is around 10% and a loss-function NQO1 polymorphism has been identified with a 40% frequency.

### Benzene, Chromosome Changes, and Leukemia

Benzene metabolites are not mutagenic, but are able to generate oxygen reactive species, which might be responsible for DNA damage, both by genetic and ► epigenetic mechanisms.

In the 1960s, the possibility of studying human chromosomes in lymphocytes, stimulated to divide in culture, and in direct preparations of bone marrow cells, raised the interest for cytogenetic studies in benzene-exposed workers with or without signs of benzene toxicity and in cases of benzene leukemia.



**Benzene and Leukemia. Fig. 1** Benzene metabolism in humans

Exposure to high concentrations of benzene was demonstrated to induce structural (► **Chromosome Translocations**, breaks, deletions) and/or numerical chromosome changes, persisting in lymphocytes also for decades after cessation of exposure and in bone marrow cells at the time of benzene poisoning or during persisting myelodysplastic syndrome.

Structural chromosome changes in benzene-exposed workers were studied more recently with special techniques and resulted to be nonrandom, involving specific chromosomes, both for breaks and for translocations.

In vitro studies of hematopoietic progenitor cells from human bone marrow or umbilical cord blood,

cultured in the presence of hydroquinone, showed specific deletions and/or numerical changes in chromosomes more frequently involved in benzene-induced myelodysplastic syndrome and leukemia.

On the basis of some clinical reports, the hypothesis is suggested that myeloid precursor cells with different chromosome changes either die by apoptosis or necrosis, or survive giving rise to atypical cell clones. One clone with selective advantage might proliferate and be responsible for the evolution into leukemia. This mechanism might be enhanced by the bone marrow microenvironment conditions and be favored by benzene-induced immunodepression.

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## Benzidine

### Definition

Is a carcinogenic ► aromatic amine that has been used in the synthesis of dyes. It has been linked to bladder cancer and ► pancreas cancer. ► Arylamine N-Acetyltransferases.

## Benzo(a)pyrene

### Definition

An environmental carcinogen belonging to the polycyclic aromatic hydrocarbon family that is primarily found in sources such as tobacco smoke. Its role in smoke-causing ► lung cancer is extensively studied.

- Sulforaphane
- Polycyclic Aromatic Hydrocarbons
- Tobacco Carcinogenesis
- Tobacco-Related Cancers

## Benzo[a]pyrene Diol Epoxide (BPDE)

### Synonyms

BPDE

### Definition

Is a potent mutagenic and carcinogenic metabolic product of *benzo[a]pyrene*, one of the most well-known combustion products in cigarette smoke and vehicle exhausts. BPDE induces DNA bulky adducts and is commonly used in epidemiologic studies as a challenge mutagen.

- DNA Adduct to DNA
- Mutagen Sensitivity

## Benzol

### Definition

A commercial form of benzene that is a mixture of benzene and its homologues (toluene and xylene).

- Benzene and Leukemia

## Benzoquinone ansamycin

- Ansamycin Class of Natural Product Hsp90 Inhibitors

## Benzpyrene

### Definition

Member of the group of ► polycyclic aromatic hydrocarbons. Benzpyrenes are present in coal tar at low levels and are considered carcinogenic (cancer-inducing). Traces of benzpyrenes are present in wood smoke, and this has given rise to some concern about the safety of naturally smoked foods.

## Berlin Breakage Syndrome

- Nijmegen Breakage Syndrome

## Beta Subunit of Human Chorionic Gonadotropin

### Synonyms

[β-hCG](#); [Beta-hCG](#)

### Definition

β-hCG is normally produced by the placenta and human fetal tissue. Elevated serum β-hCG is most commonly associated with pregnancy, gestational trophoblastic disease, and germ cell tumors. It can also be found in hypogonadal states and with marijuana use.

#### ► Serum Biomarkers

## Beta-2 Microglobulin

### Synonyms

[β2 Microglobulin](#)

### Definition

Component of MHC class I molecules present virtually on all cells except red blood cells. β2 microglobulin has no transmembrane region.

#### ► Plasmacytoma

## Beta-Catenin

### Definition

Multifunctional cytoplasmic protein that is involved in ► [E-cadherin](#)-mediated cell–cell ► [adhesion](#), linking cadherins to the actin cytoskeleton. Can also act independently as a gene regulatory protein. Has an important role in animal development as part of a ► [Wnt](#) signaling pathway.

## Beta-hCG

#### ► Beta Subunit of Human Chorionic Gonadotropin

## Betacellulin

### Definition

BTC; this has been isolated from conditioned media from a pancreatic β cell tumor cell line. BTC is also expressed in a variety of mesenchymal and epithelial cell lines and in many tissues including pancreas, liver, kidney, and small intestine. Membrane-bound proBTC is processed by ADAM10 to release soluble BTC. Transgenic chicken actin promoter-driven BTC overexpression in mice causes bony deformations of the skull, pulmonary hemorrhage syndrome, and complex eye pathology. Transgenic animals showed decrease in the weight of pancreas and increase in the weight of the eye, lung, and spleen.

#### ► ADAM Molecules

#### ► Epidermal Growth Factor (EGF)-Like Ligands

## Beta-glucosidase

A glycoside hydrolase enzyme that cleaves sugar residues from compounds.

#### ► Genistein

## Betel Quid

### Definition

Also known as *pan*, this material consists of four main ingredients: tobacco, areca nuts, and slaked lime wrapped in a betel leaf. Betel quid chewing is widely practiced in Southeast Asia, particularly in India.

#### ► Tobacco Carcinogenesis

#### ► Tobacco-Related Cancers

## Betulin

### Definition

A triterpenol from birch bark.

► [Urothelial Carcinoma](#)

## Betulinic Acid

Stephen Safe, Sudhakar Chinthrapalli and Sabitha Papineni  
Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX, USA

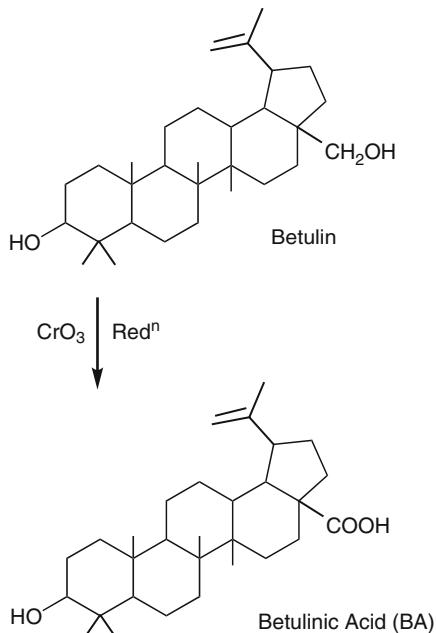
### Definition

*Betulinic acid* is a naturally occurring triterpenoid acid and a potent anticancer drug.

### Characteristics

► **Betulin** is a pentacyclic triterpenol natural product that is found in tree bark and this compound may constitute up to 30% of the bark from birch trees. Betulinic acid (BA) is a minor bark constituent but is readily synthesized from betulin by oxidation to betulonic acid followed by reduction to betulinic acid (**Fig. 1**). Birch bark extracts containing betulin and BA have been used in traditional folk medicines; however, in recent years, BA alone or some of its derivatives have been developed as pharmacological agents for treating multiple diseases. For example, these compounds are antiviral agents that inhibit HIV-1 replication and exhibit antimalarial, antihelmintic, and antibacterial activity as well as antiinflammatory and analgesic effects. Many of these responses induced by BA and its derivatives are structure-dependent and involve changes in structure of one or more regions in the molecule.

Many of the naturally occurring triterpenoid acids, such as ► [ursolic acid](#), ► [glycyrrhetic acid](#), ► [oleanolic acid](#), and *betulinic acids*, exhibit some cytotoxicity to various cancer cell lines; however,



**Betulinic Acid. Fig. 1** Betulin, a major component of birch bark, is readily oxidized by chromic acid to betulonic acid which is reduced with sodium borohydride to betulinic acid

among these natural products, BA is by far the most potent anticancer agent. Initial studies by Pisha et al. showed that BA was a highly potent drug for treatment of melanoma in mouse xenograft model. In this study, athymic mice were injected with melanoma cells (MEL-2 or MEL-1) and treated with BA at doses of 50, 250, or 500 mg/kg every third day and this resulted in significant tumor growth inhibition. Moreover, BA also decreased tumor volume in mice already bearing relatively large tumors. It was also reported that tumor growth inhibition could be observed at doses of BA as low as 5 mg/kg (X 6), whereas at doses as high as 500 mg/kg, minimal toxic side effects were observed in the animals. It was also reported that BA-induced ► [apoptosis](#) in melanoma cell lines and the high cytotoxicity of BA was observed in melanoma cells but not in squamous, breast, colon, sarcoma, prostate, lung, neuroblastoma, and glioma cancer cell lines.

Subsequent studies on the cytotoxicity of BA in cancer cell lines demonstrated that comparable effects were observed in cells derived from multiple tumor types. BA alone inhibited proliferation of various cancer cell lines, and several reports show the potential chemotherapeutic advantages of using BA in combination with other anticancer drugs such as vincristine, tumor necrosis factor (TNF)-related

apoptosis inducing ligand (TRAIL), doxorubicin, taxol, and irradiation. Interactions of these anticancer drugs with BA generally enhance the overall cytotoxicity of the combination compared to the treatments alone; however, these interactions are highly cell context-dependent. Recombinant TRAIL is now being investigated in clinical trials and the protein is a ligand for cell membrane death receptors and activates the extrinsic apoptosis pathway characterized by caspase-8-dependent PARP cleavage. Treatment of neuroblastoma cells with TRAIL plus BA (combination) clearly enhanced apoptosis compared to treatment with the individual agents. TRAIL and BA alone tend to activate the extrinsic and intrinsic apoptosis pathways and the combination of these drugs results in mutual enhancement of both pathways. Since many tumor types are highly resistant to cell death, the combination of BA and other proapoptotic agents may offer many advantages for clinical treatment of some tumors.

Although BA induces apoptosis in most cancer cell lines, there is also evidence that activation of other responses may also contribute to the anticancer activity of this drug. In human melanoma cells, BA induces reactive oxygen species (ROS) and this is accompanied by time-dependent and persistent activation of p38 and c-Jun NH<sub>2</sub>-terminal kinase (JNK). ROS acts upstream of these mitogen-activated protein kinases; however, both p38 and JNK can be involved in apoptotic pathways induced by BA in melanoma cells. Interestingly, studies in other melanoma cell lines showed that BA-induced effects on some cell cycle proteins and apoptosis (PARP cleavage and DNA laddering) were dependent on persistent activation of MAPK, since all of these responses were inhibited by the MAPK inhibitor U0126. Thus, BA-induced apoptosis is linked to activation of multiple kinases and differences in their action are highly dependent on cell context. The anticancer activity of BA may also be associated with other effects including the inhibition of topoisomerase 1 and antiangiogenic activity. This latter response was determined in ECV 304 endothelial cells in a Matrigel tube formation assay where BA and three substituted analogs exhibited angiogenic activity.

The mechanism of the anticarcinogenic activity of BA is complex and cell context-dependent and may include contributions from the direct effects of this compound on mitochondria and activation of kinase pathways. Research in our laboratory has focused on

studying some of the underlying mechanisms of cancer cell and tumor growth, survival, and angiogenesis. Using RNA interference, we have shown that specificity protein (► Sp) transcription factors are responsible, in part, for the growth and survival of cancer cells and their ability to metastasize and grow at distal sites. Sp1, Sp3, and Sp4 are overexpressed in colon and pancreatic cancer cells and tumors, and play a key role in overexpression of the ► angiogenic factors vascular endothelial growth factor (► VEGF), VEGF receptor 1 (VEGFR1) and VEGFR2, the survival gene survivin, and Sp3-dependent suppression of the cyclin-dependent kinase inhibitor p27. These results directly link overexpression of Sp proteins to the enhanced growth and survival and potential for metastasis/angiogenesis of cancer cells and tumors, and also suggest that inhibiting Sp protein-dependent gene expression or by inducing Sp protein degradation may be an important strategy for developing effective anticancer drugs. The first example of this approach was the identification and application of ► tolafenamic acid, a nonsteroidal antiinflammatory drug, which inhibited pancreatic cell growth through activation of proteasome-dependent degradation of Sp1, Sp3, and Sp4. Moreover, in an orthotopic model for pancreatic cancer, we also observed that tolafenamic acid inhibited tumor growth and metastasis and this was accompanied by degradation of Sp proteins in pancreatic tumors. Based on the reported proapoptotic/antiangiogenic effects of BA in cancer cell lines, we hypothesized that this compound may also act, in part, through Sp protein degradation. In LNCaP prostate cancer cells, we have shown that BA induced proteasome-dependent degradation of Sp1, Sp3, and Sp4 and we have observed similar responses in cell lines derived from other tumor types. Moreover, in *in vivo* studies in athymic nude mice bearing human LNCaP cells as xenografts, BA also inhibited tumor growth and this was accompanied by decreased Sp1, Sp3, and Sp4 expression in the tumors. These results demonstrate that some of the anticancer activities BA in multiple cell lines may be partially due to degradation of Sp proteins and we have observed that this process is due to the activation of both proteasome-dependent and proteasome-independent pathways. Thus, betulinic acid and some of its derivatives are part of a new class of anticancer drugs that work through targeting Sp proteins and Sp-dependent genes involved in cancer cell survival, growth, and angiogenesis.

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## Bevacizumab

### Definition

Avastin® made by Genentech/Roche.

► Monoclonal antibody against the ► vascular endothelial growth factor (VEGF). Bevacizumab has activity in ► colorectal cancer ► (Colorectal Cancer Therapeutic Antibodies), non-small-cell ► lung cancer, ► breast cancer, and ► ovarian cancer. Binding to VEGF prevents it from binding to its receptor. Originally approved by the US ► FDA for the treatment of ► colorectal cancer.

VEGF stimulates the growth of new blood vessels, a process called ► angiogenesis. The binding of bevacizumab is designed to inactivate VEGF so that it is no longer an effective stimulant for angiogenesis. As a result, new blood vessels are not formed. Cancers depend on the development of new blood vessels to grow. Without an adequate supply of blood, they cannot get larger and may even shrink. Bevacizumab does not work directly on the tumor, but prevents its growth by reducing its supply of blood. Bevacizumab does not cure cancer, but it can slow down its growth and increase survival times. It is normally given immediately after treatment with chemotherapy. Bevacizumab is thought to have great promise in slowing down the growth of inoperable tumors. As of 2005, it was being tested in more than three dozen clinical trials in combination with other drugs to treat many other types of metastatic cancer including ► non-small-cell lung cancer, ► pancreas cancer, head and neck tumors,

► ovarian cancer, malignant ► melanoma, and solid tumors in children and adults.

Bevacizumab binds to ► vascular endothelial growth factor (VEGF)-A, thus blocking its binding to the VEGF-receptor (VEGFR). It does not bind to other VEGF molecules, such as VEGF-B or VEGF-C. Its half-life in the patient is 17–21 days, making administration every 2 or 3 weeks possible. As would be expected for a successfully ► humanized monoclonal antibody, there has been no evidence of development of high titer antibodies directed against bevacizumab in treated patients. Bevacizumab is supplied as a clear to slightly opalescent sterile liquid in 100-mg and 1000-mg glass vials ready for parenteral administration. The loading dose should be infused intravenously over 90 min, and if no adverse infusion reactions occur, then the second dose can be administered over 60 min. If no adverse events occur after the second administration, then the third and all subsequent doses can be administered over at least 30 min. The principal toxicities associated with the use of bevacizumab include hypertension, arterial thrombosis, proteinuria, delayed wound healing, and rarely gastrointestinal perforation. In ► colorectal cancer (CRC) clinical trials, an increased incidence of bleeding was noted. However, life-threatening hemoptysis occurred in clinical trials evaluating bevacizumab for the treatment of ► squamous cell carcinoma of the lung.

<http://www.answers.com/topic/bevacizumab>; <http://www.answers.com/topic/bevacizumab>

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## BFGF

### Definition

► Basic fibroblast growth factor is a mitogenic and angiogenic growth factor involved in wound healing and tumor growth.

► Basic Fibroblast Growth Factor (BFGF)  
► Fibroblast Growth Factor 2 (FGF2)  
► Securin

## BGP, Biliary Glycoprotein

- [CEACAM1 Adhesion Molecule](#)

## BH

### Definition

► [BCL-2](#) homology domain. There are four BH domains, referring as BH1, 2, 3, and 4 domains.

- [PUMA](#)

## BH3

### Definition

- [Bcl2](#) homology domain-3.
- [Mcl Family](#)

## BH3 Binding Pocket

### Definition

A single  $\alpha$ -helix called the ► [BH3](#) region. Activity is proposed to be mediated through the association of the BH3 region of one protein, including Mcl-1, with a large hydrophobic pocket on the other binding partner, such as Bak. Mcl family.

- [Mcl Family](#)

## BH3-Interacting Death Domain Agonist

### Synonyms

[Bid](#)

## Definition

Bid is a 195 amino acid, 22 kDa proapoptotic BH3-only protein of the ► [BCL-2](#) family that is localized to chromosome 22q11. Upon death receptor activation, Bid is cleaved by caspase-8 into a C-terminal 15 kDa and an N-terminal 6 kDa fragment. Truncated C-terminal Bid then translocates to mitochondria to trigger cytochrome *c* release by binding to Bax and/or Bak, causing them to oligomerize.

- [Caspase-8](#)

## BHD Syndrome

- [Birt–Hogg–Dubé Syndrome](#)

## BHLH

### Definition

Basic helix-loop-helix (bHLH) is a protein motif shared by a group of transcription factors, therefore named bHLH proteins (or E proteins). For DNA binding, mono- or heterodimerization is compulsory, which is mediated by the helix-loop-helix motif. The basic region is composed of basic amino acids and determines DNA sequence-specific binding of the dimer.

- [E-box](#)
- [Myc Oncogene](#)

## BHLH-PAS Proteins

### Definition

A family of transcription factors characterized by a basic-helix-loop-helix (bHLH) structural motif and PAS sequence homology. The bHLH motif consists of an amino acid sequence in which the secondary structure has two  $\alpha$  helices connected by a loop. The PAS sequence refers to a highly homologous region found in the proteins Per, Arnt, and Sim.

## Biallelic Inactivation

### Synonyms

Biallelic mutation

## Biallelic Mutations

### Definition

Pathogenic sequence alterations, albeit not necessarily identical, are present in both copies of the same gene.

### Bias

### Definition

A flaw in the study design or method of collecting or interpreting information that leads to an erroneous result.

- ▶ [Cancer Epidemiology](#)
- ▶ [Coffee Consumption](#)
- ▶ [Epidemiology of Cancer](#)

## Bicalutamide

### Definition

Non-steroidal antiandrogen

- ▶ [Gynecomastia](#)

## Bid

Xiao-Ming Yin

Department of Pathology, University of Pittsburgh  
School of Medicine, Pittsburgh, PA, USA

### Synonyms

BH3-interacting domain death agonist

### Definition

Bid is a pro-death ► [Bcl2](#) family protein. Structurally, it contains only one Bcl-2 homology domain, the BH3 domain. Thus, it belongs to the BH3-only subfamily, which also includes Bad, Bik/Nbk/Blk, Bim, Bmf, Hrk/DP5, EGL-1, Noxa, and PUMA.

### Characteristics

#### The Bid Molecule

Bid was first cloned in 1996 from an expression cDNA library screened with recombinant ► [Bcl2](#) and Bax. Its ability to interact with both the anti-death and the pro-death Bcl2 family proteins is a distinguished feature of this molecule among the BH3-only members. Bid was re-cloned in 1998 by two other laboratories. In both cases, Bid was identified as a substrate of ► [caspase-8](#).

Bid is phylogenetically conserved. The mouse Bid gene is located at chromosome 6 (6F1; 6 54.0 cM), while the human Bid is localized in a syntenic region, chromosome 22q11.2. The major protein product is derived from the originally defined five exons with 195 amino acids (about 22Kd) in both human and mouse. The Bid molecule is widely expressed. At the protein level, the full length Bid is a long-lived protein, but caspase-8 cleaved truncated Bid (tBid) is degraded through the ubiquitination proteasome system and has a half-life of less than 1.5 h.

The structure of Bid has been resolved with ► [NMR](#). It is the only structure resolved for a BH3-only molecule. Bid is composed of eight alpha helices. The central hydrophobic helices (alpha 6 and alpha 7) are surrounded by the amphipathic helices. Such an arrangement is conserved among other Bcl2 family proteins, such as Bcl-2, Bcl-x<sub>L</sub>, and Bax. There is a non-structural loop between alpha 2 and alpha 3, which is subjected to regulatory modifications by protease cleavage or phosphorylation. Similar loops are present in Bcl-x<sub>L</sub>, Bcl2 and Bax, which play the same regulatory role.

Bid does not have a transmembrane domain. It shares sequence homology with other Bcl-2 family proteins in the BH3 domain, which is important for its interaction with other family members and for its pro-apoptotic activity. Interaction of Bcl2 or Bcl-x<sub>L</sub> suppresses Bid by preventing it from interacting with the pro-death Bax or Bak.

## Bid as a Pro-death Sensor for Specific Protease Activation

Early studies based on transient transfection or an inducible system demonstrated that overexpression of full-length Bid could induce ► apoptosis. However, in most cases, it seems that Bid may function in a truncated form. Bid was initially found to be cleaved and activated by caspase-8 following death receptor activation and thus considered to be specific to the death receptor pathway. However, studies in recent years indicate that Bid can be cleaved in a specific and limited way by several other proteases such as Granzyme B, calpains, and cathepsins. These proteases are first activated in response to a plethora of stimuli, including death receptor activation, cytotoxic T cell attack, ischemia/reperfusion injury, and lysosome damage. These observations indicate that Bid is in general a sentinel to protease activation resulted from various injury stimuli. As such Bid serves a critical role in connecting these stimuli to the ► mitochondria, allowing the death process to be advanced or amplified.

The cellular death receptor pathway is activated when the death receptors, Fas, TNF-R1, or TRAIL-R1, are engaged by their ligands or agonistic antibodies. Both in vitro cell lines and in vivo animal models have been used to study the signaling events. In a murine model of anti-Fas antibody-induced liver injury, Bid has been found to play a significant role in Fas-mediated apoptosis. Normal wild-type mice are particularly susceptible to the administration of a Fas agnostic monoclonal antibody (clone Jo2), which induces significant hepatocyte apoptosis and severe liver injury. However, *Bid*-deficient mice are resistant to such a treatment with minimal hepatocyte apoptosis and liver injury. In these mice, while caspase-8 is appropriately activated, the downstream effector caspase-3 is not. Caspase-3 activation is arrested in *Bid*-deficient hepatocytes in a pattern consistent with being suppressed by XIAP, the X-linked inhibitor of apoptosis protein. In the wild-type mice, Bid is cleaved by caspase-8 and the truncated Bid is translocated to the mitochondria to induce the release of cytochrome c and Smac. While cytochrome c could activate Apaf-1 and therefore caspase-9, Smac is able to bind to XIAP to release its suppression on caspase-3 activation. In this scenario, Bid connects the death receptor pathway to the ► mitochondria pathway, which is necessary for the prompt

activation of effector caspases and subsequent apoptosis in hepatocytes.

## Activation of Mitochondria by Bid

The ability of Bid to activate the mitochondria pathway is related to its ability to interact with the mitochondria and to permeabilize the mitochondrial outer membranes. Bid is able to induce the release of multiple mitochondrial intermembrane space proteins, including cytochrome c and Smac/DIABLO. Full-length Bid is usually much weaker than truncated Bid in this capability. Bid is also able to induce several other prominent mitochondria dysfunctions, including mitochondrial permeability transition, mitochondrial depolarization, mitochondrial cristae reorganization, and the generation of mitochondrial ► reactive oxygen species. While the mechanisms for some of the phenomena are better understood, others are not.

In the case of cytochrome c release, it seems that Bid could activate at least two different mechanisms. One is based on protein interactions and the other is based on lipid interactions. Bid can interact with either Bax or Bak, the multi-domain pro-death Bcl-2 family proteins, via its BH3 domain, to promote their oligomerization on the mitochondrial outer membranes. Indeed, mice deficient in both Bax and Bak are much like *Bid*-deficient mice and are resistant to anti-Fas induced hepatocyte apoptosis in vivo. The other important mechanism is based on the interaction of Bid with cardiolipin at the mitochondrial contact site. This interaction promotes mitochondrial cristae reorganization, which contributes to the mobilization of stored cytochrome c and its subsequent release. As the majority of cytochrome c is tightly bound to cardiolipin, a full release of this molecule would require its dissociation from cardiolipin, which is facilitated by Bid–cardiolipin interactions.

The two mechanisms activated by Bid, involving proteins and lipids, respectively, seem to be well coordinated. Bid interaction with Bak or Bax is the primary mechanism, which initiates mitochondrial leakage. This requires the BH3 domain and can be blocked by Bcl-2 or Bcl-x<sub>L</sub>. On the other hand, the serial events of Bid–cardiolipin interaction and cristae reorganization do not require the BH3 domain of Bid and could not be suppressed by Bcl-2/Bcl-x<sub>L</sub>. The mobilized cytochrome c is released through the mechanism enforced by Bid–Bak or Bid–Bax interactions.

## Bid as a Pro-life Sensor for Cell Cycle Progression and DNA Damage

Although Bid was initially defined as a pro-death molecule, recent studies have shown that Bid can possess functions important to the life of a cell. Bid has a pro-proliferation activity and can also serve as a DNA damage sensor to participate in cell cycle arrest. Other Bcl-2 family proteins, such as Bcl-2, Bcl-x<sub>L</sub>, Bax, and Bad, have also been shown to possess the function of regulating cell cycle progression. From a broad point of view, it seems that Bcl-2 family proteins do not just simply regulate cell death, but also affect other key cellular events.

Bid seems to be able to regulate the G0-G1/S transition, as shown in several types of cells entering cell cycle from the resting stage. As a result, *Bid*-deficient cells are often delayed in entering S phase upon mitogen stimulation. How Bid may promote cell proliferation is not clear at the moment. Regulation at the cyclin and cyclin-dependent kinase could be a key mechanism, although this has yet to be determined.

Another function of Bid in cell cycle regulation relates to S/G2 transition in cells with DNA damage or under replication stress. Thus *Bid*-deficient cells fail to be arrested at S/G2 boundary in these conditions. Further studies showed that Bid can be a phosphorylation substrate of ATM/ATR. Mutagenesis studies indicate that Bid phosphorylation by ATM/ATR is required for the S phase arrest following DNA damage. However, it is not clear how Bid may then contribute to the S phase arrest. It seems that this function of Bid is beneficial to cells so that they would not have to go into mitosis in the presence of DNA damage. Thus the protective effect of Bid phosphorylation in this case could be largely due to its effect in inducing S phase arrest. Finally, this ability of Bid is not dependent on its BH3 domain.

## Role of Bid in Oncogenesis

In general, neoplasia could be resulted from an uncontrolled cell proliferation owing to the activation of oncogenes, or from deregulated cell survival owing to the overexpression of anti-death molecules or the loss of pro-death molecules. For the Bcl-2 family proteins, it is generally assumed that their role in tumorigenesis is related to their ability to regulate cell death. However, other functions of the Bcl-2 family proteins can be equally important in tumorigenesis.

Bid-deficient mice develop spontaneous chronic myelomonocytic leukemia when they become aged. This may be explained by the loss of the pro-death activity of Bid. However, *Bid*-deficient mice do not have an enhanced development of liver cancers following the administration of a chemical carcinogen, diethylnitrosamine (DEN). In contrast, they manifest a delayed development of tumors despite that there is a reduced cell death in the affected livers. These observations could be better explained by the role of Bid in promoting cell proliferation. Indeed, Bid was subsequently found to have such a function. Since this ability to regulate cell cycle progression is also possessed by other Bcl-2 family proteins, it is possible that Bcl-2 family proteins can in general affect tumorigenesis via both of their functions in cell death and cell proliferation. The net effects could be specific to the affected tissue or the etiology of the tumor.

In addition, as far as Bid is concerned, one may have to also consider whether the regulation of mitosis checkpoint by Bid following DNA damage can be another key factor in affecting tumorigenesis. There is a significant presence of genomic instability in *Bid*-deficient myeloid cells. It is possible that the myeloid cells are prone to DNA damage, which in the absence of Bid would lead to an accumulation of DNA abnormalities and subsequent leukemogenesis.

## Summary

Bid is a versatile multifunction BH3-only molecule. While its function was initially defined to be pro-apoptosis, it is now clear that it can also regulate cell proliferation and genomic stability. These functions could be intimately connected and are overall responsible for the role of Bid in cell death, tissue injury, cell proliferation, and cooncogenesis. Future studies would be devoted to understand how these functions are integrated and regulated, and what the underlining mechanisms are.

### ► BH3-Interacting Death Domain Agonist

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## Bidirectional Differentiated Malignant Tumors

### Definition

Possess features of both endothelial cells and mesenchymal cells. ► [Melanoma](#), alveolar rhabdomyosarcomas, mesothelial sarcomas, synovial sarcomas, and epithelioid sarcoma belong to bidirectional differentiated malignant tumors.

### ► [Vasculogenic Mimicry](#)

## BIG

Breast International Group [1]

### References

1. <http://www.breastinternationalgroup.org>

## 2,3'-Biindolinylidene-2,3'-diones

### ► [Indirubin and Indirubin Derivatives](#)

## BIK Proapoptotic Protein

Toshi Shioda

Massachusetts General Hospital Center for Cancer Research, Charlestown, MA, USA

### Synonyms

**BIK (BCL-2 interacting killer); Bp4; NBK (Natural born killer)**

### Definition

BIK is a ► [BRAF](#) localized exclusively on the cytosolic surface of the endoplasmic reticulum (ER) membrane. Although the amino acid sequence of mouse Blk (BIK-like killer; also known as Biklk for Bik-like) has only 43% identity to that of human BIK, Blk is usually considered mouse ortholog of BIK due to their functional similarities. The official gene symbol of Blk is *bik*. Blk should not be confused with *B lymphoid* tyrosine kinase, a member of the SRC family nonreceptor protein tyrosine kinase and whose official gene symbol is BLK.

### Characteristics

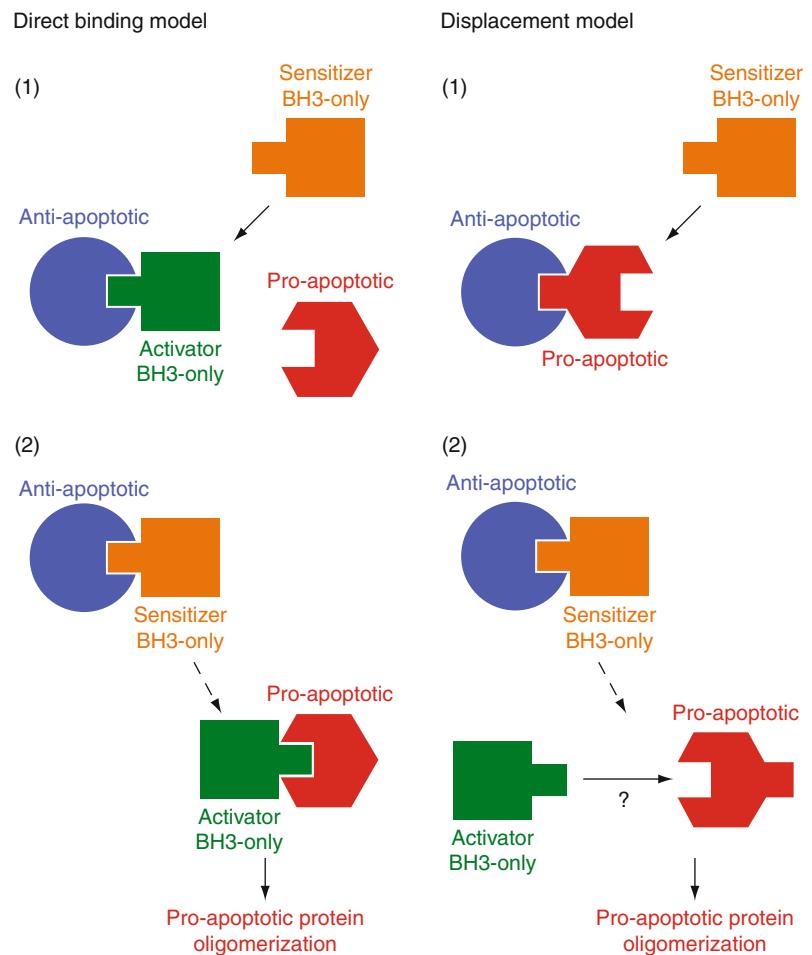
#### Discovery

BIK was originally cloned in a two-hybrid screening as a protein that interacts with cellular antiapoptotic proteins ► [BCL-2](#) and BCL-xL as well as viral antiapoptotic proteins ► [Epstein–Barr virus BHRF1](#) and the ► [32P-postlabeling](#), both of which were viral homologues of human ► [BCL-2](#). In an independently performed two-hybrid screening using the 19-kDa adenovirus E1B protein as bait, BIK was isolated as an E1B-binding protein and described as cDNA clone Bp4, which was later renamed NBK. These early studies characterized BIK/NBK as an apoptosis-inducing protein. Later, a DNA microarray study performed by an independent investigator identified BIK as a protein induced by the ► [Adenovirus E1A protein](#) in human KB epithelial cells.

#### Protein Structure and Molecular Functions

Human BIK consists of 160 amino acids, and its ► [BH3 domain](#) spans from amino acid 57(Leu) to 71(Ser). The strongly hydrophobic, leucine-rich C-terminal transmembrane domain (amino acids 136–159) selectively anchors BIK to the ER membrane, thus almost entire BIK protein including its N-terminus is exposed to cytosol. In human cells, BIK is phosphorylated at 33(Thr) and 35(Ser) by an unidentified, casein kinase II-like enzyme. Substitutions of these amino acids with alanines reduce the proapoptotic activity of BIK without significantly affecting its protein stability or ability to heterodimerize with BCL-2. Conversely, substitutions of these amino acids with aspartic acids enhance the proapoptotic activity.

**BIK Proapoptotic Protein.**  
**Fig. 1** Mechanism of the proapoptotic actions of the BH3-only proteins



When expressed in mammalian cells, either endogenously or from exogenous vectors, BIK potently induces apoptosis. This activity involves the release of cytochrome c from mitochondria and is entirely dependent on BAX, a proapoptotic member of the BCL-2 family. However, BIK does not directly bind to BAX, nor affect mitochondrial membrane potential or voltage-dependent anion channel activity. Instead, BIK directly binds to BCL-2, BCL-xL, and BCL-w antiapoptotic members of the BCL-2 family. Therefore, BIK is a typical sensitizer-type BH3-only protein. In contrast, the activator-type BH3-only proteins bind directly to BAX or BAK, the proapoptotic members of the BCL-2 family that possess multiple BCL-2 homology (BH) domains and oligomerize on the mitochondrial outer membrane to form a channel that release cytochrome c to the cytosol. The antiapoptotic members of the BCL-2 family bind to the activator-type BH3-only proteins and thus sequester them from

interacting with BAX or BAK (Fig. 1). Since each sensitizing and activator BH3-only protein has discrete binding specificity and affinity to the antiapoptotic members of the BCL-2 family, the apoptotic signal is activated only when correct combinations of these three groups of proteins are expressed (Table 1). Reflecting its weak affinity to MCL-1, an antiapoptotic member of the BCL-2 family, BIK cannot kill cells in the presence of sufficient expression of MCL-1. When MCL-1 expression is weak and BCL-2/BCL-xL/BCL-w expression is high, BIK can kill cells in the presence of tBID as an activator BH3-only protein, showing specificity similar to that of BMF. However, BIK also has to cooperate with another, weak BH3-only protein such as NOXA to cause rapid release of mobilized cytochrome c and subsequent activation of caspases.

The activator BH3-only proteins such as tBID or BIM directly bind to BAK or BAX, the proapoptotic

**BIK Proapoptotic Protein. Table 1** Effects of combinations of anti-apoptotic, sensitizer BH3-only, and activator BH3-only proteins on induction of cell apoptosis

Anti-apoptotic BCL-2 family	Activator BH3-only	Sensitizer/inactivator BH3-only proteins				
		None	BAD	NOXA	BMF	BIK
BCL-2	tBID	n	Y	n	Y	Y
BCL-xL	BIM	n	Y	n	n	n
	PUMA	n	Y	n	n	n
MCL-1	tBID	n	n	Y	n	n
	BIM	n	n	Y	n	n
	PUMA	n	n	Y	n	n

Modified from Kim et al. [2]

n no apoptosis, Y apoptosis

multi-BH domain members of the BCL-2 family. These activator BH3-only proteins are sequestered by the antiapoptotic members of the BCL-2 family proteins BCL-2, BCL-xL, BCL-w, and/or MCL-1. When the sensitizer BH3-only proteins bind to the antiapoptotic members, the activator BH3-only proteins are released from them and instead bind to BAK or BAX, which then oligomerizes to form channels through the outer membrane of mitochondria to release cytochrome c to the cytosol. Thus, the sensitizer and activator BH3-only proteins compete for binding to the antiapoptotic proteins.

At the ER, BIK can initiate early release of Ca<sup>2+</sup> from the ER lumen to cytosol in response to apoptotic stimuli. This BIK-activated Ca<sup>2+</sup> release requires BAK recruitment to the ER membrane. The reuptake of cytosolic Ca<sup>2+</sup> by mitochondria causes recruitment and activation of the fission enzyme DRP1 at discrete sites on the mitochondrial tubular network, resulting in mitochondrial fragmentation and cristae opening but minimal release of cytochrome c. Since loss of the GTPase activity of DRP1 results in suppression of mitochondrial fission and cytochrome c release during apoptosis, the BIK-dependent early morphological changes in mitochondria may enhance cytochrome c release, which is later induced by activation of BAX/BAK in the presence of both BIK and NOXA.

### Gene Structure, Expression, and Phenotypes

The *BIK* gene is found in human, bovine, rat, and mouse genomes but not in frog or fish. Therefore, among the BH3-only proteins, BIK seems a relatively new, mammalian-specific member. The human *BIK*

gene is localized to chromosome 22q13.3 and is comprised of five exons spanning in about a 19-kb region. The minimal BIK promoter is localized to a region between -211 and +153 bp relative to its transcription initiation site. Although it was originally reported as a TATA-less promoter, a later study and the EST data suggest possible involvement of a TATA-like sequence in its transcriptional activity. No evidence of alternative promoters or splicing has been found in the EST database or literature.

The BIK mRNA transcripts are strongly expressed in lymphatic tissues and endothelial cells of the venous (but not arterial) lineages. Normal adult mammary and prostate glands also express significant amounts of BIK mRNA. C57BL/6 mice express Bik mRNA in the liver, lung, heart, and kidneys; weaker expression was also detected in spleen, skeletal muscle, and salivary gland. Mechanisms of the BIK tissue-specific distribution are unknown. At least one strain of *bik* gene knockout mice was generated, but no significant phenotype has been observed with them. However, simultaneous knockdown of *bik* and *bim* genes causes male infertility with severely perturbed spermatogenesis. Bik and Bim may share the role of eliminating supernumerary germ cells during the first wave of the permatogenesis, a process critical for normal testicular development.

The A/WySnJ mice, which have 90% fewer peripheral B cells than normal animals and fail to make significant immunoglobulin memory response, overexpress the Bik mRNA transcripts, and their transitional B cells rapidly succumb to apoptosis in vitro. During the transition from naïve B cell to centroblast B cell, expression of the BIK mRNA transcripts increases by about 8.5-fold, and the high level of BIK mRNA expression is maintained in memory B cells. These observations suggest possible roles of BIK during B-cell maturation.

Expression of the BIK mRNA transcripts and protein is inducible in human cells by overexpression of wild type ► p53 protein. Adenovirus E1A protein also induces BIK in a manner dependent on p53 protein. Apoptosis-inducing stimuli that involve p53 protein activation, such as doxorubicin or gamma-irradiation, induce BIK expression as well. Doxorubicin may be able to activate *BIK* gene transcription by a mechanism independent of p53 protein but involving the E2F transcription factors. In MCF-7 human ► breast cancer cells, antiestrogens such as ► tamoxifen and ► fulvestrant induce BIK expression in a manner

dependent on p53 protein, but its mechanism may be independent of the transcription factor activity of p53 protein.

### Relevance to Cancer Genetics and Therapy

Significant frequency of missense mutations in the *BIK* gene was observed in peripheral B-cell lymphomas. Chromosomal deletions causing loss of heterozygosity (LOH) of the *BIK* locus has been reported for head and neck tumors, colorectal cancers, glioblastomas, and clear-cell renal cell carcinomas. Epigenetic silencing of *BIK* mRNA expression was reported for the KAS-6/1 multiple myeloma cell line and in a number of cell lines of renal cell carcinoma. Although these data suggests possible roles of *BIK* in human carcinogenesis, this remains to be established by further studies.

Since the strong proapoptotic activity of *BIK* potently induces ► apoptosis even in malignant cells, a number of studies reported the possible application of *BIK*-expressing vectors in the context of ► gene therapy. A chimeric protein consisting of gonadotropin releasing hormone (GnRH) and *BIK* specifically killed adenocarcinoma cell lines expressing plasma membrane GnRH receptor in vitro. In vivo growth of a human melanoma cell line stably transfected with a doxycycline-inducible expression plasmid for *BIK* as nude mice xenograft was strongly inhibited by doxycycline administration in drinking water. When administered systematically by intravenous injection, a cationic liposome-based gene delivery system of a *BIK* expression plasmid effectively suppressed growth of human breast cancer xenografts in nude mice. An adenoviral expression system of *BIK* induced apoptosis in glioma cell lines, and intratumoral injection of an ► adenovirus vector expressing *BIK* significantly suppressed growth of prostate (PC-3) and colon (HT-29) tumor xenografts in nude mice. A liposome-based, systematic intravenous delivery of a plasmid that expressed *BIK* using the pancreatic cancer-specific, cholecystokinin type A receptor promoter completely suppressed growth of human PANC-1 cells as xenograft in nude mice. These results suggest promise for *BIK* as a cytotoxic protein agent in gene therapy.

Because of the apoptosis sensitizing activity of *BIK*, low-level expression of exogenously introduced *BIK* that cannot induce apoptosis by itself may still be able to enhance cellular sensitivity to apoptotic stimuli. Thus, *BIK*-enhanced sensitivity of the H9

human T-cell leukemia cell line to chemotherapeutic agents increased apoptosis by 10- to 39-fold. Breast cancer cell lines selected for doxorubicin resistance were also effectively sensitized by expression of exogenously introduced *BIK*. These results suggest possible use of *BIK* in adjuvant gene therapy in combination with apoptosis-inducing chemotherapy.

The intracellular half-life of *BIK* protein is very short because of its rapid degradation by a proteasome-dependent mechanism. Therefore, when cells are exposed to proteasome inhibitors such as lactacystin, MG-132, or ► bortezomib, strong intracellular accumulation of *BIK* protein is often observed. Bortezomib does not cause significant accumulation of other BCL-2 family proteins except for NOXA. The bortezomib effect to resensitize malignant cells resistant to the death receptor ligand ► TRAIL is dependent on *BIK* protein accumulation. Bortezomib-induced ZR75-1 breast cancer cell apoptosis is also largely dependent on the accumulated *BIK* protein. Thus, proteasome inhibitors might be useful as adjuvant therapy agents for the purpose of increasing expression of the endogenous *BIK* protein in malignant cells to enhance their sensitivity to apoptosis-inducing chemotherapy.

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### Bilateral Acoustic Neurofibromatosis

- Neurofibromatosis 2

## Bilateral Salpingo-Oophorectomy

### Definition

A bilateral salpingo-oophorectomy is a surgery in which both a woman's ovaries are removed, along with the fallopian tubes. This surgery is used primarily to treat gynecological cancers such as ► [ovarian cancer](#), fallopian, and ► [uterus cancer](#), although it is used in the treatment of some other gynecological conditions as well. One of the major consequences of the bilateral salpingo-oophorectomy surgery is that the woman becomes infertile, and she also stops producing a variety of hormones, which triggers the onset of menopause.

This surgery is usually only recommended in cases where it is really needed, because of the very serious consequences. In addition to being included in the treatment plan for some cancers, the bilateral salpingo-oophorectomy is sometimes also recommended in the case of severe infections related to Pelvic Inflammatory Disease (PID), or in patients with extreme endometriosis. The gynecological or general surgeon who performs the procedure will usually discuss the issues involved and all of the available options with the patient before scheduling the surgery.

## Bile

### Definition

Bile is a yellow, green digestive juice produced by the liver and stored in the gallbladder. It passes through the bile ducts into the duodenum where it aids in digestion and absorption of dietary fat. Bile is predominately composed of bile salts, cholesterol, phospholipids, bicarbonate, and waste products such as bilirubin. The electrolytes present in bile especially bicarbonate which neutralize acid secretions from the stomach as they enter the duodenum.

- [Bile Duct Neoplasms](#)
- [Gallbladder Cancer](#)

## Bile Acids

Toshiya Soma and Yutaka Shimada

Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

B

### Definition

Bile acids are complex physiological molecules that are essential for solubilization, absorption, and transport of dietary lipids in the intestine. On the other hand, bile acids are potentially toxic to cells.

### Characteristics

Primary bile acids such as cholic acid and chenodeoxycholic acid are derived from cholesterol in the liver and are secreted and stored in the gallbladder as glycine or taurine conjugates. For example, cholic acid is stored as either glycocholic acid or taurocholic acid, and chenodeoxycholic acid is stored as either glycodeoxycholic acids or taurodeoxycholic acids. Bile acids are then released into the intestinal tract when fat enters the proximal portion of the intestine. About 90–95% of released bile acids are absorbed in the terminal ileum. Bile acids are transported via the portal vein to the liver and extracted for re-use in the so-called enterohepatic circulation. However, about 5–10% of secreted bile acids reach the colon, where conjugated cholic acid and chenodeoxycholic acid undergo deconjugation and 7 $\alpha$ -dehydroxylation by the anaerobic bacterial flora, forming the secondary bile acids deoxycholic acid and lithocholic acid, respectively. The tertiary bile acid ursodeoxycholic acid is subsequently formed by epimerization of chenodeoxycholic acid. In the colon, deoxycholic acid is partly absorbed and enters the enterohepatic circulation. Consequently, about 2–5% of secreted bile acids, consisting mainly of lithocholic acid, are excreted in the stool.

### Toxicity of Bile Acids

Bile acids are probably not genotoxic but may be cytotoxic (► [Toxicological Carcinogenesis](#)). Specifically, unconjugated bile acids are known to be

toxic. Conjugation reduces the pKa of bile acids, thereby increasing solubility at low pH. Thus, at a pH lower than the physiological value, unconjugated bile acids precipitate easily, whereas conjugated bile acids remain soluble. Taurine conjugates are especially soluble even at more acidic pH. Hence, at physiological pH, bile acids usually remain as ionized forms and may be termed bile salts, which cannot pass through the cell membrane. However, at acidic pH, unconjugated bile acids are nonionized and can accumulate inside mucosal cells and potentially cause damage. An acidic pH thus does not affect the toxicity of conjugated bile acids, but may potentiate the toxicity of unconjugated bile acids. On the other hand, both conjugated and unconjugated bile acids reduce the pH sensitivity of cells. Decreased pH sensitivity results in the induction of cyclooxygenase-2, which is rapidly induced in response to tumor promoters, cytokines, and growth factors. Furthermore, cholic acid and chenodeoxycholic acid are considered to be tumor promoters and increase the incidence of benign adenomas and malignant adenocarcinomas when administered after carcinogens. It is thus likely that pH and bile acids are dual drivers of metaplasia, acting in combination to fuel inflammation and mediate cellular change.

### Effects of Bile Acids on Organs

#### Upper Gastrointestinal Tract

Several studies indicate that duodenogastroesophageal reflux leads to esophagitis or Barrett esophagus and may be related to esophageal adenocarcinoma. Although the pathogenesis of ► [esophageal cancer](#) remains to be fully elucidated, bile acids are somehow involved, probably by being cytotoxic rather than genotoxic. As such, bile acids stimulate the development of esophageal squamous cell carcinoma, not to mention esophageal adenocarcinoma or ► [gastric cancer](#), by promoting ► [angiogenesis](#) via the cyclooxygenase-2 pathway (► [Arachidonic acid-pathway and cancer](#)). In contrast, unconjugated bile acids, which are more toxic than conjugated forms, appear more frequently in the bile acid profiles of patients with severe esophagitis. Although reflux of unconjugated bile acids has not been demonstrated in patients with an intact stomach, such reflux has been found in the stomach after partial gastrectomy and in the esophagus after total gastrectomy. However, further studies are

required to establish whether bile acids have a role in gastric cancer.

#### Lower Gastrointestinal Tract

Several studies indicate that ► [colorectal cancer](#) is associated with higher fecal levels of secondary bile acids. Deoxycholic acid and lithocholic acid appear to promote carcinogenesis and tumorigenesis by activating multiple oncogenic signaling pathways (► [Cyclooxygenase-2 in colorectal cancer](#)). Furthermore, a high fat diet and cholesterol are implicated in the pathogenesis of human colorectal cancer, presumably because both of these factors promote the synthesis and secretion of bile acids. Carcinogenesis is also associated with conditions such as ileal resection, cholecystectomy, or ileal inflammation such as that accompanying ► [Crohn's disease](#), which can alter intestinal exposure to bile. These conditions can result in incomplete active reabsorption of bile acids from the distal ileum and interrupt the enterohepatic circulation of bile acids. In addition, increased colonic concentrations of bile acids are also associated with diarrhea, which may respond to bile acid sequestrants.

#### Liver, Gallbladder, and Bile Ducts

Several etiological studies indicate that bile acids might induce carcinogenesis in the gallbladder (► [Gallbladder cancer](#)). The gallbladder and bile ducts are exposed to high concentrations of bile acids, most of which are unconjugated. If retained for a long time in the gallbladder and bile ducts, bile acids may induce carcinogenesis in the biliary tree, although the mechanism remains unclear. Furthermore, numerous studies have shown that elevated concentrations of bile acids in the liver induce hepatocyte apoptosis. Some evidence suggests a relation between the hydrophobicity of bile acids and the induction of apoptosis. Thus, the degree of hepatocellular damage may be related to bile acid hydrophobicity, and lithocholic acid, the major constituent of hydrophobic bile acids, is the most hepatotoxic. These findings suggest a possible mechanism for bile-acid-mediated liver injury, but since most hepatic bile acids are in conjugated form, the hydrophobicity of bile acid is reduced, thereby decreasing its entry into cells. Moreover, the hepatotoxicity of chenodeoxycholic acid treatment in patients with cholelithiasis appears to be caused by secondary increases in lithocholic acid production.

## Pancreas

Epidemiological studies have demonstrated a positive correlation between the incidence of pancreatic cancer and a high fat diet in association with the secretion of bile acids (► [Pancreas cancer, clinical oncology](#)). Pancreatic adenocarcinoma tends to develop in the head of the gland, which is more exposed to bile. These findings suggest that bile acids participate in carcinogenesis of the pancreas, although underlying mechanisms remain to be clarified.

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## Bile Duct

### Definition

Bile ducts are long tube-like structures lined by epithelial cells that carry bile (originating from the bile canaliculus) from the liver to the hepatic duct, which joins with the cystic duct to form the common bile duct opening into the duodenum. Bile ducts within the tissue of the liver are termed intrahepatic, whereas those outside the liver are termed extrahepatic.

- [Bile Duct Neoplasms](#)
- [Gallbladder Cancer](#)

## Bile Duct Adenoma

- [Bile Duct Neoplasms](#)

## Bile Duct Carcinoma

- [Cholangiocarcinoma](#)
- [Klatskin Tumors](#)

## Bile Duct Hamartoma Biliary Cystadenoma

- [Bile Duct Neoplasms](#)

## Bile Duct Neoplasms

Shannon S. Glaser<sup>1</sup> and Gianfranco Alpini<sup>2</sup>

<sup>1</sup>Department of Internal Medicine, Texas A&M Health Science Center; Central Texas Veterans Health Care System, Temple, TX, USA

<sup>2</sup>Departments of Medicine and Systems Biology and Translational Medicine, Central Texas Veterans Health Care System, Scott & White Hospital and Texas A&M, Texas A&M Health Science Center College of Medicine, Temple, TX, USA

### Synonyms

Bile duct adenoma; Bile duct hamartoma; Biliary cystadenoma; Carcinoid; Cholangiocarcinoma Cholangiosarcoma; Intraductal papillary mucinous tumors

### Definition

Bile duct neoplasms are classified as benign and malignant tumors of the cells comprising the ► [bile ducts](#) of the liver, which include the bile duct epithelial cells and the connective tissues supporting the bile duct structure.

### Characteristics

The biliary system is comprised of intra- and extrahepatic ► [bile ducts](#). The function of this system is to

transport ► **bile** from the liver to the duodenum where bile aids in the digestion of dietary fats. Bile ducts have a tube- or vessel-like appearance. The interior of the ducts is lined with columnar epithelial cells, which have been termed ► **cholangiocytes**. Cholangiocytes are surrounded by a subepithelial layer of tough connective tissue, which contains a scant number of smooth muscle cells. Also, within this connective tissue layer, resides a population of mucous cells. The biliary system is surrounded by a network of nerves, blood vessels, and lymphatics. Bile duct neoplasms are an extremely rare condition that includes both benign and malignant growth of cholangiocytes and the surrounding supporting tissues of the bile duct.

### Benign Bile Duct Neoplasms

Benign tumors of the intrahepatic and extrahepatic biliary system are exceedingly rare as the large majority of bile duct neoplasms are malignant. These benign tumors include adenomas and papillomas and neoplasms of the supporting structure of the bile duct, such as bile duct hamartomas, carcinoids, leiomyomas, and fibromas. Some benign biliary lesions, such as papillomas, adenomas/carcinoids, and cystadenomas, result in biliary obstruction and symptoms of ► **cholestasis** and ► **jaundice**. The most commonly diagnosed benign neoplasms are bile duct hamartomas (von Meyenburg complexes), carcinoids, and cystadenomas.

Bile duct hamartomas are characterized by the growth of many tiny noncancerous nodules in the intrahepatic bile ducts, which are the result of the malformation of the ductal plates of the liver during embryonic development. Pathologically hamartomas are characterized by cysts dilated embedded in a fibrous, collagenous ► **stroma**. Hamartomas have for the large part been defined to be innocuous. Hamartomas have been associated with increased neoplastic transformation resulting in biliary adenocarcinoma (i.e., ► **cholangiocarcinoma**). Biliary cystadenomas arise from von Meyenburg complexes and are also a rare neoplasm of the bile duct that is difficult to diagnose preoperatively. Biliary cystadenomas occur more often in females. The predominant treatment for cystadenoma is surgical ablation due to the high potential for malignant transformation.

Benign carcinoid tumors are also extremely uncommon. These neoplasms arise from enterochromaffin cells of the biliary tract. Due to the rarity of this type

of tumor, carcinoids have been poorly characterized. Biliary carcinoids have not been associated with the production of functional hormones that has been reported for carcinoids in other areas of the gastrointestinal tract. Patients with carcinoids often present with symptoms mimicking cholangiocarcinoma and/or choledocholithiasis. Biliary carcinoids are slow growing and thus, have a low potential for malignant transformation. The predominant treatment for carcinoids is surgical removal.

Diagnostically, benign bile duct neoplasms are virtually impossible to differentiate from malignant neoplasms. Since these neoplasms are extremely rare, there is a lack of understanding of the potential for these tumors to become malignant. In certain cases, benign neoplasms are thought to contribute to ► **inflammation** of the liver from damage due to cholestasis. Therefore, surgical resection is the current and predominant treatment course.

### Malignant Bile Duct Neoplasms

Over 95% of bile duct neoplasms are malignant. Recent reports indicate that there is an increase in global incidence of malignant bile duct tumors. These malignant tumors include cholangiocarcinoma (an ► **adenocarcinoma**), cholangiosarcoma, malignant carcinoids, and intraductal papillary mucinous adenocarcinoma.

Cholangiocarcinoma is the predominant cancer of the bile ducts. Cholangiocarcinoma results from the malignant transformation of cholangiocytes, which are epithelial cells that line the biliary system. Cholangiocarcinoma occurs in ~2 per 100,000 people. Approximately 13% of primary ► **liver cancers** are cholangiocarcinomas. Cholangiocarcinoma is divided into two types: (1) intrahepatic that occurs in the bile ducts residing within the liver; and (2) extrahepatic that arises in the right and left hepatic ducts, common hepatic and common bile duct. Risk factors for this cancer share long-standing inflammation of the liver and chronic damage of the biliary epithelium. Increased proliferation of biliary epithelium due to chronic damage of the liver is thought to play a key role in the pathogenesis of malignant bile duct neoplasms. The list of risk factors includes: ► **gallstones** or gallbladder inflammation, ► **chronic ulcerative colitis**, or chronic infection of the parasitic worm, ► **Clonorchis sinensis**, and ► **primary sclerosing cholangitis (PSC)**. The prognosis for cholangiocarcinoma is grim due to lack of early

diagnostic modalities and effective treatment paradigms. Cholangiocarcinomas are slow growing, metastasize late during the cancer's progression, and present with symptoms of cholestasis due to the blockage of the bile duct by tumor growth. In most cases, the tumors are well advanced at the time of diagnosis, which results in limited treatment options. Many of these tumors are too advanced to be removed surgically and chemotherapy and radiation therapy usually are not effective. In addition to cholangiocarcinoma, cholangiosarcoma is a tumor arising from the cells constituting the connective tissue layer of the bile ducts. Cholangiosarcoma is rarely reported and information is lacking on prevalence.

A subset of cholangiocarcinoma tumors have been defined as papillary cholangiocarcinoma or interductal papillary mucinous neoplasms. These tumors are characterized by frond-like, papillary projects that occasionally produce large amounts of mucus. The excessive mucus secretions may disturb bile flow and cause dilation of the bile ducts, which results in symptoms of obstructive cholestasis or bile duct stones. Interductal papillary tumors have low-grade malignancy penetrating the bile duct wall in the late stages of pathogenesis. Diagnostically, these tumors are often confused with bile duct stones due to the constant sloughing of tumor debris into the bile. The predominant treatment is surgical removal.

Malignant carcinoids are also extremely rare neoplasms. Similar to benign carcinoids, the treatment of choice is surgical removal with chemotherapy used when the tumors are metastatic.

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## Biliary Glycoprotein

### Definition

A member of the ► [CEA gene family](#) first found in bile. The BGP group of molecules consists of seven members formed by alternative splicing. There are differences in the number of Ig domains and there are two types of cytoplasmic domains present. The forms with longer cytoplasmic domains can participate in signaling.

### ► CEA

## Bilirubin

### Definition

Is formed by the breakdown of heme present in hemoglobin, myoglobin, cytochromes, catalase, peroxidase, and tryptophan pyrrolase. Eighty percent of the daily bilirubin production (250–400 mg in adults) is derived from hemoglobin; the remaining 20% being contributed by other hemoproteins and a rapidly turning-over small pool of free heme. Enhanced bilirubin formation is found in conditions associated with increased red cell turnover such as intramedullary or intravascular hemolysis (e.g., hemolytic, dyserythropoietic, and megaloblastic anemias) and in syndromes where bilirubin metabolism is impaired, such as ► [Gilbert syndrome](#) or ► [Crigler–Najjar syndrome](#).

## Bin1

George C. Prendergast

Department of Pathology, Anatomy and Cell Biology,  
Jefferson Medical School, Lankenau Institute for  
Medical Research, Wynnewood, PA, USA

## Synonyms

[Amph II](#); [Amphiphysin II](#); [Amphiphysin-like](#); [AMPL](#); [SH3P9](#)

## Definition

Bin1 is a cancer suppression gene that functions in membrane dynamics, ► vesicle trafficking, and nucleocytosolic signaling processes. The Bin1 gene maps to human chromosome 2q14–2q21.

## Characteristics

Bin1 encodes a set of BAR adapter proteins that bind and tubulate curved membranes in the cytosol and that can restrict gene expression in the nucleus. Bin1 protein structure is varied by alternate RNA splicing events that determine its cancer suppression activity. All Bin1 proteins include an N-terminal ► BAR domain and a C-terminal ► SH3 domain. Two isoforms found in all cells localize to the nucleus and cytoplasm and both display cancer suppression activity. Several other tissue-specific isoforms found mainly in neurons include specialized membrane targeting sequences that prevent nuclear entry. These isoforms lack cancer suppression activity. In fact, Bin1 suppression activity is often inactivated in cancer cells by a specific RNA missplicing event that adds a neuron-specific exon (12a) preventing nuclear entry by the aberrant Bin1 protein generated. Studies of RNA splicing patterns in cancer cells indicate that this aberrant event is among the most common missplicing events occurring in human cancer.

Loss of heterozygosity at the Bin1 locus occurs with some frequency in ► Prostate Cancer, but in general deletions of Bin1 seem to be rare in human cancer. In contrast, Bin1 is often attenuated at the level of missplicing or loss of expression, including in breast (► Breast Cancer), prostate, lung (► Lung Cancer), and ► colon cancer and in ► astrocytoma, ► neuroblastoma, and malignant ► melanoma. In breast cancer, loss of nuclear Bin1 protein may predict poor prognosis. Restoring normal expression in cancer cells can restrict cell proliferation and/or survival, including by eliciting a caspase-independent mechanism of cell suicide. Thus, attenuation of the nuclear function(s) of Bin1 is important during cancer development or progression.

Genetic and cell biological studies in animal model systems indicate that Bin1 acts at several levels to suppress cancer, including by blocking cell proliferation, survival, motility, and ► immune escape. Bin1

was initially identified through its ability to interact with and inhibit the transcriptional and oncogenic activity of the ► Myc oncogene. There is also some evidence that Bin1 may also facilitate Myc-mediated ► apoptosis in certain settings. Furthermore, genetic ablation of Bin1 in the mouse mammary gland drives the progression of lesions initiated by activation of the Ras pathway, which cooperates with Myc in triggering neoplastic cell transformation. Thus, Bin1 may act in part to suppress cancer by restraining the oncogenic activity of Myc. In animals where Bin1 is more widely ablated, inflammation (► Inflammation), premalignant lesions, and tumors occur with a markedly increased incidence during aging. In particular, lung or liver tumors occur within 18 months in most animals where Bin1 is ablated. Mouse model studies further indicate that Bin1 acts to restrict immune escape, an important trait of cancer which is highly relevant to the emergence of clinical disease. At this level, Bin1 acts by restricting expression of ► indoleamine 2, 3-dioxygenase, an important modulator of T-cell immunity in cancer. Thus, Bin1 loss during tumor development influences the immune ► microenvironment as well as the cancer cell itself.

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## BING2

- Daxx

## Bioactivation

### Definition

Is the transformation of a compound within an organism into a more biochemically active metabolite.

- [Arylamine N-Acetyltransferases \(NAT\)](#)

## Bioactive Lipids

- [Lipid Mediators](#)

## Bioavailability

### Definition

Bioavailability of nutrients: Refers to the fraction of a mineral nutrient intake that is biologically available to meet the essential metabolic and/or structural functions associated with that mineral nutrient in the body. Bioavailability incorporates the concepts of absorption, distribution, metabolic transformation (where necessary to meet biological function), and excretion. In some circumstances the main component of bioavailability is absorption across the gastrointestinal wall, so that sometimes the term bioavailability may be used interchangeably with absorption.

Bioavailability of drugs: Refers to the percentage of drug that is detected in the systemic circulation after its administration. Losses can be attributed to an inherent lack of absorption/passage into the systemic circulation and/or to metabolic clearance. Detection of drug can be accomplished

- [pharmacodynamically](#) or ► [pharmacokinetically](#).

Oral bioavailability is associated with orally administered drugs.

- [Genistein](#)
- [Lead Optimization](#)
- [Mineral Nutrients](#)
- [Personalized Cancer Medicine](#)

## Biochip

- [Proteinchip](#)

## Bioconjugate

### Definition

A covalent or non-covalent coupling two or more distinct molecules together to confer a specific functionality. For example, the coupling of aptamer targeting molecules to drug encapsulated nanoparticles can result in a nanoparticle drug delivery bioconjugate that is targeted to specific cells or tissues.

- [Aptamer Bioconjugates for Cancer Therapy](#)

## Biodribin

- [Cladribine](#)

## Biogenic Amines

### Definition

A group of naturally occurring, biologically active amines, such as monoamines (norepinephrine, histamine, tyramine, dopamine, and serotonin) and polyamines (putrescine, spermidine, and spermine).

- [Amine Oxidases](#)

## Bioinformatics

### Definition

Is a discipline covering all aspects of biological information acquisition, processing, storage, visualization, distribution, as well as analysis and interpretation that

combines the tools of mathematics, computer science, and biology to advance the scientific understanding of the biological significance of huge amount of data. It involves the creation and advancement of algorithms, computational and statistical techniques, as well as the theories to solve formal and practical problems inspired from the management and analysis of biological data. Genomics and proteomics analyses generate expression information from tremendous amount of genes or proteins, which have to be organized, stored, and analyzed with the aid of bioinformatics.

- ▶ [Drug Design](#)
- ▶ [Personalized Cancer Medicine](#)

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## Biologic Therapy

### Definition

Treatments for autoimmune disease or cancer that comprise proteins such as antibodies and cytokines or fragments of proteins or synthetic peptides are called biologic therapy. The term also encompasses the use of cells such as bone marrow-derived cells for treatment.

- ▶ [Sjögren Syndrome](#)

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## Biological Clock

### Definition

The mechanism within an organism that generates repeated cycles (rhythms, oscillations) in behavioral, biochemical, metabolic, and/or physiological activity that can be synchronized by environmental stimuli, primarily light.

- ▶ [Melatonin](#)

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## Biological Markers

- ▶ [Clinical Cancer Biomarkers](#)

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## Biological Monitoring

- ▶ [Biomonitoring](#)

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## Biological Response Modifiers

### Definition

Substances, either natural or synthesized, that boost, direct, or restore normal immune defenses, BRMs include interferones, interleukins, thymus hormones, and monoclonal antibodies.

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## Biological Therapy

- ▶ [Immunotherapy](#)

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## Biologically Effective Dose

### Definition

BED is the quantity of a drug that results in a therapeutic benefit. The accurate measurement of the BED is essential for the clinical evaluation of cytostatic molecularly targeted drugs.

- ▶ [Drug Design](#)

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## Biologicals

### Definition

Biologicals are large drug compounds with atomic mass units typically in the tens of thousands, e.g., vaccines, monoclonal antibodies, fusion proteins, Pegylated proteins (proteins chemically modified with long chains of polyethylene glycol).

- ▶ [ADMET Screen](#)

## Bioluminescence Imaging

Scott K. Lyons

Molecular Imaging Group, CRUK Cambridge Research Institute, Li Ka Shing Centre, Cambridge, UK

### Synonyms

BLI

### Definition

A noninvasive imaging approach that relies upon the detection of light resulting from the oxidation of a specific substrate by luciferase enzyme expressing cells.

### Characteristics

Bioluminescence imaging (BLI) comprises a relatively new preclinical imaging modality that has become popular with researchers across a broad range of biological disciplines. From an oncology perspective, the high sensitivity, versatility, and speed afforded by BLI has made it a particularly attractive modality for measuring many aspects of in vivo tumor biology within cohorts of animals.

In general terms and in comparison to the other noninvasive imaging modalities, the hardware and consumable reagents needed for BLI are relatively cheap and safe (i.e., non-radioactive). In vivo images of multiple subjects can be acquired quickly (typically ranging between 1 and 180 s), so throughput is high. Moreover, when used in conjunction with small animal models of disease, where tissue depths seldom exceed 1–2 cm, BLI can exhibit sensitivity limits that compare very favorably with ► PET (► Positron Emission Tomography) (currently deemed the most sensitive noninvasive imaging approach). Unlike ► CT (► Computed Tomography) or ► MRI (► Magnetic Resonance Imaging), BLI does not generate images with a high degree of anatomical detail. The wavelengths of light generated in this approach are prone to scattering and absorption as they pass through

tissue, resulting in an imaging resolution of approximately 1 mm. As BLI is reliant on the expression of a luciferase transgene, it is also a highly versatile technique that can be used to noninvasively determine diverse aspects of in vivo tumor biology, ranging from the relative quantification of tumor burden to measuring the activation states of various cellular processes or detecting specific protein–protein interactions. In addition, as only viable labeled cells generate bioluminescence in the vast majority of cases, BLI has proven particularly useful for testing the in vivo efficacy of experimental cancer treatments using tumor cell lines and small animal models of cancer.

### About Luciferases

As stated in the definition, BLI relies upon the external detection of light produced by cells that express a luciferase enzyme. These are not endogenously expressed by mammalian cells, therefore, luciferase transgene expression must first be introduced prior to the direct imaging of tumor cells by this method. The most commonly used luciferase enzymes for such purposes have been the ► codon-optimized (Codon-optimization) forms of firefly luciferase (derived from the North American firefly, *Photinus pyralis*) and renilla luciferase (derived from the sea pansy, *Renilla reniformis*). Also validated for expression in mammalian cells are the click beetle luciferases (green or red; derived from the Jamaican click beetle *Pyrophorus plagiophthalmus*) and gaussia luciferase (derived from a copepod called *Gaussia princeps* and unique amongst the enzymes mentioned in that it is naturally secreted by expressing cells).

These individual luciferase enzymes share little homology and vary in the efficiency that they produce light. To generate bioluminescence, firefly and click beetle luciferases specifically catalyze the oxidation of D-luciferin in the presence of O<sub>2</sub>, ATP and Mg<sup>2+</sup>. Renilla and gaussia luciferases, however, specifically catalyze the oxidation of a different substrate, coelenterazine, in the presence of O<sub>2</sub> and independently of ATP. Neither of these substrates are produced endogenously by mammalian cells and so must be administered either to tissue culture medium or by injection/► osmotic pump to enable in vitro or in vivo bioluminescence imaging respectively. Both substrates are relatively small molecules that are broadly taken up by cells throughout the body and can be administered repeatedly without eliciting an

immune reaction. The *in vivo* use of coelenterazine is slightly more complicated than luciferin, however, as it is less soluble and prone to auto-oxidation (resulting in bioluminescent “noise”) and deactivation in serum. It has also been shown that coelenterazine is actively pumped out of cells that express high levels of ► **P-glycoprotein**. Therefore, renilla or gaussia luciferases are likely not ideal reporters to directly image multidrug resistant (i.e., P-glycoprotein overexpressing) tumor cells *in vivo*. Similarly, it has recently been shown that luciferin is a substrate for the ABCG2/BCRP transporter, therefore the relevant luciferin-oxidizing luciferases are likely not ideal reporters for imaging models that overexpress this transporter.

The emission spectra produced by these luciferases are also characteristically broad (spanning >100 nm) and differ. For example, the peak emission of renilla luciferase is 480 nm, whereas firefly luciferase is 610 nm. Collectively, these factors ensure that bioluminescent signals from selected pairs of luciferase enzyme can be discerned upon the basis of substrate exclusivity as well as their spectral signature. This is highly useful as it enables the employment of powerful dual-labeled BLI studies, where the light generated by different luciferase enzymes can be detected sequentially to measure multiple parameters within the same cell or individual animal (e.g., viable tumor burden measured by renilla luciferase and the activation of a cellular process by firefly luciferase). The development of increasingly sophisticated ► **spectral unmixing** image analysis techniques should soon make it possible to routinely discern the optical spectra from two different luciferases when both substrates are administered simultaneously.

### How Is In Vivo Bioluminescence Detected?

The intensity of light generated by luciferase-labeled cells in a typical bioluminescence imaging experiment is sufficiently low that a highly sensitive light detector is needed to measure it. Such detectors are commercially available and typically comprise a cryogenically cooled CCD camera (Charge-Coupled Device; cooled to  $\leq -90^{\circ}\text{C}$  to reduce thermal noise and increase light sensitivity) that is housed behind a lens within a completely light tight box. The non-visible levels of light associated with bioluminescence imaging can be detected by the pixels of the cold CCD, which results in a fully digitized and quantifiable 2-D map of light

intensity across the field of view. An image of this light intensity map is then superimposed over a digital photograph of the subject (taken in normal light conditions immediately prior to bioluminescence acquisition) to indicate the regions of the subject where labeled cells reside (Fig. 1).

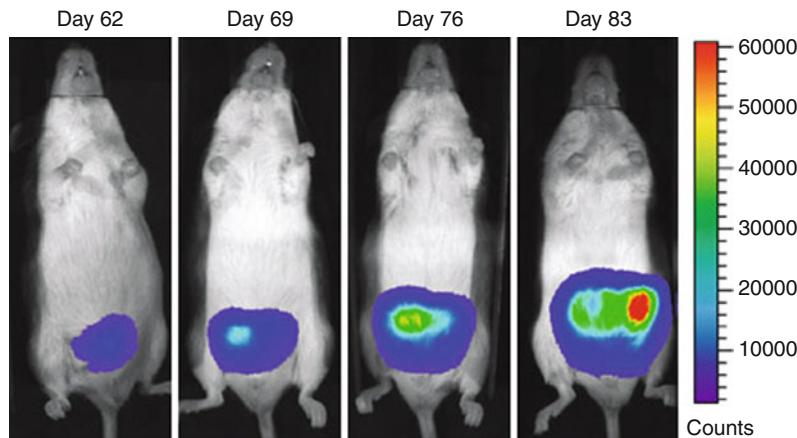
In a manner analogous to conventional photography, the exposure time and aperture settings of the CCD camera can be adjusted to modify the sensitivity of bioluminescence acquisitions. This ensures that CCD pixels do not become saturated when imaging relatively bright subjects and maximizes sensitivity when imaging relatively dim subjects. Computer software can be used to add together (or “bin”) the signals detected by adjacent CCD pixels to further increase imaging sensitivity, but this gain is made at a cost to image resolution. Software tools are also used to create “regions of interest” to quantitatively measure light emission from any area of the image in fully calibrated physical units (i.e., photons/s/cm<sup>2</sup>/► **steradian**).

### Considerations to Maximize In Vivo Imaging Sensitivity

Currently, BLI is considered one of the most sensitive noninvasive preclinical imaging modalities when used in conjunction with small animal models of disease. There are, however, several important factors that will affect the sensitivity of any *in vivo* BLI approach and so influence the minimum number of cells that can be detected or the ability to visualize the activity levels of a cellular process above noise.

One obvious issue relates to the extent of luciferase enzyme expression in the target cell; labeled cells that express relatively low levels of luciferase will be harder to detect than an equivalent number of labeled cells that express greater amounts of luciferase.

Another key issue is the depth of signal, as the wavelengths of light produced by the commonly employed luciferases are prone to scatter and absorption as they pass through mammalian tissue. Red wavelengths of light (>600 nm) pass through tissue with greater efficiency than relatively bluer wavelengths (<500 nm). Therefore, in principal, luciferases that produce light with the highest proportion of red light should be better-suited for *in vivo* imaging. The quantum yield or relative brightness of the different luciferases also varies however, and can compensate for issues relating to the color of emitted light. For example, even though gaussia luciferase generates



**Bioluminescence Imaging. Fig. 1** The above figure shows a series of bioluminescent images of an individual mouse taken at weekly intervals and shows the development of a spontaneous and bioluminescent prostate tumor. Note that the colors

predominantly blue/green light, imaging sensitivity has been reported to be roughly comparable to that associated with firefly luciferase as the relative efficiency of light production is sufficiently high. The absorbance of in vivo bioluminescence is increased when overlying tissues, skin, or fur are darkly pigmented. Indeed, whenever BLI sensitivity issues become prevalent for any given in vivo application, the use of albino mouse strain variants or the local removal of pigmented fur is highly recommended.

The fact that different colors of light have inherently different tissue transmission properties has led to the development of algorithms that can predict the depth of firefly luciferase expressing cells in tissue. As red light passes through tissue with greater efficiency than green, the ratio of red to green light on the surface of the animal is proportional to tissue depth (i.e., the presence or absence of green light in relation to the amount of measured red light is indicative of a shallow or deep bioluminescent origin, respectively).

Another key factor that influences the sensitivity of BLI is the extent of bioluminescent background. In terms of imaging labeled tumor ► [xenograft](#) models, this is not a serious issue as non-labeled host tissue does not emit light at appreciable levels. This issue does become pertinent when working with luciferase-labeled transgenic mice (as certain lines may express significant levels of luciferase in a nonspecific manner) or when attempting to detect micro-metastases that reside near to the primary tumor. Attempts to image the activation of a molecular pathway in a population

associated with these images and accompanying scale bar correspond to light intensity and do not reflect the color of detected light (This figure is reproduced with modification from Fig. 4A [5] by copyright permission of the AACR)

of cells can also prove challenging in transgenic mice, if basal levels of luciferase expression are already high.

### Oncology Model Applications

A tremendous degree of versatility is afforded by the fact that the direct imaging of mammalian cells by BLI relies upon the expression of a luciferase transgene. A wide range of validated transgenic strategies currently exist to control transgene expression at the transcriptional level or reporter gene functionality following translation. As a consequence, a diverse range of tumor biology related parameters can be imaged in vivo using BLI.

One of the most common applications of BLI in cancer research is the detection and repeated measurement of in vivo tumor burden within the same subject over time.

For tumor xenograft based studies, this can be achieved by introducing stable constitutive luciferase expression into the cell line of choice prior to implantation. Derivatives of strong viral promoters such as CMV or SV40 have been frequently employed for such purposes, but promoter sequences from eukaryotic housekeeping genes (e.g.,  $\beta$ -actin or GAPDH) can also be used and may be preferable for ensuring robust luciferase expression in certain cell types.

It is now well established that when luciferases are constitutively expressed in tumor cells (with the exception of secreted gaussia luciferase), overall light emission is proportional to tumor cell viability. At the early to mid-stages of tumor development, this is typically

reflected by a strong correlation between measured bioluminescence and tumor volume. This correlation becomes less pronounced when tumors near end-stage, as extensive regions of tumor necrosis (which contribute to tumor volume but not bioluminescence), variable tissue depth, and tumor perfusion/substrate bioavailability (affecting measured bioluminescence but not volume) become more prevalent.

BLI can also measure spontaneously arisen tumor burden in transgenic mice. Imaging such tumors is more complicated than xenograft models as, in order to maintain sensitivity, strategies must be implemented to ensure that luciferase expression is maximized in the tumor, yet kept to a minimum in proximal non-transformed tissues. Tissue specific promoters can be useful when tumorigenesis occurs in a target organ or cell-type population that is relatively small (tumors arising spontaneously in the pituitary and prostate glands have been successfully imaged in this way). The growth dynamics of spontaneous tumors arising in other more elaborate conditional (► *Cre/loxP* dependent) tumor models can also be measured using a generally expressed but conditional (*Cre/loxP* dependent) luciferase allele.

Constitutive promoter strategies can also be used to detect the appearance of tumor ► **Metastases** *in vivo*. As the primary tumor may be relatively large (and consequently bright) at the time that metastases appear, imaging sensitivity is maximal when metastases develop at sites that are spatially distinct from the primary tumor. Tissue depth will also vary between the metastatic sites within a cohort of subjects. Therefore, longitudinal BLI measurements indicate only the presence and relative growth dynamics of each metastatic lesion as opposed to the absolute quantification of tumor burden at every location. BLI can also be performed on freshly excised organs at necropsy (i.e., *ex vivo* BLI) to quickly validate the presence of metastases at the end of an experiment.

The sensitivity of BLI is such that many other tumor associated processes (and the effects of drug treatment upon them) can also be imaged noninvasively when luciferase expression or functionality is regulated in different ways. For example, the relative levels of tumor cell proliferation can be imaged before and after drug treatment using a luciferase allele that is only expressed at the onset of ► **S-phase** in replicating cells. Tumor cell ► **apoptosis** has also been imaged *in vivo* by employing engineered luciferase alleles

that have extra peptide domains fused to them that cause either reporter instability or impair function in normal cells. The activation of specific caspase enzymes, which mark the onset of apoptosis, specifically cleave this interfering peptide domain from the reporter, resulting in reporter functionality and the generation of bioluminescence. Several approaches have also been devised to image specific protein-protein interactions in tumor cells *in vivo*. One involves the splitting of firefly or renilla luciferase into two separate but complementary domains. When these intrinsically inactive N- and C-terminal reporter fragments are fused to two different proteins, bioluminescent activity is only reconstituted when the proteins that they are fused to bind each other. Again, the ability to garner this type of information noninvasively *in vivo* is incredibly useful for characterizing fundamental aspects of tumor biology or examining the effects of experimental therapeutics.

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## Bioluminescent Reporter Gene Assays

### ► Luciferase Reporter Gene Assays

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## Biomarkers

### Definition

Biomarkers are distinctive and relatively specific biological indicators (in the form of altered gene, protein,

carbohydrate, or lipid expression) of physiological or disease processes. They are used in determining the risk of exposure of ► **xenobiotic** agents, in assessing success of ► **chemoprevention**, or in disease prognosis and prediction.

- Biomarkers in Detection of Cancer Risk Factors and in Chemoprevention
- Biomarkers in Prognosis and Prediction
- Clinical Cancer Biomarkers

viral infections. Therefore, the majority of all human tumors are considered to be preventable by avoiding exposure to risk factors. ► **Biomarkers** may be used in human trials and in studies of ► **molecular cancer epidemiology** to study these types of exposures and to identify measures to reduce cancer risks.

### Types of Biomarkers

The most straightforward determination of risk is to identify people already carrying the disease on account of having tumor cells in their body. These markers are of diagnostic value. In the context of exposure and health, however, other parameters that can be detected prior to manifestation of tumors are considered more feasible. These include:

- Susceptibility biomarkers (predetermining damage) to identify people at high risk, since they carry cancer prone genetic alterations (mutations, gene amplifications, or recombination) in cancer target genes (e.g., ► **APC** deletions; hMSH mutations, K-ras amplification).
- Susceptibility biomarkers (predisposing alterations) to identify people at different degrees of risk because they carry frequent alterations in genes that are more indirectly related to the process of carcinogenesis. These indirect mechanisms include features of carcinogen metabolism (► **Metabolic Polymorphisms**) or pharmacological variations (e.g., receptors for micronutrients, sensory dispositions). There is some evidence available that single genetic polymorphisms, or a combination of these, can be associated with cancer risk.
- Biomarkers of early effects in cells and tissues to identify past exposure to risk factors by determining genetic damage (► **DNA adducts**, DNA breaks, ► **oxidative DNA damage**, genome instability) in somatic cells. This is based on the assumption that increased DNA damage is the result of a higher load of genotoxic agents that will cause the complex process of carcinogenesis. Additional cellular processes that may serve as biomarkers are cell proliferation or ► **apoptosis** (intermediate end points). These may also be decreased on account of exposure to protective factors. Furthermore, the modulation of gene expression, such as induction of phase II enzymes may render the cell less vulnerable and more resistant to risk factors, and the measurement of these effects are thus novel biomarkers of chemoprevention.

## Biomarkers in Detection of Cancer Risk Factors and in Chemoprevention

Beatrice L. Pool-Zobel

Nutritional Toxicology, Friedrich-Schiller-University of Jena, Jena, Germany

### Definition

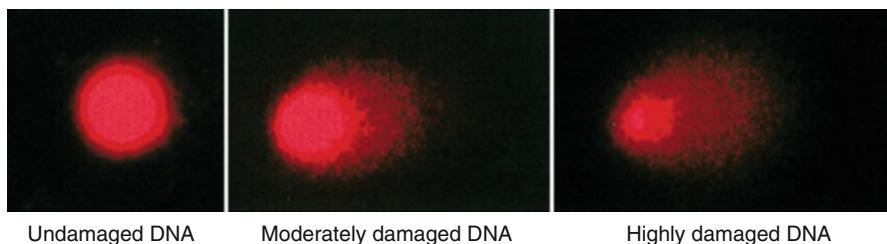
► **Biomarkers** are parameters that provide information on exposure to xenobiotics and to chemopreventive compounds, or on the effects of that exposure in an individual or in a group.

### Characteristics

Biomarkers used for the detection of cancer risk factors and in studies of chemoprevention can reveal an overall body load of genotoxins (which should be avoided) or of chemoprotective components (which should be enhanced).

### Background

Most forms of cancer are due to somatic alterations (► **mutation**, ► **amplification**, recombination) in proto-oncogenes, in tumor suppressor genes or in ► **DNA repair** genes. These are acquired in the tumor target tissues during lifetime, accumulate and produce a clonal selection of cells with aggressive and invasive growth properties. Only ~1% of all cancers are due to inheritance of these types of genetic alterations. Most other cancers are dietary related, are due to inhalation of tobacco smoke (Tobacco Carcinogenesis), or may be a consequence of ► **inflammation** or



Undamaged DNA

Moderately damaged DNA

Highly damaged DNA

**Biomarkers in Detection of Cancer Risk Factors and in Chemoprevention.** **Fig. 1** Images of undamaged to damaged DNA from single human peripheral lymphocytes in the “comet assay.” Cells were embedded into agarose on microscopical

slides, lysed, subjected to alkaline electrophoresis, and stained with ethidium bromide. Usually the proportion of damaged cells and degree of damage is quantified for 50–100 cells per slide, using an image analyzer

- Biomarkers of exposure (risk and protective factors) to identify current exposure to risk compounds (e.g., carcinogens from tobacco (► [Tobacco Carcinogenesis](#)) or food, reactive oxygen species, products of lipid peroxidation) or protective compounds (e.g., antioxidants, metabolites of chemopreventive agents, ► [fermentation products](#) of the gut flora) by measuring their concentrations in urine or blood. For complex associations such as ► [diet and cancer](#), the shift of these groups of substances relative to each other can then be evaluated as contributing to an increase or to a decrease of risk.

### Biomarker Techniques and Fields of Application

Depending on the source of body fluid or cells analyzed, the biomarkers will reveal systemic or tissue-specific exposures. Specific end points will be more suited for molecular cancer epidemiology studies, whereas nonspecific end points are also of value for occupational types of exposure assessment or for dietary intervention studies. Noninvasive methods should be better suited for large-scale studies, whereas invasive methods will be employed more selectively. In this context, largely depending on the degree of invasiveness, biomarkers may be categorized as follows:

- Noninvasive methods using body fluids or exfoliated cells include techniques such as the analytical detection of single compounds or of their metabolites. The methods are indicators of exposure. Also, a functional determination of mutagenic or genotoxic effects of body fluids using cultured cells as target organisms (e.g., determination of fecal water genotoxicity) are biomarkers for determining exposure. Other noninvasive methods are directed at analyzing genetic alterations in isolated

exfoliated cells from these body fluids. Examples are the analysis of micronuclei in sputum or urinary and buccal cells.

- Relatively noninvasive methods using cells of the peripheral blood stream are aimed at detecting exposure-related genotoxic damage. The end points include DNA-strand breaks, oxidized DNA bases (using the single cell microgel electrophoresis assay, also referred to as the ► [comet assay](#)), DNA adducts (detected with ► [32P-postlabeling](#)), and cytogenetic end points (micronuclei, sister chromatid exchanges, chromosomal aberrations). The development of the techniques for genetic damage has been largely based on their utilization as methods to assess exposure in occupational, environmental settings or subsequent to tobacco smoke inhalation. They have only sporadically been used to study associations of diet and cancer.
- Invasive methods using cells from tumor target tissues make use of cells from biopsies (e.g., colon, breast, kidney) to determine functional parameters in potential tumor target tissues. The parameters indicate cellular responses and genetic alterations (proliferation, K-ras-, p53-mutations, APC-alterations, and DNA damage). The end points are indicators of very early response to risk factors and are biomarkers of effect. However, they are invasive, and thus may be limited to studies on special exposures or in specific groups of patients. In any case, their utilization and development will serve as basis for the refinement of noninvasive methods with exfoliated cells as outlined above (Fig. 1).

In conclusion, a variety of biomarkers to assess the impact of risk and of protective factors is available. Already research has provided evidence that

biomarkers can measure the efficacy of exposure as well as of exposure reduction. Many of the techniques, however, need to be further validated for their applicability, reliability, and predictivity of potential tumor risks in human studies. Another set of techniques is available that can serve as a meaningful basis for the development of potentially new biomarkers. Altogether these methods are of value to serve as indicators of effects and indicators of exposure by risk and protective compounds. Depending on the specificity of the end point or on the technical feasibility, individual methods will be more or less suited for use in dietary intervention studies, in occupational exposure settings and/or in larger scale trials of molecular epidemiology.

- Biomonitoring
- BORIS
- Carcinogen Macromolecular Adducts
- CCCTC-Binding Factor (CTCF)
- Clinical Cancer Biomarkers
- Molecular Pathology
- Oncopeptidomics
- Tissue Inhibitors of Metalloproteinases

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## Biomarkers in Prognosis and Prediction

### Definition

Are distinctive and relatively specific biological indicators (in the form of altered gene, protein,

carbohydrate, or lipid expression) of physiological or disease processes. Cancer biomarkers have been broadly categorized into:

1. Prognostic biomarkers, which aid in determining the disease outcome (prognosis)
2. Predictive markers, which predict response to therapy

Identification of prognostic biomarkers would enhance the management of breast cancer patients by helping clinicians make better decisions with regard to the mode of treatment for each patient, such as which group of patients would benefit from chemotherapy after surgical excision of the tumor. Prognostic biomarkers also form the basis for the development of effective targeted therapy against cancer.

- Breast Cancer Prognostic and Predictive Biomarkers

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## Biomonitoring

Ari Hirvonen

Finnish Institute of Occupational Health, Helsinki, Finland

### Synonyms

[Biological monitoring](#)

### Definition

Biological monitoring (i.e., biomonitoring) has conventionally been defined as “the periodic measurement of ► [xenobiotic](#)(s) or their ► [metabolite](#)(s) in accessible biological media for the comparison with an appropriate reference.” At present, a broader definition could be used that included effect ► [biomarkers](#) and biologically relevant dose, as well as biomarkers of susceptibility.

### Characteristics

Biomonitoring is mainly aimed at

- Defining the existence of an occupational or environmental exposure

- Quantifying the ► internal dose
- Verifying that exposure limits are respected

The most commonly used matrices for biomonitoring are blood (and its components, e.g., serum and plasma) and urine.

Biological monitoring is complementary to the two other monitoring programs that are carried out to evaluate the health risk associated with exposure to pollutants, that is, ambient monitoring and health surveillance. The basis of these monitoring programs is defined by following up the fate of a chemical from the environment to the target molecules in the organism. The main characteristics of biological and ambient monitoring are summarized in **Table 1**.

Once absorbed and present in the circulation, the chemical may be eliminated unchanged, mainly in urine or in expired air, or distributed to different compartments of the body. Organic chemicals usually undergo a ► biotransformation to more water-soluble compounds that are more easily excreted via urine or bile than the parent compound. If not excreted, the chemical or its ► metabolites may bind to different sites on the target molecules. Binding on critical sites may give rise to adverse health effects at least when the amount bound has reached a certain level and the protective mechanisms are inadequate or insufficient.

Biological monitoring may offer several advantages over environmental monitoring to evaluate the internal dose and hence to estimate the health risk. One of the main advantages is that biological monitoring takes into consideration all routes of absorption (inhalation, skin, ingestion) in both occupational and leisure activities, accounting for individual differences in absorption rate due variations in, for example, work load or coexposure to additional components of complex mixtures. It also takes into account the variations in individual ► metabolic capability, due to either genetically determined or acquired changes in gene expression and enzyme activity. The greatest advantage of biomonitoring, however, is the fact that the biological parameter of exposure is more directly related to the adverse health effects that one attempts to prevent than any environmental measurement.

On the other hand, biomonitoring is more complex in terms of ► standardization and interpretative efforts as compared with ambient monitoring. Since biomonitoring rely on the use of biomarkers, rational biological monitoring is only possible when sufficient

**Biomonitoring.** **Table 1** Main characteristics of biomonitoring and ambient monitoring

	Biomonitoring	Ambient monitoring
Quantifies	Dose	External exposure
Routes of absorption	All routes	Inhalation
Measurement	Biomarkers	Direct
Interpretation	Complicated	Easy
Variability	High	Usually low
Confounding	Metabolic ► phenotype	Protection devices
Cost	Usually high	Usually low

toxicological information has been gathered on the mechanism of action and/or the metabolism (absorption, biotransformation, distribution, excretion) of ► xenobiotics to which people may be exposed. When a biomonitoring method is based on the determination of chemical or its metabolite in biological media, it is essential to know how the substance is absorbed via the lung, the gastrointestinal tract, and the skin, and subsequently how it is distributed to the different compartments of the body, ► biotransformed, and finally eliminated. It is also important to know whether the chemical can accumulate to the body.

According to the National Research Council (NRC), biomarkers can be classified as (1) biomarkers of exposure, (2) biomarkers of effect, and (3) biomarkers of susceptibility. The use of biomarkers rather than their intrinsic properties may define their classification.

### Biomarkers of Exposure

Exposure biomarkers are widely used, for example, in occupational ► toxicology for a more accurate risk assessment. In workers exposed to similar air concentrations of chemical pollutants, various factors can determine the actual absorbed dose, including physical workload; additional skin absorbance due to bad working practice or, on the contrary, the use of personal protection devices; and differences in individual uptake and metabolism.

The meaning of the marker may depend on the sampling time. Therefore, the choice of the biomarker should rely on a number of considerations, but mainly on ► kinetic parameters and on the knowledge of the mechanistic basis of adverse effects. An ideal biomarker of exposure should be (1) specific for the

exposure of interest, (2) detectable in small quantities, (3) measurable by noninvasive techniques, (4) inexpensive, (5) associated with prior exposure, and (6) able to provide an excellent positive predictive value to a specific health status. Several biomarkers of exposure are often available for the same chemical, for example, the parent compound itself, a metabolite, or a macromolecular ► **adduct** (to DNA or protein).

A great majority of the currently available biomonitoring tests are based on determination of the chemical or its metabolite in a biological media. According to their selectivity, these tests can be classified into two subgroups: (1) the selective tests based on the direct measurement of the unchanged chemicals or their metabolites in biological media and (2) the nonselective tests used as nonspecific indicators of exposure to a group of chemicals.

DNA and protein ► **adducts** are primary measures of exposure to carcinogenic compounds. DNA adducts are mechanistically linked to cancer formation, as they may cause gene mutations and chromosomal alterations in growth controlling genes. Measurement of DNA adduct levels allows insight into the impact of metabolic variations, the interactions between components of complex mixtures, and coexposure to compounds that enhance the effect of carcinogens. In human studies, DNA adduct levels in the target organ are consistent with the excess risk noted for populations with specific exposures. For instance, significant differences in DNA adduct levels have been detected in persons exposed to passive tobacco smoke in the face of only modest differences in exposures. Moreover, simple interventions have been shown to reduce DNA adduct levels in the target organ of an exposed population. So, while analysis of carcinogen DNA adducts remains primarily a research tool, these research studies have begun to validate its wider use in biological monitoring of exposed human.

Biotransformation is obviously a central issue for any biomarker used in biomonitoring of xenobiotic exposure. Variation in individual metabolism is expected to be an important contributor to variation in biomarker levels. Metabolic differences among individuals can stem from acquired factors, such as enzyme induction or inhibition, or from inherited polymorphisms of xenobiotic-metabolizing enzymes (XMEs).

Most absorbed xenobiotic chemicals undergo biotransformation that eventually aims at disposal of the

chemical with as little harm as possible. For indirectly toxic chemicals, phase I reactions mediated by cytochrome P-450 (CYP)-dependent monooxygenases usually comprise metabolic activation, while phase II conjugation reactions are part of detoxification and lead to excretion. In many cases, the metabolism is, however, complicated, and metabolic activation and detoxification does not follow this simple model.

During the last decade, many of the XME-genes have been shown to be polymorphic, resulting in individual differences in the metabolic capability related to these enzymes. For some enzymes, the polymorphism involves ► **genotypes** that are associated with no enzyme activity, while in other cases ► **phenotypic** differences between the genotypes are subtler. The phenotypic consequences of many metabolic polymorphisms are, however, inadequately known. Accordingly, we are just beginning to understand the possible toxicological impacts of genetic polymorphisms in environmental exposures.

### **Biomarkers of Effect**

Biomarker of effect has been defined as “any measurable biochemical, physiological, or other alteration within an organism that, depending on the magnitude, can be recognized as an established or potential health impairment or disease.” Research on biomarkers of effect is rapidly generating a large amount of data measuring intermediate end points occurring probably after exposure and possibly before illness. Such biomarkers are expected to reflect early modifications preceding progressive structural or functional damage at the molecular, cellular, and tissue level. A wide spectrum of biomarkers may be used for this purpose. Cytogenetic (► **Cytogenetics**) tests involving scoring of microscopic chromosomal damage are the oldest of biomarkers used and are still applied in a wide variety of exposures.

The main conceptual basis for using cytogenetic assays for biological monitoring is that genetic damage in a nontarget tissue, most often peripheral blood lymphocytes, reflects similar events in cells involved in carcinogenic process. The conventional chromosomal damage assessments include determination of (1) structural chromosomal aberrations (CAs), (2) sister chromatid exchanges (SCEs), and (3) micronuclei (MN). Recently, *in situ* fluorescence techniques (FISH) have been used in order to score specific chromosomes and

chromosomal loci. Rigorous study design is necessary in all cytogenetic biomonitoring methods, since many interindividual factors that are not related to the specific chemical exposure(s) of interest may affect the parameters studied. Experimental confirmation of the chromosome damaging potential of the test agent(s) is therefore a prerequisite in performing human cytogenetic studies.

A good example of the potential applicability of chromosomal damage as surrogate for disease comes from recent prospective studies on cytogenetic biomarkers and cancer risk that followed several European cohorts; subjects in the group of highest frequency of CAs were at a more than doubled overall risk for cancer with comparison to the lowest frequency group. The use of MN as a measure of chromosomal damage, on the other hand, has become a widely used assay in both genetic toxicology testing and human biomonitoring studies. Analysis of results from European cohorts indicated that subjects with cancer had a significant increase in MN frequency.

### Biomarkers of Susceptibility

The concatenation of environmental exposure, genetic effect, and ► individual susceptibility is a key issue in assessment of risks for populations exposed to environmental pollutants. In view of the interindividual differences in susceptibility to xenobiotics, one might consider the detection of increased susceptibility to a chemical hazard. A biomarker of susceptibility is defined as an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance. For instance, the ability to acetylate aromatic amines has been shown to be genetically determined and it has been suggested that carriers of allelic variants of the *N*-acetyltransferase (NAT2) gene that result in decreased *N*-acetylation capacity are at increased risk for colorectal cancer when exposed to carcinogenic aromatic amines.

Genetic polymorphisms in the activity of the aryl hydrocarbon hydroxylase (AHH) has also been suggested as an example of the relationship of metabolic variation to individual susceptibility to develop lung cancer in case of exposure to polycyclic aromatic hydrocarbons (PAHs).

The genetic polymorphisms potentially important for a particular biomarker largely depend on the exposing agent and biological material examined.

As ► genotype effects have only occasionally been considered in ► Biomarker studies, much basic research is needed, and no general conclusions can yet be drawn on their real importance. It is, however, expected that genotype differences exist in biomarker response to many exposures. In such cases, information on this effect will be very valuable for correct assessment of exposure and effect biomarkers. If genotyping can be shown to markedly improve routine biomarker reliability, the ethical question whether this tool should be utilized only in setting standards or incorporated as part of the analysis must be addressed.

### Ethical and Social Implications

Biological monitoring has been a major tool of medical health surveillance in most EU member states already for several decades. Biomonitoring data can also be used to fine tune or even launch environment and health policies; it allows policy makers to identify priorities and provides early warning on potential threats and enables them to assess how effective the strategies are (time trends analysis).

For many pollutants, however, interpretation of health significance is still hampered by the lack of toxicological and medical information. Moreover, ► internal doses cannot be directly linked to the external exposure source. And because human biomonitoring has to do with people, an ethical and communication framework has to be further developed in order to ensure that the biological monitoring surveys respect ethical and privacy considerations.

Much work has therefore still to be done, especially with respect to proper interpretation of human biomonitoring data and its translation into policy actions. In line with this, developing a coherent approach to human biomonitoring is one of the main priorities of the European Union Environment and Health Action Plan 2004–2010.

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## Biopsy

### Definition

Sample of tissue obtained for diagnostic examination by a special surgical intervention.

- [Fine Needle Aspiration Biopsy](#)

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## Bioreductive Drug

### Definition

Is an agent that is reduced in the state of oxygen deficiency, usually to produce a more active metabolite that can be cytotoxic or that can be used for the detection of O<sub>2</sub>-depleted tissue areas.

- [Oxygenation of Tumors](#)

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## Biosensor

### Definition

A sensor that detects a biological component or a sensor that provides bioanalytical information by means of applying biological molecules such as nucleic acids, enzymes, or antibodies which function as biological recognition elements.

- [Surface Plasmon Resonance](#)

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## Biosynthesis

### Definition

Is the production (usually enzymatic) of components in living cells.

- [Estrogenic Hormones](#)

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## Biotechnology

### Definition

The use of living organisms or their products to make or modify a substance. Biotechnology includes recombinant DNA techniques (genetic engineering) and hybridoma technology.

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## Biotechnology Derived Therapeutic Proteins

- [Recombinant Therapeutics](#)

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## Biotin

### Definition

(vitamin H or B7) A water-soluble B-complex vitamin which is composed of an ureido (tetrahydroimidazalone) ring fused with a tetrahydrothiophene ring. Biotin is important in the catalysis of essential metabolic reactions to synthesize fatty acids, in gluconeogenesis, and to metabolize leucine.

- [Anti-HER2/Neu Peptide Mimetic \(AHNP\)](#)

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## Biotransformation

### Definition

Chemical conversion of substances by living organisms or enzyme preparations.

- [Biomonitoring](#)

## BiP/GRp78

### Definition

BiP (binding protein for immunoglobulins)/GRp78 (glucose-regulated protein of 78 kDa) is a molecular chaperone of the Hsp70 family, and expressed in the endoplasmic reticulum. It recognizes nonnative folding intermediates through hydrophobic amino acids in proteins.

### ► Calreticulin

## BIR Domain

### Definition

► **Baculovirus** IAP Repeat Domain is an approximately 70 amino acid zinc-binding domain, first identified by sequence homology among proteins belonging to the ► **Inhibitors of Apoptosis** (IAP) family. Present in one to three tandem copies per protein, the BIR domain has been identified in over 80 different proteins in eukaryotic organisms. Most of what is known about BIR domains come from their role in IAP proteins. IAPs bind to and inhibit caspases, a class of cysteine proteases involved in propagating ► **apoptosis** signals within the cell.

## BIRADS

### Definition

Acronym for ► **Breast Imaging Reporting and Data System**

## Birbeck Granule

### Definition

Is a Langerhans' cell specific rod-shaped or tennis racquet-shaped organelle detected by electron microscopy, whose function is unknown.

### ► Langerhans Cell Histiocytosis

## BIRC5

### ► Survivin

## Birt-Hogg-Dube Syndrome

### ► Birt–Hogg–Dubé Syndrome

## Birt–Hogg–Dubé Syndrome

Shree Ram Singh

Mouse Cancer Genetics Program, National Cancer Institute at Frederick, Frederick, MD, USA

### Synonyms

BHD syndrome; Fibrofolliculomas with trichodiscomas and acrochordons; Folliculin (FLCN); Hornstein–Knickenberg syndrome

### Definition

Birt–Hogg–Dubé syndrome is a rare, autosomal dominantly inherited genodermatosis characterized by multiple, benign cutaneous hair follicle tumors (fibrofolliculomas), trichodiscomas, and acrochordons (skin tags), lung cysts, spontaneous pneumothorax (lung wall collapse), colon polyps and colon carcinoma, lipomas, angiopelmas, parathyroid adenomas, parotid oncocyomas, and an increased risk for developing kidney tumors such as oncocytomas, chromophobe, papillary, and clear renal cell carcinoma (RCC).

### Characteristics

BHD syndrome was originally described in 1977 by Birt, Hogg, and Dubé as a rare form of inherited ► **autosomal dominant** syndrome in large kindred, wherein 15 of 37 members were older than 25 years of age. Originally, it was characterized as a triad of multiple, skin hamartomatous lesions (fibrofolliculomas,

trichodiscomas, and acrochordon). The fibrofolliculomas and trichodiscomas appear as multiple, small, dome-shaped, smooth, 2–4 mm, yellowish, or skin-colored papules scattered over the forehead, face, neck, nose, chest, scalp, and upper trunk. The onset of skin lesions typically begins during the third or fourth decade of life. Skin lesions tend to increase in size and number with age. The acrochordons appear as small and soft skin tags (furrowed, 1–2 mm soft papules) composed of loose connective tissues. Histogenesis of skin lesions confirmed that trichodiscomas originated from the mesenchymal component of the pilar complex, acrochordons from epithelial components, and fibrofolliculomas from both epithelial and mesenchymal proliferation. Since its initial description, more than 60 families have been identified with BHD syndrome and a number of other features of BHD have been recognized, including an increased incidence of ► renal carcinoma, most commonly, chromophobe and hybrid oncocytic/chromophobe ► renal cell carcinomas (RCCs), lung cysts, pleural blebs, spontaneous pneumothorax, developing colonic adenomas and carcinoma, neurothekeomas, meningiomas, flecked chorioretinopathy, parathyroid adenomas, multiple lipomas, intraoral papules, parotid oncocytoma, and other cutaneous tumors such as, collagenomas, perivascular fibromas, angiomyomas, and ► melanomas.

Individuals with BHD syndrome were found to have sevenfold higher risk of developing kidney neoplasm, 50-fold higher risk of developing spontaneous pneumothorax, and 80-fold higher risk of developing pulmonary cysts over the general population. The first report of BHD syndrome with renal pathology when examined showed bilateral kidney tumors with one clear RCC and one chromophobe RCC. Further, in a study of 13 patients with BHD syndrome, seven had renal neoplasms, including renal oncocytomas and papillary RCCs. BHD patients with renal ► neoplasia display multifocal, bilateral tumors of several histopathological variants, including chromophobe RCC (34%), oncocytic hybrid (50%) with features of chromophobe RCC and renal oncocytoma, and less frequently, clear cell RCC (9%), renal oncocytoma (5%), and papillary RCC (2%). Oncocytoma and chromophobe RCC originate from the intercalated cells of renal collecting tubules and share overlapping histologic features. A study on 98 patients with BHD syndrome described the occurrence of both oncocytoma and chromophobe RCC with predominance of

chromophobe RCC in renal cancer, found in 7 of 14 histologically examined tumors. Chromophobe RCCs are slowly progressive, locally invasive, and average 7–9 cm in diameter but rarely metastasize. Mean age at diagnosis of kidney tumors is 50.7 years. Recent findings suggest that microscopic oncocytic lesions may be precursors of hybrid oncocytic tumors, chromophobe RCCs, and perhaps clear cell RCCs in patients with BHD syndrome. Strong associations between renal neoplasms and pulmonary cysts and spontaneous pneumothorax have been observed in BHD families. The lung cysts in BHD affected individual are mostly bilateral and multifocal and have a high risk of developing spontaneous pneumothorax. Pneumothorax likely occurs in younger individuals with BHD syndrome. Male gender and older age has been associated with increased risk of renal tumors, whereas the risk of spontaneous pneumothorax is inversely associated with age. Based on these clinical manifestations, penetrance of BHD syndrome is considered to be very high. Thus, the BHD syndrome conferred an increased risk for the development of renal tumors, spontaneous pneumothorax, and lung cysts.

### Diagnostic Criteria

The following diagnostic features may be considered in a patient with BHD syndrome: the presence of 10–100 cream to flesh-colored, smooth, firm skin papules on the face, neck, or upper torso, with at least one histologically confirmed fibrofolliculoma with or without family history of BHD or a single renal tumor or history of spontaneous pneumothorax; a patient with multiple and bilateral chromophobe, oncocytic, and/or oncocytic hybrid renal tumors; single oncocytic, chromophobe, or oncocytic hybrid tumor and a family history of renal cancer with any of above renal cell tumor types; and a family history of autosomal dominant primary spontaneous pneumothorax without a history of chronic obstructive pulmonary disease.

### BHD Gene Mutations

The genetic defect responsible for BHD syndrome has been mapped to the pericentromeric region of chromosome 17p11.2 by linkage analysis and the gene in this region has been cloned and is believed to be responsible for the BHD syndrome. This region is interesting because of the presence of low copy number repeat elements, unstable, and associated with a number of diseases. Several heterozygous ► Germline mutations

have been identified in a novel gene, *BHD*, in BHD families. The human *BHD* gene encodes a tumor-suppressor protein, folliculin (*FLCN*), a cytoplasmic protein with an open reading frame of 579 amino acids, 64-kDa protein. Human *FLCN* consists of 14 exons. Folliculin contains a glutamic acid rich, coiled-coil domain with no significant homology to any known human protein. Folliculin homologs have been identified in many species, including *Drosophila*, *Caenorhabditis elegans*, mouse, dog, and rat, implying a critical biological role for folliculin. Although the function of the *BHD* gene is unknown, germline mutations in *FLCN*, with somatic mutations and loss of heterozygosity in tumor tissue, suggest that loss of function of the folliculin protein is the basis of tumor formation in BHD syndrome. Recently, it has been shown that *FLCN* binds with *FNIP1* (folliculin interacting protein 1) and may be involved in energy and/or nutrient sensing through AMPK and mTOR signaling pathways. Further, a recent study demonstrates that the *Drosophila* homolog of gene *BHD* regulates male germline stem cell maintenance and functions downstream of the JAK/STAT (janus kinase/signal transducer and activator of transcription) and Dpp (decapentaplegic) signal transduction pathways. This study suggests that the *BHD* may regulate tumor formation through modulating stem cells in human.

The germline mutations identified in BHD families so far are frameshift or nonsense mutations, predicted to truncate folliculin, including insertions or deletions (44%) of a hypermutable tract of eight cytosines (C8) in exon 11. Initially, the distinct germline mutations on exon 11 of the folliculin gene (c.1733insC and c.1733delC) in three of four families with BHD syndrome, was identified. Later, mutations along the entire length of the coding region of the folliculin gene has been identified, including 16 insertion/deletion, 3 nonsense, and 3 splice site mutations in 51 of 61 families with BHD syndrome. Interestingly, among patients with a mutation in the exon 11 hot spot, significantly fewer renal tumors were observed in patients with the C-deletion than those with the C-insertion mutation. Two unique features of renal tumors in patients with BHD syndrome are the variable expression of the phenotype among members of a given family who carry the same germline mutation, and between families who carry the “hot spot” mutation in exon 11. Mutational hot spot is also reported to

be a target of mutation in ► **microsatellite instability** (MSI) sporadic colorectal cancer. Five of 32 (16%) sporadic colorectal cancers with MSI were found to have insertion/deletion mutations in the poly(C) 8 tract of the *BHD* exon 11. In addition, mutations truncating folliculin have been described in patients with 4-bp deletions in *BHD* exon 4, dominantly inherited lung cysts and/or spontaneous pneumothorax without skin lesions or kidney tumors. Moreover, germline mutation in the rat and dog homologs of the *BHD* gene also resulted in inherited kidney tumors, suggesting that the *BHD* gene has a tumor suppressor function. Furthermore, recent evidence of somatic second “hit” mutations in renal tumors from BHD patients, in which 53% showed a second somatic mutation and 17% showed loss of ► **heterozygosity** (LOH) of the wild type allele, strongly supports the Knudson “two-hit” tumor suppressor model for *BHD* and suggesting that BHD is a new ► **tumor suppressor gene** with roles in both human and animal carcinogenesis.

*BHD* mRNA is expressed in a wide variety of normal tissues, including the differentiated epidermal layers of the skin, the outer and inner root sheath supporting structures of the hair follicle, lung, and kidney and also expressed in a variety of secretory cell types, including acinar cells of the parotid gland and pancreas, brain, lymphocytes and ductal cells of the breast and prostate. Tissues with reduced expression of folliculin mRNA included heart, muscle, and liver. Folliculin immunoreactivity also occurred in the nucleolus of normal cells and was associated with mitosis. In addition, folliculin mRNA was expressed strongly in fibrofolliculomas but loss of folliculin expression was seen in oncocytoma (3.3%), chromophobe RCC (60.7%), papillary RCC (36.4%), and clear cell RCC (21.1%). Abnormal accumulation in the cytoplasm was also observed in oncocytoma (76.7%), chromophobe RCC (3.6%), and clear cell RCC (14.7%). Thus, the protein may have important biological functions in a variety of tissues and organisms. Furthermore, the defective protein in BHD patients may affect the cell’s cytoskeleton, disrupting the extracellular matrix and affecting the regulation of cellular proliferation.

### Screening and Possible Treatment for BHD

BHD syndrome is inherited in an autosomal dominant manner. A child having a parent with mutation on *BHD* has a 50% chance of inheriting that mutation.

No specific screening guidelines for BHD syndrome have been described. However, due to the risk of kidney cancer and other associated abnormalities, it has been suggested that individuals with BHD syndrome or a family history of BHD syndrome should have yearly ultrasounds of their kidneys from the age of 25 and abdominal computerized tomography (CT) scan or magnetic resonance imaging (MRI) every 2 years. Further, BHD syndrome can be identified by skin biopsies to confirm the fibrofolliculomas and X-rays to look for lung cysts and previous spontaneous pneumothorax. Individuals with BHD syndrome should avoid smoking because of increased risk of kidney cancer associated with smoking. No curative medical treatment is currently available for the cutaneous lesions associated with BHD syndrome. However, surgery and electrode desiccation have provided definitive treatment of solitary perifollicular fibromas and multiple lesions, respectively. Treatment of folliculoma/trichodiscoma shows substantial improvement after laser ablation but can be reverted. Renal tumors can be treated with nephron sparing surgical approaches, depending on the size and location of the tumors. Individuals with spontaneous pneumothorax may avoid high ambient pressures, which can precipitate spontaneous pneumothorax. Consider colonoscopy for colonic polyps and colonic adenocarcinoma. Genetic testing for BHD syndrome is also available. Use of molecular genetic testing for early identification of at-risk family members before disease-causing mutations are manifested may improve diagnostic certainty and reduce costly screening procedures. Methods of using BHD encoding sequence also allow for a differential genetic diagnosis of spontaneous pneumothorax or collapsed lung.

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## Bispecific Antibodies

Roland E. Kontermann

Institute of Cell Biology and Immunology,  
University of Stuttgart, Stuttgart, Germany

### Definition

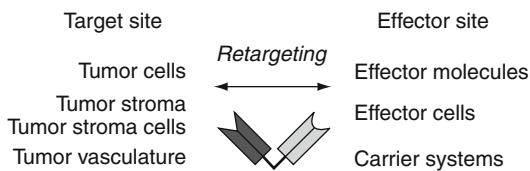
Bispecific ► antibodies are antibodies possessing antigen-binding sites with specificity for two different structures (dual specificity).

### Characteristics

Bispecific antibodies are molecules able to simultaneously bind to two different ► epitopes on the same or different antigens. Bispecific antibodies act as mediators or adaptors bringing two different structures into close contact. In cancer therapy, possible applications include the retargeting of effector molecules (e.g. radionuclides, drugs, enzymes, ► Cytokines), effector cells (e.g. cytotoxic T lymphocytes, ► Natural Killer Cells), or carrier systems (e.g. drug-loaded liposomes, genetic vehicles) to tumor-associated target sites, such as tumor cells, tumor stroma cells, and extracellular components as well as cells and structures associated with the tumor vasculature. Thus, potential applications of bispecific antibodies cover the areas of immunotherapy, ► chemotherapy, radiotherapy (► radioimmunotherapy), and ► gene therapy. Bispecific antibodies can lead to increased selectivity and improved efficacy of natural effector functions and are able to expand therapeutic effects to those not exerted by normal immunoglobulins used in the clinic (e.g. IgG molecules) (Fig. 1).

### Generation of Bispecific Antibodies

Bispecific antibodies are not found in nature and hence have to be generated in vitro. Various methods have

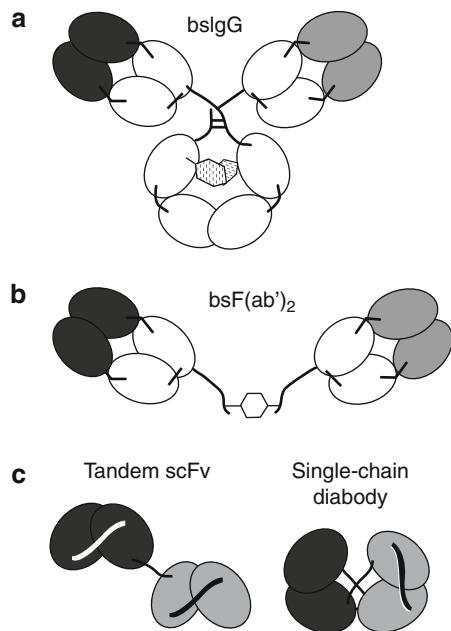


**Bispecific Antibodies.** **Fig. 1** Possible applications of bispecific antibodies in cancer therapy. Bispecific antibodies can act as mediators to retarget effector molecules, effector cells or carrier systems to tumor-associated target sites

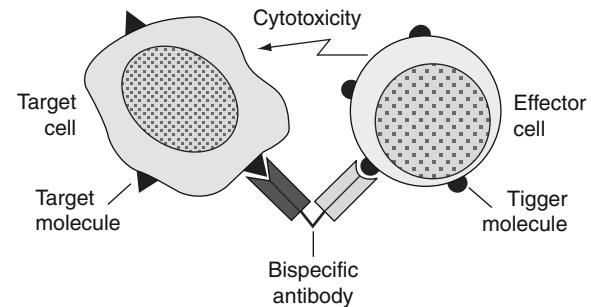
been established including somatic hybridization of two antigen-producing cells, chemical cross-linking of two Fab' fragments derived from different antibodies, and genetic approaches leading to recombinant antibody molecules. Somatic hybridization, for example, of two ► **hybridoma** cells, leads to hybrid-hybridomas or quadromas. These quadromas produce light and heavy chains of both antibodies within one cell, which assemble into bispecific antibodies. However, also nonfunctional or monospecific antibodies are produced due to random association of light and heavy chains. Thus, this approach results in a heterogeneous population of antibodies. Alternatively, Fab' fragments produced by proteolytic cleavage of two antibody molecules with different specificity can be chemically conjugated to form bispecific F(ab')<sub>2</sub> fragments. More recent approaches utilize genetic engineering to combine two different antigen-binding sites within one molecule. A large variety of different formats have been developed. Currently, the most widely used formats are tandem scFv molecules linking two ► **single-chain Fv fragments** (scFv) by a flexible linker and diabodies or single-chain diabodies with a more rigid structure. Compared to whole antibodies or F(ab')<sub>2</sub> fragments these recombinant formats are much smaller with a molecular weight of 50–60 kDa (Fig. 2).

### Effector Cell Retargeting

Preclinical and clinical developments of bispecific antibodies for cancer therapy have a strong focus on the retargeting of effector cells of the immune system to tumor cells. Suitable effector cells of the immune system include cytotoxic T lymphocytes (CTL), Natural killer cells (NK), macrophages, and neutrophils, which can efficiently kill target cells by antibody-independent or ► **antibody-dependent cellular cytotoxicity** (ADCC). Retargeting of these effector cells to target cells requires binding of the bispecific



**Bispecific Antibodies.** **Fig. 2** Various forms of bispecific antibodies. Bispecific antibodies can be generated (a) by somatic hybridization of two antibody-producing cells, (b) by chemical cross-linking of two Fab' fragments, or (c) by genetically combining two different antigen-binding sites, e.g., as tandem scFv or as single-chain diabody format



**Bispecific Antibodies.** **Fig. 3** Bispecific antibodies for the retargeting of effector cells. Bispecific antibodies are able to retarget effector cells such as cytotoxic T lymphocytes or natural killer cells to target cells by simultaneous binding to a target molecule on the target cell and a trigger molecule on the effector cell leading to killing of the target cell

antibody to one or more trigger molecules on the effector cell (Fig. 3).

Cytotoxic T cells are among the most potent effector cells of the immune system. Trigger molecules on CTLs are molecules associated with the T cell receptor (TCR) such as CD3. Bispecific antibodies thus bypass normal ► **MHC**-mediated T cell activation. This is of

**Bispecific Antibodies.** **Table 1** Effector cells and trigger molecules

Effector cell	Main trigger molecule	Costimulus	Activating cytokine
Cytotoxic T lymphocytes (CTL)	TCR/CD3	B7, anti-CD28	IL-2
Natural killer cells (NK)	CD16	–	IL-2
Macrophages	CD64, CD16, CD89	–	GM-CSF, IFN- $\gamma$
Neutrophils	CD89, CD64	–	G-CSF, GM-CSF, IFN- $\gamma$

special interest since many tumor cells escape from a T cell response by down-regulation or loss of MHC expression during tumorigenesis. T cell activation, however, depends on a costimulatory signal, for example, through binding of B7 to CD28 on CTLs. In a therapeutic setting this costimulatory signal can be provided by anti-CD28 monoclonal antibodies or by bispecific or bifunctional antibodies (Table 1). Interestingly, some anti-CD3 antibodies are able to activate T cells without the need for costimulation. One recombinant bispecific antibody (MT103) directed against CD19 and CD3 is based on such a costimulation independent anti-CD3 antibody. This antibody is currently in a phase I trial for the treatment of non-Hodgkin lymphoma (NHL).

Fc receptors are the trigger molecules employed for retargeting of NK cells, macrophages, and neutrophils. Fc $\gamma$  receptor III (CD16) represents the main trigger molecule on NK cells, while Fc $\gamma$  receptor I (CD64) or the Fc $\alpha$  receptor (CD89) have been utilized for retargeting of macrophages and neutrophils, respectively. The expression of these trigger molecules on effector cells can be increased by activating cytokines such as interleukin-2 (IL-2), granulocyte/macrophage-colony stimulating factor (GM-CSF), or ► *Interferon- $\gamma$*  (INF- $\gamma$ ) (Table 1).

### Clinical Experience with Bispecific Antibodies

Various bispecific antibodies have entered clinical trials. However, as to yet none has been approved for therapeutic applications. Initial problems were associated with the use of whole bispecific immunoglobulins derived from murine hybridomas, especially Fc-mediated toxicity due to the release of inflammatory cytokines (cytokine storm) and a neutralizing immune response against the murine antibodies

(human-anti-mouse antibodies, HAMA). Further studies using bispecific F(ab')<sub>2</sub> fragments in combination with activating cytokines such as GM-CSF could demonstrate some biological effects, however, clinical responses remained vague. Current research focuses on the development of novel antibody formats including costimulation-independent bispecific tandem scFv molecules for the retargeting of CTLs, the retargeting and activation of CTLs as well as Fc $\gamma$  receptor expressing effector cells by combination therapy with two bispecific antibody molecules or trispecific antibodies, but also on bispecific antibody molecules with improved pharmacokinetic properties.

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## Bisphosphoglycerate

### Definition

BPG is a molecule able to inversely modulate the affinity of hemoglobin for oxygen.

#### ► Polycythemia

## Bisphosphonates

Valentina Guarneri

Department of Oncology and Hematology, University of Modena and Reggio Emilia, Policlinico, via del Pozzo, Modena, Italy

### Definition

Bisphosphonates are potent inhibitors of ► *osteoclast* mediated ► *bone resorption*. These compounds are

stable analogues of the inorganic pyrophosphate (PPi), which is an endogenous regulator of bone mineralization.

## Characteristics

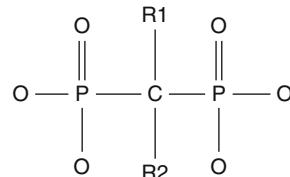
Bisphosphonates were developed in the nineteenth century for industrial use, in particular as “water softeners.” The first clinical use of bisphosphonates in humans was in the 1960s for the treatment of ► **Paget disease**, a focal disorder of bone remodeling due to abnormally increased osteoclast-mediated bone resorption. So far, bisphosphonates have been successfully studied in several clinical disorders characterized by an alteration in bone resorption, such as metastatic and osteolytic bone diseases, hypercalcemia of malignancy and ► **osteoporosis**.

All bisphosphonates share a common structure which consists of two phosphate groups attached to a single carbon atom (P-C-P) (See Fig. 1).

The P-C-P group is responsible for the affinity of these drugs for the bone, since it is essential for binding to ► **hydroxyapatite or hydroxylapatite**. The substitution in the R1 and R2 side chains give rise to a variety of compounds with different potency and biological effects. For instance, the presence of a ► **hydroxyl group** in the R1 side chain confers a higher affinity for the bone mineral. The R2 side chains directly influence the potency of bisphosphonates for inhibiting osteoclast-mediated bone resorption. In particular, the bisphosphonates containing a basic primary nitrogen atom in an ► **alkyl** chain (such as pamidronate and alendronate) are 10–100 times more potent than non-nitrogen bisphosphonates. Indeed, the higher antiresorptive potency is obtained when the R2 side chain contains a nitrogen atom within a heterocyclic ring (as in risendronate and zoledronate).

Thus, according to the chemical structure of the R2 side chain, bisphosphonates are generally classified as follows:

- Non-nitrogen-containing bisphosphonates:
  - Etidronate (Didronel®)
  - Clodronate (Bonefos®, Loron®)
  - Tiludronate (Skelid®)
- Nitrogen-containing bisphosphonates:
  - Pamidronate (Aredia®)
  - Neridronate
  - Olpandronate



**Bisphosphonates.** **Fig. 1** Generic structure of bisphosphonates

- Alendronate (Fosamax®)
- Ibandronate (Bonduromat®)
- Risendronate (Actonel®)
- Zoledronate (Zometa®)

## Mechanism of Action

Bisphosphonates bind to the bone mineral in particular at sites of active bone metabolism, where they achieve therapeutic concentration. During the process of bone resorption, since the acid environment osteoclasts causes dissolution of the hydroxyapatite bone mineral, bisphosphonates are released in this sub-cellular space, and are internalized by osteoclasts. At this point, osteoclasts lose the ruffled border and show cytoskeleton alterations, and eventually become apoptotic.

The toxic effect of bisphosphonates on osteoclasts can be explained in at least two different ways, according to their chemical structure.

First-generation, non-nitrogen-containing bisphosphonates, such as clodronate and etidronate, are metabolized by osteoclasts to nonhydrolyzable adenosine triphosphate (ATP) analogues, with subsequent inhibition of ATP-dependent intracellular enzymes. The intracellular accumulation of these metabolites inhibits osteoclast function and can induce apoptosis. On the other hand, aminobisphosphonates, following internalization in the osteoclasts, inhibit the farnesyl diphosphonate (FPP) synthase, affecting the biosynthetic mevalonate pathway. This pathway is involved in the production of sterols such as cholesterol, and isoprenoid lipids such as isopentenyl diphosphate, farnesyldiphosphate (FPP) and geranylgeranyldiphosphate (GGPP). FPP and GGPP are essential for the posttranslational modification of small ► **GTPases** (Ras, Rab, Rho, and Rac). These signaling proteins are involved in the regulation of cell proliferation, cytoskeletal organization, membrane ruffling, intracellular vesicle transport, and apoptosis. In addition,

aminobisphosphonates can also induce the formation of intracellular ATP analogues which may directly induce osteoclast apoptosis.

More recently, a growing number of preclinical data have consistently demonstrated the direct antitumor effect of bisphosphonates. The mechanisms responsible for this effect are not still fully elucidated. However, it has been shown that bisphosphonates can inhibit angiogenesis, cell proliferation and adhesion. Moreover, the effects of bisphosphonates on osteoclasts result in an inhibited release of growth factors in the bone microenvironment, thus rendering the bone less hospitable to cancer cell homing.

### Pharmacokinetic

Bisphosphonates are characterized by very low absorption from the gastrointestinal tract (less than 6% for clodronate and etidronate). The plasma half-life ranges between 20 min and 2–3 h, depending on the type of bisphosphonates and the individual rate of clearance. However, because of the high affinity for the bone matrix, half-life in bone is very long, ranging from months to years.

### Clinical Use

#### Hypercalcemia of Malignancy (HCM)

HCM is a severe clinical condition that can occur in up to 20% of patients with advanced cancer, in the presence or absence of bone metastases. HCM is a consequence of osteoclast activation due to the presence of cancer cells in the bone (metastatic bone disease), or the production by cancer cells of parathyroid hormone-related protein (humoral hypercalcemia). Tumors more frequently inducing episodes of HCM include non-Hodgkin lymphomas, myeloma, lung cancer, breast cancer, ► renal cancer and ► Prostate Cancer.

HCM occurs when total serum calcium is above 10.2 mg/dl (2.55 mmol/l), and causes a variety of symptoms including gastrointestinal manifestations (anorexia, vomiting, and constipation), renal function deterioration, alteration of cardiac rhythm (EKG abnormalities and arrhythmias), and neurological disorders (from asthenia to lethargy and coma).

Treatment of HCM includes iv hydration and diuretics to facilitate renal calcium excretion, and bisphosphonates to inhibit calcium resorption from

the bone. A single bisphosphonate iv infusion can obtain a sustained serum calcium normalization in about 80% of the patients.

### Treatment of Bone Metastases

Bone metastases represent a major health problem and can occur in a significant proportion of patients with solid tumors. Bone metastases are frequent (up to 70–80% of the patients) in common tumor types such as prostate cancer, breast cancer, and lung cancer. Bone metastases develop when circulating tumor cells home in the bone marrow and stimulate the activation of osteoclasts that eventually initiate bone matrix resorption. Bone metastases can be lytic, sclerotic, or mixed, depending on the balance between bone resorption induced by osteoclasts and new bone formation by osteoblasts.

Metastatic bone disease causes considerable morbidity, leading to several complications including pain, pathologic fractures, spinal cord compression, ineffective hematopoiesis, and HCM. In addition to the specific anticancer therapy (e.g. chemotherapy, hormonal therapy, or biologic agents), the current options to treat bone metastases are radiation therapy, orthopedic surgery, radiopharmaceuticals, and bisphosphonates. Currently, bisphosphonates are considered the mainstay of the treatment for metastatic bone disease from myeloma, breast cancer, prostate cancer, and other solid tumors including lung and renal cancer.

In clinical trials, the efficacy of bisphosphonates has been measured on the basis of their capacity to reduce or delay the skeletal related events (SRE). An SRE is defined as the occurrence of pathologic fractures, radiation therapy for bone pain or to treat/prevent a fracture, surgery to stabilize bone fractures, hypercalcemia of malignancy, or spinal-cord compression.

The following sections will briefly summarize the clinical experience with different bisphosphonates according to tumor types.

#### Multiple Myeloma

► **Multiple myeloma** (MM) is associated with relevant skeleton morbidity, since lytic lesions are present in more than 90% of the patients. The lytic process in MM is different from bone metastases from other cancers, where bone destruction is generally followed by new bone formation. Several bisphosphonates including clodronate, pamidronate, and ► **zoledronic acid** are effective in preventing or delaying skeletal

complications. Oral clodronate have shown a significant reduction in non-vertebral and vertebral fracture rates over a placebo. Again compared to placebo, intravenous pamidronate significantly reduce the proportion of patients with any SRE. It was also associated with significant decrease of bone pain. More recently, ► **zoledronic acid** was shown not only to be as effective as iv pamidronate, but to also produce an additional 16% risk reduction of skeletal complication as measured by multiple event analysis.

#### Bone Metastases from Breast Cancer

Several bisphosphonates have been approved in the United States and Europe for the treatment of skeletal metastases from ► **breast cancer**.

The efficacy of pamidronate has been known since the early 1990s. In two pivotal, phase III randomized trials, pamidronate significantly reduced the incidence and delayed the onset of SREs as compared to placebo. It was also effective in the reduction of pain scores.

The more potent bisphosphonate ► **zoledronic acid** has been directly compared to pamidronate. The pivotal trial, including breast cancer and multiple myeloma patients, was designed as a non-inferiority trial, the primary end point being the percentage of patients with at least 1 SRE at 25 months. Zoledronic acid was at least as effective as pamidronate according to the primary end point. Furthermore, the multiple events analysis demonstrated that zoledronic acid was significantly more effective in reducing the risk of SREs in the subset of breast cancer patients.

Zoledronic acid was also compared to placebo in a trial conducted in Japan, where pamidronate is not approved for the treatment of bone metastases from breast cancer, showing a clear superiority in reducing the SRE rate ratio, the percentage of patients with at least 1 SRE, and in delaying the time to first SRE. The multiple event analysis showed a 44% reduction in the risk of developing an SRE, and significantly reduced mean pain scores from baseline over 12 months.

Ibandronate is a single-nitrogen bisphosphonate available in both intravenous and oral formulations. In terms of reduction of SREs and pain control, the efficacy of iv and oral ibandronate has been confirmed in three placebo-controlled phase III randomized trials. Ibandronate is currently approved in more than 40 non-US countries for the treatment of patients with breast cancer and bone metastases. The approval of the US Food and Drug Administration is still

pending. A direct comparison between ibandronate and zoledronic acid is ongoing. Recently, in a review of 21 randomized controlled trials of bisphosphonates in breast cancer by the Cochrane Collaboration, it has been shown that zoledronic acid reduces the risk of SRE by 41%, compared with 33% by pamidronate, 18% by iv ibandronate, 14% by oral ibandronate, and 16% by oral clodronate.

#### Bone Metastases from Prostate Cancer

Bisphosphonates have also been studied in patients with ► **prostate cancer** and bone metastases. In patients with symptomatic bone disease, pamidronate failed to show any advantage over placebo in pain scores, analgesic use, and SREs. On the contrary, as compared to placebo, zoledronic acid significantly reduced the proportion of patients with an SRE over 2 years and delayed the time to first SRE by approximately 6 months. Therefore, zoledronic acid was approved for the treatment of patients with prostate cancer metastatic to bone and progression of disease despite first line hormonal therapy.

More recently, ibandronate has shown some palliative benefit in a small open-label study, but its efficacy over placebo in a randomized trial is still to be demonstrated.

#### Bone Metastases from Lung Cancer and Other Solid Tumors

The first bisphosphonate with proven efficacy in the treatment of bone metastases from ► **lung cancer** and solid tumors is zoledronic acid. In a placebo-controlled randomized trial in more than 700 patients, zoledronic acid significantly reduced the proportion of patients experiencing at least one SRE, and delayed the median time to first SRE as well. Currently, zoledronic acid is the only bisphosphonate approved for the treatment of metastatic bone disease from solid tumors other than breast cancer.

#### Prevention of Bone Metastases

Because of their mechanism of action, bisphosphonates have the potential to prevent cancer cells homing in the bone, thus changing the entire process of metastatic spread. The data from the earlier, weaker generation of bisphosphonates are conflicting, and to date bisphosphonates are not recommended for

the prevention of bone metastases. However, ongoing studies with the newer, more potent intravenous bisphosphonates will establish the role of these compounds in the prevention of bone metastases in several tumor types such as breast, prostate, and lung cancer.

B

### Osteoporosis

In both men and women, bone mass decreases with age. In men, this process is constant over time while women usually experience a significant increase in the rate of bone loss after menopause. The most standardized method to evaluate the bone mineral density (BMD) is the dual energy X-ray absorptiometry (DXA) at the recommended site of the proximal femur. Patients are classified as osteoporotic when the BMD (as expressed as T-score) is 2.5 standard deviation (SD) or more below the average value for premenopausal women. When the T-score is between  $-1$  and  $-2.5$  SD patients are classified as osteopenic. For each SD reduction in BMD, there is a doubling in the risk of fracture.

Bisphosphonates have been successfully used to treat osteoporosis. In particular, alendronate and etidronate can increase the BMD and almost halve the fracture rates in postmenopausal women, representing the most frequent agents used worldwide in this setting.

Besides the risk of bone metastatization, the bone health of cancer patients can be further affected by cancer therapies. This particular condition, known as cancer-treatment-induced bone loss (CTIBL), reflects the effects of cancer therapy (both chemotherapy and endocrine therapy) on bone mineralization. In brief, all cancer therapies that directly or indirectly antagonize the effect of estrogen or androgen significantly enhance the loss in bone mineral density, thus dramatically increasing the risk of fractures. Because of the higher severity of CTIBL, the common strategies to treat benign osteoporosis, such as oral bisphosphonates, calcium/vitamin D supplements, and calcitonin, might not be sufficient. In early breast cancer, both daily oral clodronate and intermittent oral risendronate have shown superiority over placebo, although clodronate was unable to completely prevent bone loss in patients with chemotherapy-induced ovarian dysfunction. The use of more potent intravenous bisphosphonates is under investigation in three large trials. In prostate cancer patients receiving androgen deprivation therapy, zoledronic acid was able to

reverse CTIBL, and even increase bone density at multiple sites. Several trials in prostate cancer are under way to confirm these results. With these potent agents, a less frequent schedule (every 3 or 6 months) seems to be effective, while the monthly dose is used for metastatic bone disease.

### Side Effects

The safety profile of bisphosphonates varies depending on the route of administration. Treatment with intravenous bisphosphonates is usually well tolerated, with transient side effects such as mild to moderate flu-like symptoms following initial infusions, generally self-limited. However, iv bisphosphonates have the potential to adversely affect renal function, and sporadic episodes of both acute and chronic renal failure have been described. The risk of renal failure is directly related to dose and to the drug infusion time: when bisphosphonates are administered at the recommended doses and infusion rates, the incidence of elevated serum creatinine is generally low ( $<10\%$ ), and severe adverse renal events are rare. Nevertheless, accurate renal-function monitoring is recommended in the use of iv pamidronate and zoledronic acid. In breast cancer patients, iv ibandronate has shown a renal safety profile similar to a placebo, and because no case of renal failure has been described at the time of writing this, the monitoring of serum creatinine prior to each ibandronate administration is not mandatory.

Oral administration of bisphosphonates can cause esophagitis and other gastrointestinal side effects such as mucositis, nausea, vomiting, and diarrhea.

In the past few years, a growing number of cases of ► [jaw osteonecrosis](#) have been associated with the use of aminobisphosphonates, prompting labeling changes for pamidronate and zoledronic acid. Several reports have described a frequency of jaw osteonecrosis ranging from 0.6% to 4.3% for patients with breast cancer and from 3% to 9.9% for those with multiple myeloma. The exact mechanism underlying jaw osteonecrosis has not yet been fully elucidated. Dental disease, dental surgery, periodontal disease, trauma, and poor oral hygiene are the most often reported precipitating factors. Several reports have also identified a relationship between dose and duration of treatment and the development of this complication. Because jaw osteonecrosis is not reversible in the majority of the

cases, physicians should focus on the prevention of this complication. It is therefore recommended to assess the dental status of patients before starting bisphosphonates, and avoid invasive dental procedures while on bisphosphonate therapy.

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## BL

### Definition

Burkitt lymphoma.

► [Childhood Cancer](#)

### Bladder Cancer

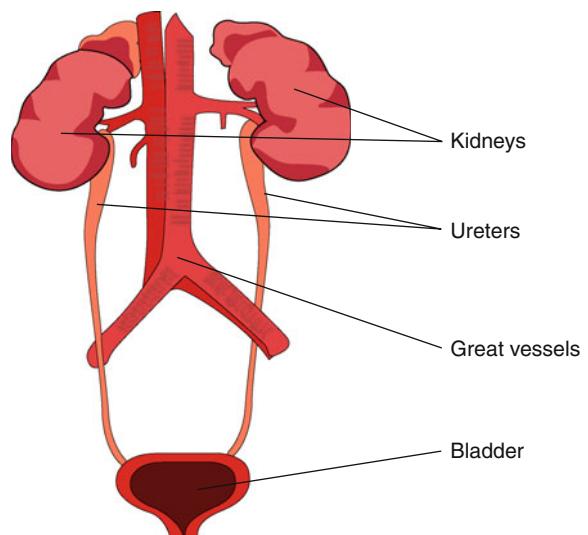
Dan Theodorescu<sup>1</sup> and Behfar Ehdaie<sup>2</sup>

<sup>1</sup>University of Colorado Cancer Center, University of Colorado, Aurora, CO, USA

<sup>2</sup>Urology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

### Definition

Bladder cancer is a malignant neoplasm which arises from the epithelial lining of the bladder (Fig. 1). Several histological forms have been identified. Cancers



**Bladder Cancer. Fig. 1** Anatomy of the urinary tract

with urothelial histology (UC) comprise more than 90% of the neoplasms, while ► [squamous cell carcinoma](#) (SCC) and adenocarcinoma account for 5% and 2%, respectively. In areas with endemic schistosomiasis, SCC is the predominant histological form. It is also not uncommon for urothelial cell malignancies to have minor elements of adenomatous or squamous cell histology. However, from the clinical management standpoint, urinary neoplasms with minor components of these two histologic types are treated for their primary component. The clinical relevance of these minor components or the percentage at which a minor component becomes significant is unclear. An important prognostic criterion in ► [urothelial cell carcinoma](#) is tumor grade. Tumor grading most commonly follows World Health Organization (WHO) guidelines in which malignant tumors are classified as papillary urothelial neoplasm of low malignant potential (PUNLMP), low grade or high grade, regardless of invasion status.

### Characteristics

#### Clinical Epidemiology and Risk Factors

Carcinoma of the urinary bladder is the second most common urologic malignancy. The worldwide incidence of bladder cancer is approximately 200,000

patients per year with 120,000 annual deaths, accounting for 3.2% of all malignancies. It affects more males than females by a 3:1 ratio. In the United States, the incidence is higher in Whites than Blacks, although survival is longer in Whites and men than in Blacks and women. The disease can affect all ages (even children) but the median age at presentation is 70 years. It is rarely found as an incidental finding at autopsy suggesting that these cancers do not have a long latent or subclinical course. Bladder cancer incidence has increased 50% between 1985 and 2005; however, the mortality rate has decreased by 33% in the past four decades.

Environmental risk factors for ► **urothelial cell carcinoma** include cigarette smoking (► **Tobacco-Related Cancers**), aniline dyes, pelvic radiation, benzidine, 2-naphthylamine, and other aromatic amines (► **Aromatic Amine**). Acrolein, a metabolic product of cyclophosphamide, can increase the risk of bladder cancer ninefold. Smoking increases the risk of bladder cancer fourfold, and at least one quarter of cases can be attributed to smoking. Chronic cystitis and long-term bladder catheters increase the risk of squamous cell carcinoma. *Schistosoma haematobium* infection not only increases the risk of SCC significantly, but also increases the risk of urothelial cell carcinoma. Epidemiologic evidence does not exist for a hereditary etiology for bladder cancer.

### Tumor Biology and Genetics

Urothelial cell carcinoma is a field change disease rendering the entire urothelium susceptible to malignant transformation. Polyclonotopicity refers to the propensity of tumors to arise at different times and sites in the urothelium. Both the ► **TP53** and ► **RAS** genes are known targets of chemical carcinogens. The most frequent genetic alterations in urothelial cell carcinoma are monosomies of chromosome 9 (57%), and losses on chromosome arms 11p (32%), 17p (32%), 8p (23%), 4p (22%), and 13q (15%). Deletions specifically associated with higher grades and stages of cancer, indicative of tumor progression to muscle invasive disease, have been identified at 3p, 4q, 8p, 10, 15, 17p, and 18q among many others. Other studies utilizing immunohistochemical techniques have suggested that overexpression of p21Ras protein, mutated TP53, and the epidermal growth factor receptor (EGFR) in bladder tumors are related to bladder tumor progression. In addition, loss of RB1, DCC, and ► **E-cadherin** (CDH1)

expression has also been related to this transition. Tumors with p53 mutations tend to exhibit more aggressive behavior when present in both noninvasive and invasive disease, while chromosome 9 alterations and ► **fibroblast growth factor receptor** 3 (FGFR3) mutations are associated with low grade noninvasive disease. In fact, FGFR3 mutations are found in up to 90% of non-muscle invasive cancers while only found in 10% of muscle invasive or metastatic cancers.

### Characteristics of Nonurothelial Cell Carcinomas

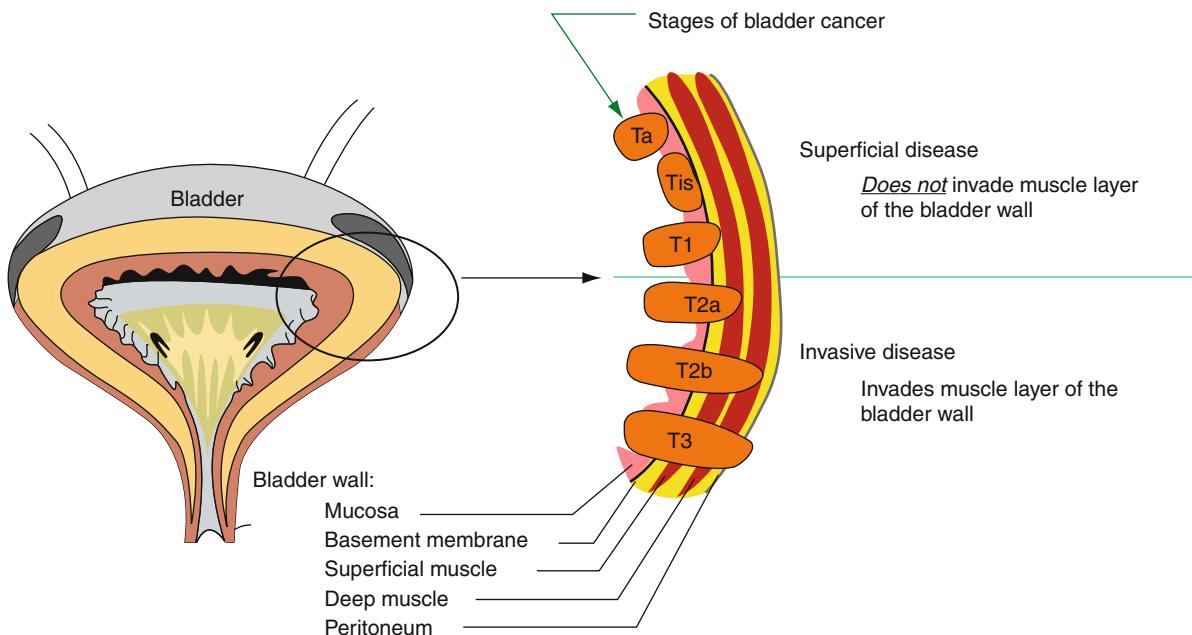
SCC comprises only 1–3% of bladder tumors in the United States and Britain but represents 75% of tumors in Egypt. Most of the SCCs found in Egypt are due to *S. haematobium* (“bilharzial” bladder cancer) and are well differentiated with lower risk of metastases than urothelial cell carcinoma. Non-bilharzial squamous cell tumors are caused by chronic inflammation from infection, stones, indwelling catheters, or bladder diverticuli. Although these tumors’ prognosis is similar to urothelial cell carcinoma by stage, non-bilharzial tumors tend to present with late-stage disease. Primary bladder adenocarcinomas represent approximately 2% of bladder tumors and are more common in extrophic bladders, urachi and intestinal conduits or augmentations. They may produce mucus and can be associated with cystitis glandularis. Most are poorly differentiated and present with advanced disease.

### Clinical Presentation

Bladder cancer frequently presents with painless hematuria, although urinary frequency, urgency, and dysuria can occur as well. Gross hematuria is common, and bladder cancer is rarely diagnosed in the absence of at least microscopic hematuria although this can be intermittent. Bladder cancer can also present with flank pain and hydronephrosis if the tumor obstructs the ureteral orifice.

### Diagnosis and Staging

The diagnostic evaluation of bladder cancer begins with a history and physical examination including bimanual pelvic exam, urinalysis, cytology, and cystoscopy. In the past, intravenous urography (IVU) was indicated in all patients with bladder tumors to evaluate the upper urinary tracts. Retrograde ureteropyelograms (IVP) can also be performed at the time of cystoscopy if IVU does not provide an



**Bladder Cancer. Fig. 2** Stages of bladder cancer

adequate view of the upper tracts. Currently, IVP has been replaced by CT scanning of the abdomen and pelvis with 3D reconstructions allowing for both the assessments of extravesical spread (contiguous and metastatic) and urography (CT urogram). This is discussed in more detail later. Cytologic examination of bladder cells that slough off into urine is useful in the diagnosis of carcinoma in situ (CIS) or high grade tumors, but low grade tumors are more difficult to detect by cytology. Cytology is primarily used in the diagnosis and follow up of patients at risk for recurrent disease. Novel biomarkers such as the nuclear matrix protein (NMP-22) assay, survivin, BLCA4, and FISH analysis (UroVysion) offer promise for enhanced detection of symptomatic patients, screening of high risk populations, and treatment follow up and monitoring. Most recently, a novel technique called COXEN has been applied to translate signatures of in vitro chemosensitivity for prediction of clinical outcome and drug discovery. In addition, somatic tumor genetic signatures have been applied and validated to predict lymph node micrometastases.

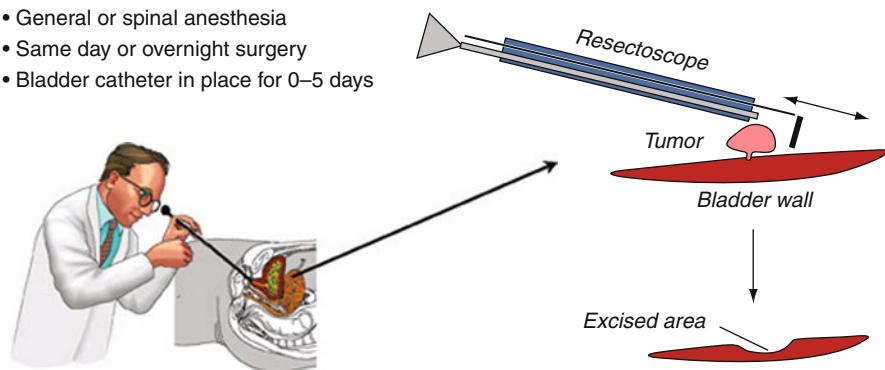
At presentation, 85% of patients with urothelial cell carcinoma of the bladder have disease limited to the organ, while 10% have regional disease and 5% have metastatic disease. Of the 85% with localized disease,

80% have non-muscle invasive disease (stages Tis/CIS, Ta, T1) and 20% have muscle invasive disease (stages T2–T4) (Fig. 2). These stages are according to the UICC/AJCC system, the most common staging system in use today. Urothelial cell carcinoma may grow in papillary, sessile, nodular, or flat (Tis) forms. Papillary tumors with orderly cellular arrangement and minimal nuclear atypia are designated PUNLMP. Such tumors rarely progress to invasive disease and are considered benign. Tis/CIS is sessile, poorly differentiated urothelial cell carcinoma involving only the urothelium. Although CIS can cause irritative voiding, it is often asymptomatic. Cystoscopy may be normal or exhibit erythematous patches, and urine cytology is 80–90% sensitive.

Transurethral resection (TUR) of bladder tumors not only provides tissue for pathologic diagnosis, but can represent definitive therapy in the most clinical stage Ta and T1 tumors as long as the whole tumor is resected (Fig. 3). After the intraluminal portions of the tumor are resected, the tumor base is frequently resected as a separate pathologic specimen to ensure complete resection and accurate staging. T1 tumors undergo a second resection 3–6 weeks later to reduce the risk of understaging. It is critical that muscularis propria is included in the specimen to exclude the

**Bladder Cancer. Fig. 3** The transurethral resection (TUR)

- Electrical scraping of tumor
- General or spinal anesthesia
- Same day or overnight surgery
- Bladder catheter in place for 0–5 days



presence of muscle invasion by the tumor. Routine performance of random biopsies of the bladder or prostatic fossa mucosa remains controversial. However, these may be indicated to evaluate for CIS in patients with positive cytology or in patients that are candidates for orthotopic neobladder or partial cystectomy. If the tumor appears invasive (i.e., sessile, solid configuration), the resection is tailored so as to accurately determine clinical stage and to optimize subsequent definitive therapy. For example, if the patient is likely to choose radical cystectomy as the treatment of choice then complete TUR is not necessary. Conversely, if the patient is likely to select bladder sparing therapy with radiation and chemotherapy, then resection of as much tumor as safely possible should be carried out.

As alluded to earlier, the staging of bladder cancer is based primarily on the specimen generated by the TUR and is classified according to the 2010 UICC/AJCC system (Table 1) and revised by the World Health Organization/International Society of Urological Pathology WHO/ISUP Consensus Classification (Table 2). In 1998, the WHO/ISUP Consensus Classification of urothelial neoplasms of the urinary bladder was developed to unify the numerous diverse grading schemes for noninvasive bladder cancer and provide detailed histological criteria for papillary urothelial lesions. In addition, the new classification system allows for designation of a lesion papillary urothelial neoplasm of low malignant potential, which biologically has a very low risk of progression, but is not entirely benign. Therefore, an intermediate classification enables these patients to avoid the label of having cancer with its psychosocial and financial implications

**Bladder Cancer. Table 1** UICC/AJCC consensus classification

UICC/AJCC 2010	Description
Ta	Papillary tumor, epithelium confined
Tis	Carcinoma in situ: “flat tumor”
T1	Lamina propria invasion
T2a	Tumor invades non-muscle invasive muscle (inner half)
T2b	Tumor invades deep muscle (outer half)
T3a	Tumor invades perivesical fat microscopically
T3b	Tumor invades perivesical fat macroscopically (extravesical mass)
T4a	Tumor invades prostate or uterus or vagina
T4b	Tumor invades pelvic wall or abdominal wall
N1	Single node
N2	Multiple nodes
N3	Nodal metastases above bifurcation of common iliac vessels
M1	Distant metastasis

**Bladder Cancer. Table 2** WHO/ISUP Consensus Classification

World Health Organization/International society of urological pathology classification	
Hyperplasia	Flat hyperplasia
	Papillary hyperplasia
Flat lesions with atypia	Reactive (inflammatory) atypia
	Dysplasia
	Carcinoma in situ (CIS)
Papillary neoplasms	Papilloma
	Papillary neoplasm of low malignant potential (PUNLMP)
	Papillary carcinoma, low grade
	Papillary carcinoma, high grade

and prevent them from being diagnosed as having a benign lesion, whereby they might not be followed as closely.

The pathologic exam of bladder specimens may be complicated by difficulty in differentiating muscularis propria from the more non-muscle invasive and thin muscularis mucosa, the latter which does not represent “true muscle” invasion and thus direct communication between urologist and pathologist is essential. If pathologic examination reveals tumor invasion into muscularis propria, CT or MRI of the abdomen and pelvis is used to evaluate for gross extravesical spread, lymphadenopathy, or hepatic metastases. In general, these methods fail to detect lymph node spread in as many as 30% of patients. A radionucleotide bone scan can be obtained to evaluate for bony metastases, but the yield of a bone scan in the face of a normal alkaline phosphatase is low. Chest X-ray or CT scan is obtained to rule out pulmonary metastases.

### Management of Noninvasive Disease

The therapy of noninvasive (Ta/T1) bladder cancer consists of TUR and fulguration. Because approximately 30% of these tumors tend to recur and 10% may progress to muscle invasion, follow-up cystoscopy at regular intervals is mandatory. Tumors that invade the lamina propria (T1) should be considered potentially more aggressive, particularly if high grade. Argon and Nd:Yag lasers have also been used successfully for ablation of noninvasive bladder tumors especially those that are multifocal or difficult to access via the resectoscope used for TUR. The disadvantage of these techniques is the lack of tumor specimen to be analyzed by pathology and thus only lesions with a high likelihood of being noninvasive should be treated in this way.

Patients with recurrent, high grade Ta, T1 tumors, or CIS may benefit from intravesical therapy with ► **Bacillus Calmette–Guerin (BCG)** or ► **mitomycin C**. These treatments can be given in two clinical contexts. They can be given for the treatment of residual disease which could not be removed at TUR. Alternatively, they can be used to reduce the incidence of recurrence and progression in patients that have completely resected tumors. Furthermore, Mitomycin C delivered perioperatively in one dose has successfully reduced the incidence of tumor recurrence following TUR. BCG is a live attenuated strain of

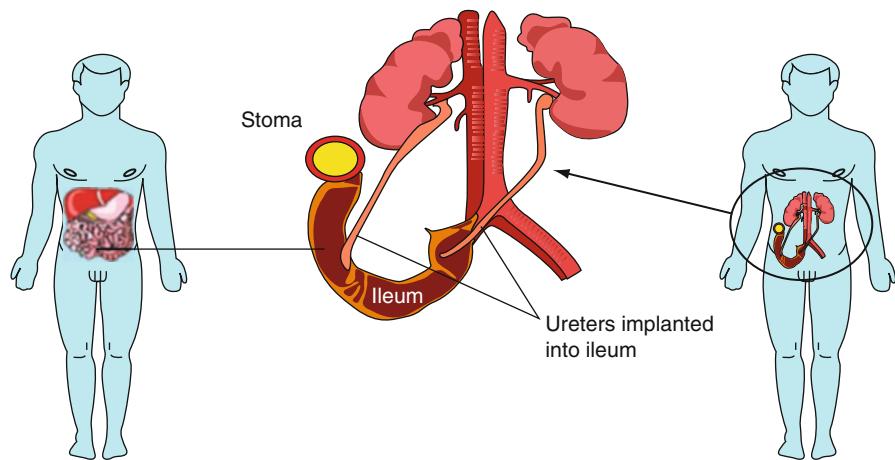
*Mycobacterium bovis*, which stimulates a local and possibly systemic immune response. BCG can often delay recurrence and progression of high grade noninvasive disease and CIS. Side effects of BCG include bladder irritability, granulomatous prostatitis, systemic disseminated infection requiring antitubercular agents, and rarely death. Contraindications for intravesical delivery include: active tuberculosis, immunosuppression, traumatic catheterization, gross hematuria, and prior severe reaction to BCG. Mitomycin C is an alkylating chemotherapeutic agent that inhibits DNA synthesis. BCG is superior to Mitomycin C in reducing the risk of progression in high grade tumors in some but not all studies. BCG can also be used for maintenance therapy where it has been shown to reduce recurrence even further. Other intravesical compounds include interferon, keyhole-limpet hemocyanin, bropirimine, mycobacterial cell wall DNA extract, doxorubicin and its derivatives, thiotepa, and ► **gemcitabine**. The effectiveness of these agents compared with the two mentioned earlier in delaying progression and recurrence in initially treated patients is generally less and thus their use has been reserved for the salvage setting. Similarly, the addition of interferon gamma to BCG has been used to treat patients who have recurred after initial BCG therapy. Among patients with Ta, T1, or CIS, radical cystectomy is reserved for diffuse, symptomatic, recurrent high-grade, or unresectable papillary tumors unresponsive to intravesical therapy.

Recurrence polychronotropism (multiple recurrences in space and time) in noninvasive bladder tumors is uniquely elevated when compared to other organ sites. Twenty to seventy percent of patients suffer disease recurrence. While in the absence of progression recurrence per se is not life threatening, this phenomenon nonetheless constitutes a cause of significant morbidity and treatment expense. While less common, the progression of noninvasive tumors to muscle invasion is associated with a marked decrease in 5-year disease-specific survival. Progression risks vary widely by stage and grade, ranging from less than 5% for low grade papillary tumors and greater than 50% for T1 lesions with associated CIS.

### Management of Invasive and Metastatic Disease

Radical cystectomy with urinary diversion or bladder sparing protocols, using a combination of radiation

**Bladder Cancer. Fig. 4** The ileal conduit urinary diversion



and chemotherapy, are the treatments of choice for patients who have resectable muscle invasive bladder cancer. Radical cystectomy includes wide excision of bladder and prostate in male patients and typically bladder, uterus, ovaries, and anterior vaginal wall in females. Perioperative mortality from cystectomy is approximately 1% in most centers. The 5-year disease-free survival is 65–80% for pT2 tumors and 37–61% for pT3 tumors. Microscopic involvement of local lymph nodes decreases 5-year survival to approximately 5–20% depending on the number and extent of nodal involvement. Pelvic recurrence rates after cystectomy range from 2% to 10% and depends on the stage of the primary tumor as well as the presence of pelvic nodal involvement. In addition, an interval longer than 12 weeks between the diagnosis of muscle invasive bladder cancer and radical cystectomy is associated with decreased survival. Recently, the use of ► **neoadjuvant therapy** consisting of a four drug regimen MVAC (methotrexate, vinblastine, doxorubicin, and ► **cisplatin**) has demonstrated a survival advantage for patients with localized bladder cancer undergoing cystectomy. The use of ► **adjuvant therapy** has been suggested by some authors; however, there is limited evidence of benefit for this approach. Ongoing clinical trials are addressing this question.

Recurrence or persistence rates after bladder sparing protocols approach 50%. By careful patient selection, these latter protocols can achieve comparable disease-specific survival rates to those obtained by radical cystectomy. Large tumors that are only minimally resectable by TUR and those causing hydronephrosis have a significantly worse response

rate with such bladder sparing protocols. Complications of radiotherapy include dysuria, frequency, or diarrhea in up to 70% of patients.

Following cystectomy, multiple options in urinary diversion exist, most of which utilize intestinal segments. An ileal conduit using a short portion of the terminal ileum to carry urine from the ureters to the anterior abdominal wall is the simplest most commonly performed diversion and the one associated with the least number of complications. Patients wear an external appliance on the stoma. Possible complications include parastomal hernia, stoma stenosis, or stricture at the ureteroileal anastomosis. A cutaneous continent urinary diversion such as the Indiana (ileocecum) pouch forms an internal reservoir which can then be intermittently catheterized via a small cutaneous stoma (Fig. 4). In selected patients, such continent reservoirs can be anastomosed to the native urethra and in this setting are called “orthotopic neobladders.” Such continent diversions are technically more difficult and require motivated patients to manage the postoperative care required. Ureterosigmoidostomies are now rarely performed because of difficulties with reflux, urolithiasis, electrolyte imbalance, and increased risk of adenocarcinoma of the colon.

Recent advances in laparoscopic and robotic surgery have enabled minimally invasive techniques to be applied for treatment of various benign and malignant conditions of the urinary bladder. Multiple centers worldwide are reporting their initial experience with laparoscopic radical cystectomy and urinary diversion. The majority of centers perform an intracorporeal

laparoscopic cystoprostatectomy and complete the urinary diversion extracorporeally through a mini-laparotomy incision.

Metastatic urothelial cell carcinoma has traditionally been treated with MVAC with a response rate of 15–35%. Complete remission is seen in approximately 13% of patients and mean survival can be improved from 8 to 12 months. However, MVAC is associated with significant toxicity, as 20% experience neutropenic fever and sepsis-associated mortality approaches 3–4%. Newer agents have recently been used with a significantly lower morbidity and mortality than MVAC. Gemcitabine is an antimetabolite chemotherapeutic agent. The combination of gemcitabine and cisplatin has demonstrated similar effectiveness to that of MVAC with a better safety profile and tolerability. However, larger randomized trials are needed to conclusively prove this point.

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### Bladder Tumor

#### Synonyms

#### Bladder Cancer

### Blast Crisis

Christine M. Morris

Cancer Genetics Research Group, University of Otago at Christchurch, Christchurch, New Zealand

### Definition

Blast crisis is the aggressive and rapidly fatal terminal phase of ► **BCR-ABL1** positive ► **Chronic Myeloid Leukemia** (CML). This phase of the disease is characterized by accumulation of immature myeloblasts or lymphoblasts similar to those found in patients with acute leukemia.

### Characteristics

#### Clinical Features

When left untreated, CML is a biphasic disease. Patients typically present in a relatively benign chronic phase which is characterized by symptoms of fatigue and lethargy, bleeding, moderate weight loss, an enlarged palpable spleen, and a high white blood cell (WBC) count. The increased WBC population in large part constitutes cells of the myeloid compartment, with overrepresentation of the granulocyte series. Within a period of 3–5 years, the natural course of the disease is to accelerate, then to transform to an aggressive and rapidly terminal acute phase or blast crisis of 4–6 months duration. Features associated with this transformation include an increasing number of leukocytes, particularly immature blasts, in the blood and bone marrow, progressive anemia, thrombocytopenia, and lack of response to therapy. In a small proportion of patients, the blast transformation may occur outside the bone marrow (extramedullary) in sites such as the lymph nodes, spleen, skin, or meninges. There is no

known cure for blast crisis CML. However, transition to accelerated disease may be postponed for several years or prevented by treatment early during chronic phase CML with the BCR-ABL1 tyrosine kinase inhibitor ► **imatinib mesylate** or allogeneic bone marrow transplant.

Blast crisis can be divided into two general forms: lymphoid and myeloid. Lymphoid blast crisis develops in about 30% of patients, and the blast cells are phenotypically similar to the common form of ► **acute lymphoblastic leukemia** (ALL). In rare cases, T-cell morphology has been described. Myeloid transformation is heterogeneous, where myeloblasts are the usual blast cell type, but megakaryoblasts or erythroblasts have been frequently identified. Occasional patients show blasts with myelomonocytic, monocytic or very rarely, basophilic blast differentiation. It is important to differentiate between myeloid and lymphoid blast cells, as patients in lymphoid blast crisis respond better to treatment.

### Biological Basis

The precise molecular events that determine blast crisis are still poorly understood. However, the destabilized proliferation status that is imposed by expression of the ► **BCR-ABL1** fusion gene in a self-renewing leukemic stem cell is a likely mitigating influence. Much evidence has shown that there is lineage-specific selection for, and accumulation of, cytogenetic and molecular gene rearrangements in the affected myeloid or lymphoid cell compartments. Cytogenetic evolution of the BCR-ABL1 fusion gene positive clone occurs in ~80% of cases with CML that transform to blast crisis, and a change in karyotype is considered to be a poor prognostic sign, heralding or accompanying the acute transition. Diverse karyotype abnormalities are observed, both structural and numerical, either singly or in combination, and there is marked nonrandom involvement of certain chromosomes. Duplication of the Ph chromosome (and therefore the BCR-ABL1 fusion gene), i(17q), +8 or +19 are observed alone or in various combinations in 60–80% of cases having additional abnormalities. Recurring molecular changes have also been identified at transformation in some cases, and include mutation of the ► **TP53** and ► **retinoblastoma** 1 (RB1) genes, activation of ► **RAS**, and, in lymphoid blast crisis cells, homozygous loss of the tumor suppressor gene

► **CDKN2A** (p16). These and other studies suggest that BCR-ABL1 cells are genetically unstable, and preferentially accumulate nonrandom genomic mutations that are compatible with the BCR-ABL1 oncogene product and provide a proliferative advantage. Similar nonrandom accumulation of genomic aberrations is also observed in advanced leukemias of BCR-ABL1 transgenic mice. New evidence suggests that the biological characteristics of blast crisis CML will alter in the post-imatinib mesylate era.

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### Blast Phase

#### Definition

Last phase of CML, similar to an acute leukemia (poor prognosis). Characterized by >20% myeloblasts or lymphoblasts in blood or bone marrow.

► **Nilotinib**

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### Blastocyst

#### Definition

A mammalian embryo before implantation. Blastocysts consist of outer trophectoderm cells, which allow the embryo to implant, surrounding the inner cell mass, within which are found the pluripotent epiblast cells.

## Bleomycin

### Definition

A chemotherapeutic drug categorized as a cytotoxic/antitumor antibiotic. As an anticancer drug, it is typically used in the treatment of cervical cancer, head and neck cancer, Hodgkin disease, non-Hodgkin lymphomas, and testicular cancer. Bleomycin interferes with cell growth by damaging DNA and preventing DNA repair. An anticancer antibiotic that can induce DNA strand breaks. It is the first mutagen applied to

► [mutagen sensitivity assay](#).

► [Hyperthermia](#)

► [Malignant Lymphoma, Hallmarks and Concepts](#)

► [Mutagen Sensitivity](#)

## BLI

► [Bioluminescence Imaging](#)

## BLL

### Definition

Blood lead level.

► [Lead Exposure](#)

## Blood Stasis due to Vital Energy Stagnancy

### Definition

Is a pathogenesis and syndrome in Chinese medicine. Various pathogenic factors such as emotional depression, unhealthy diet, infection, and injury obstruct the circulation of vital energy and result in vital energy stagnancy. Chronic or severe stagnation of vital energy may lead to blood stasis, with syndrome characterized by distention, pain, ecchymosis, and even mass

formation. The principle of its treatment is to supplement vital energy, promote blood circulation, and resolve blood stasis.

► [Chinese Versus Western Medicine](#)

## Blood–Brain Barrier

Shalom Avraham, Hava Karsenty Avraham and Tzong-Shi Lu

Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, Boston, MA, USA

### Synonyms

Blood–brain barrier; Brain capillaries; Brain microvascular endothelial cells

### Definition

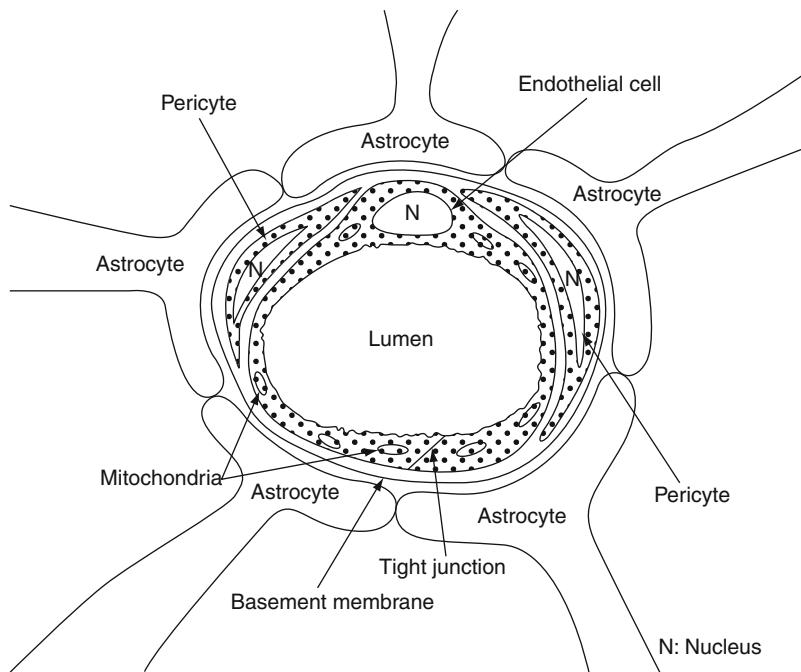
The blood–brain barrier (BBB) is formed by brain capillary endothelial cells. The BBB is composed of cerebral endothelial cells, astrocyte end-feet, and pericytes and regulates the homeostasis of the central nervous system (CNS).

### Characteristics

The BBB was identified by Paul Ehrlich in 1885 [2]. However, the biology of the BBB, its importance in health and disease, and its anatomical definition were mostly revealed over the last 30 years. The BBB is a well-differentiated network of brain microvessels that maintains the homeostasis of the brain microenvironment. The BBB regulates the interface between the peripheral circulation and the CNS. It restricts the nonspecific flux of ions, proteins, and other substances into the CNS environment, thereby protecting neurons from harmful components of the blood, and also allows the uptake of essential molecules from the blood to the CNS. The BBB is a selective diffusion barrier at the level of the cerebral microvascular endothelium. The anatomy of the brain microvascular endothelial

**Blood–Brain Barrier.**

**Fig. 1** Anatomical view of major components of the blood–brain barrier (BBB). The BBB is formed by endothelial cells, pericytes, basement membrane, and astrocytes. The BBB forms a highly restricted barrier that controls the exchange of materials between brain tissue and the circulatory system to maintain brain homeostasis. Tight junctions are more abundant than in other vessel systems and play a major role in regulating the permeability changes of the BBB. N: nucleus



cells (BMECs) of the BBB, which are a major component of the BBB, are distinguished from other types of endothelial cells in the periphery by increased mitochondrial content, a lack of fenestration, minimal pinocytotic activity, and the presence of ► **tight junctions** (TJs). The tight junctions create a barrier in the BBB that helps to maintain brain homeostasis and provide high transendothelial electrical resistance ( $100\text{--}2,000 \Omega/\text{cm}^2$ ), resulting in decreased paracellular permeability. BMECs are surrounded with pericytes, often divided into granular and filamentous subtypes, and astrocytic end-feet, which play an essential role in maintaining the structure of the BBB (Fig. 1). Astrocytes confer protection to the BBB against hypoxia and aglycemia.

The development of the BBB involves brain angiogenesis and BBB differentiation. First, brain endothelial cells derived from permeable vessels invade the vascular neuroectoderm and form intraneuronal vessels. Next, during the late embryonic and early postnatal periods, brain capillaries, in concert with astrocytes, differentiate, gradually mature, and are remodeled into the BBB, with impermeable properties.

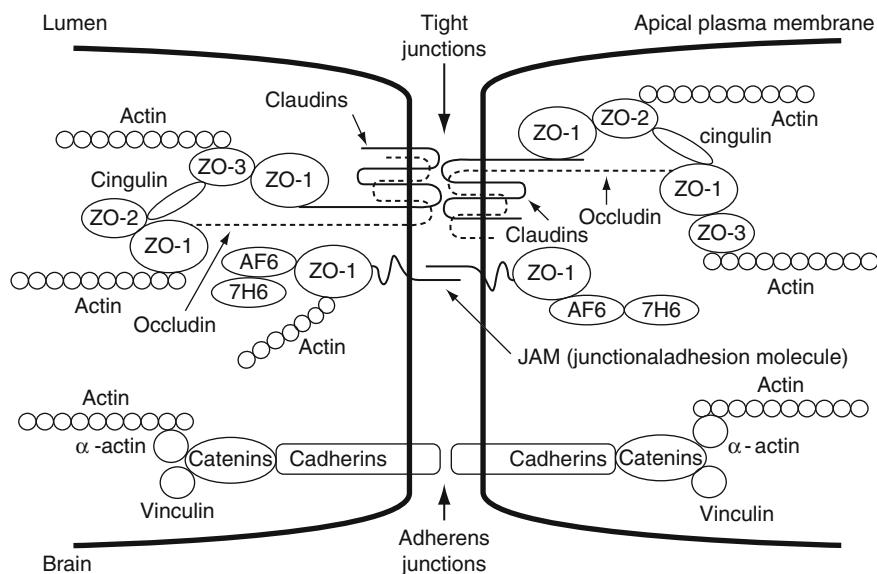
Failure to maintain BBB integrity can have profound effects on the CNS. The disruption of the BBB may result in many brain disorders, including brain

tumors. Changes in BBB function are associated with several neurological disorders, including stroke, multiple sclerosis, and Alzheimer's disease as well as inflammatory diseases such as chronic relapsing multiple sclerosis. Many of these changes have been linked to alterations in the tight junctions of the BBB.

### The BBB Junctional Complexes

The interendothelial space of the cerebral microvasculature is characterized by the presence of a junctional complex that includes adherens junctions (AJs), tight junctions (TJs), and ► **gap junctions** (Fig. 2) [3]. While the gap junctions mediate intercellular communications, both AJs and TJs act to restrict permeability across the endothelium.

The TJs are dynamic structures. The physiological and pathological conditions of the BBB affect TJ organization and function in the BBB. Disruption of the TJs by disease or drugs can lead to impaired BBB function and thus compromise the CNS microenvironment. Changes in TJ expression, subcellular localization, and/or posttranslational modification or changes in the protein–protein interactions of TJs can lead to alterations in BBB permeability and integrity.



**Blood–Brain Barrier. Fig. 2** Major tight junction and adherens junction proteins in the blood–brain barrier (BBB). Three transmembrane proteins, claudin, occludin, and junctional ► adhesion molecule (JAM), form integral tight junctions between adjacent endothelial cells. They provide the primary seal and regulate the paracellular permeability of the BBB. Other accessory proteins, such as ► zonula occludens

(ZO-1, ZO-2, and ZO-3), AF6, 7H6, and cingulin, are involved in structure support, regulation, location recognition, and signal transduction for the tight junctions. Adherens junctions consist of one transmembrane protein, cadherin, and three structure support proteins, catenin,  $\alpha$ -actinin, and vinculin, that link to the major cytoskeletal protein, actin

AJs are ubiquitous in the vasculature and mediate the following functions: (1) adhesion of endothelial cells to each other; (2) contact inhibition during vascular growth and remodeling; (3) initiation of cell polarity; and (4) partial regulation of paracellular permeability. The components of AJs include VE-cadherin, alpha-actinin, and vinculin which all link to the actin cytoskeleton, thus stabilizing the AJ complex.

The TJs form a continuous network of parallel, interconnected, intramembrane strands of protein arranged as a series of multiple barriers. It is the TJs that confer the low paracellular permeability and the high electrical resistance. The TJs are composed of transmembrane proteins that form a primary seal linked via accessory proteins to the actin cytoskeleton. The proteins of the tight junctions include: junctional adhesion molecule (JAM-1), occludin, and the claudins.

1. JAM-1 is a 40-kDa member of the IgG superfamily and is believed to mediate the early attachment of adjacent cell membranes via homophilic interactions. JAM-1 is composed of a single membrane-spanning chain with a large extracellular domain.

2. Occludin is a 60–65-kDa protein that has four transmembrane domains with the carboxyl and amino terminals oriented to the cytoplasm and two extracellular loops which span the intercellular cleft. It is highly expressed along the cell margins in the cerebral endothelium. Occludin increases electrical resistance in TJ-containing tissues, and has multiple sites for phosphorylation on serine and threonine residues. In addition, the cytoplasmic C-terminal domain is likely involved in the association of occludin with the cytoskeleton via accessory proteins, such as the ► zonula occludens ZO-1 and ZO-2.
3. Claudins are a family of 20–24-kDa membrane proteins that includes 24 members. The assumption is that claudins form the primary seal of the TJs and that occludin acts as an additional support structure. In the brain endothelium, claudin-5 is the most critical for BBB permeability.
4. In addition to the transmembrane components of the TJs, there are several accessory proteins that associate with them in the cytoplasm. These include members of the membrane-associated guanylate

kinase-like (MAGUK) homolog family. MAGUK proteins are involved in the coordination and clustering of protein complexes to the cell membrane and in the establishment of specialized domains within the membrane. Three MAGUK proteins have been identified at the TJs: ZO-1, ZO-2, and ZO-3. ZO-1, which is abundantly expressed in BMEC, is a 220-kDa protein that links transmembrane proteins of the TJs to the actin cytoskeleton. This interaction is critical to the stability and function of the TJs and is important for the integrity and permeability of the BBB.

5. Additional accessory proteins of the BBB include cingulin, AF6, and 7 H6.

## Permeability Properties of the BBB

The BBB significantly impedes entry from the blood into the brain of virtually all molecules, except those that are small and lipophilic. However, there are sets of small and large hydrophilic molecules that can enter the brain, and they do so by active transport. One of the important transporters is P-glycoprotein (Pgp), which is present in relatively high concentrations in brain capillaries and is also part of the barrier. Pgp is associated with multidrug resistance (MDR) in numerous tumors. The discovery of Pgp on the BBB has contributed to an understanding of the penetration of various drugs into the brain.

Net fluid influx and brain extracellular fluid homeostasis are regulated by hormones produced in the CNS that affect blood–brain transport. Transcytosis of insulin and transferrin has been well defined and these pathways have been utilized for targeted delivery to the brain and brain tumors. The presence of active efflux transporters in the BBB prevents many systemically administered drugs from entering the brain and is a major obstacle in designing drugs to treat neurological disorders.

## In Vitro BBB Models

Research on BBB functionality has been facilitated by the availability of in vitro BBB culture systems [4]. Culturing of the in vitro BBB involves the isolation of capillaries and culture of BMEC alone or in combination with astrocytes or astrocyte-conditioned medium.

## BBB in Disease

The BBB is sensitive to the pharmacodynamic effects of compounds and disease mediators that may result in changes in BBB integrity and function [5]. Alterations of the barrier tight junctions are a hallmark of many CNS pathologies, including tumor, stroke, HIV, encephalitis, and bacterial meningitis. BBB breakdown or TJ protein rearrangement seems to be involved in both the direct and indirect effects of stress responses and inflammatory mediators. Traumatic brain injury leads to an upregulation of ► **vascular endothelial growth factor** (VEGF) and the VEGF receptors, VEGFR-1 and VEGFR-2. Although a compromised BBB has been reported under some pathologic conditions, the precise role of a disrupted BBB in the pathogenesis of neurological diseases is not well defined.

In addition, the BBB presents a major obstacle to the treatment of malignant brain tumors and other CNS diseases. Delivery of therapeutics to the CNS is critical for the successful treatment of brain tumors and other neurological diseases. In this context, the current view is that the BBB, BMEC along with glia cells, pericytes, and neurons, should be viewed as a neurovascular unit for drug delivery.

Future studies should be aimed at understanding BBB dysfunction and the factors that regulate its recovery as well as at designing new approaches for the prevention and treatment of neurological diseases including brain tumors.

### ► Pharmacogenomics in Multidrug Resistance

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## Bloom Syndrome

Mounira Amor-Gueret

Institut Curie; UMR 2027 CNRS, Orsay Cedex, France

### Synonyms

Bloom–Torre–Mackacek syndrome; Congenital telangiectatic erythema

### Definition

Bloom syndrome (BS) is a rare human autosomal recessive disorder that belongs to the group of “chromosomal breakage syndromes,” and is characterized by marked ► chromosomal instability associated with a greatly increased predisposition to a wide range of ► cancers commonly affecting the general population. BS was first described by David Bloom in 1954 as “congenital telangiectatic erythema resembling lupus erythematosus in dwarfs.” The predominant and constant clinical feature of BS is proportionate pre- and postnatal growth retardation. Additional clinical features are described below. The hallmark of BS cells is an approximately tenfold increase in the rate of ► sister chromatid exchanges (SCEs) compared to normal cells. This increased level of SCE is the only objective criteria for BS diagnosis (Fig. 1). SCEs frequency averages 0.24 per chromosome in normal cells and 2.12 per chromosome in BS cells.

### Characteristics

#### Clinical Description

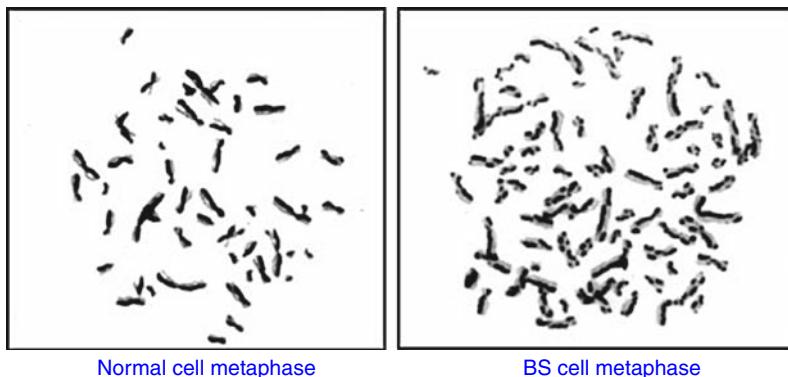
A surveillance program, the Bloom Syndrome Registry, was established in 1960 by James German and Eberhard Passarge, in which the follow-up of 168 BS patients (93 males, 75 females) was reported until 1991. From the data in this Registry, it appears that the two constant clinical features associated with BS are growth retardation starting in utero and persisting throughout life with normal proportioning and accompanied by dolichocephaly, and predisposition to all types of cancers. The mean adult height for men is 147.5 cm (range 130–162), and for women is

138.6 cm (range 122–151). Eleven additional clinical features that are not constant and that vary in severity among BS patients were also reported by James German: (1) a “bird-like” facies with a narrow face and prominent nose, and malar and mandibular hypoplasia, (2) sun-sensitive erythema affecting the butterfly area of the face (similar to that caused by lupus erythematosus), and sometimes affecting the dorsa of the hands and forearms, (3) spots of hyper- and hypopigmentation of the skin (“café au lait” spots), (4) a high-pitched voice (Mickey Mouse voice), (5) a variable degree of “vomiting and diarrhea” during infancy, (6) diabetes mellitus (diagnosed at a mean age of 24.9 years in 20 of the 168 BS patients in the Registry), (7) small testes accompanied by a total failure of spermatogenesis in men and early cessation of menstruation accompanied by reduced fertility in women, (8) immunodeficiency manifested by recurrent respiratory tract infections complicated by otitis media and pneumonia (life-threatening ear and lung infections are common), and manifested by the gastrointestinal problems mentioned in (5), (9) some minor anatomic abnormalities such as obstructing anomalies of the urethra, which were of major clinical importance in several cases, (10) average intelligence (sometimes mental deficiency), (11) clinical features that occurred in only one or a few BS patients and that are not to be considered part of BS itself, such as congenital thrombocytopenia, mild anemia, asthma, or psoriatic arthritis.

The 100 cancers that arose in 71 of the 168 BS patients recorded in the Bloom Syndrome Registry have been reported, and the distribution of the sites and types of these cancers is similar to that found in the general population. The main conclusions of this report are that nearly half of the registered BS (71/168) patients have had at least one cancer by the mean age of 24.7, and of those patients, 40% have had more than one primary cancer (29/71). ► Acute myeloid leukemias (21% of cancers), lymphomas (23%), and rare tumors (5% including ► medulloblastoma, ► Wilms tumor, osteogenic sarcoma) predominate in the first two decades of life, whereas carcinomas (51%) start to appear late in the second decade of life.

#### BLM-Deficient Cells

BS cells display an increase in chromosome breaks, a spontaneous ► mutation rate ten times higher than that in normal cells, an increased frequency of spontaneous symmetric quadriradial interchanges and



**Bloom Syndrome. Fig. 1** Increased sister chromatid exchange in Bloom syndrome cells. The sister chromatids in the images are differentially labeled so that regions of chromatid exchange can be seen as regions of light and dark staining. Little chromatid

sister chromatid exchanges, and increased ► [Loss of heterozygosity](#) (► LOH). BS cells also display replication abnormalities, including retarded replication-fork elongation and abnormal replication intermediates, and a general delay in the timing of replication associated with an increased level of constitutive ► [DNA damage](#) in mid- to late-S-phase. BS cells bud out large number of micronuclei during S phase and have constitutively high levels of RAD51-containing nuclear foci. Chronic overproduction of the superoxide-free radical O<sub>2</sub><sup>-</sup> (► [Reactive oxygen species](#)) has also been reported in BS cells.

### BLM Gene

BS arises through mutations in both copies of the BLM gene, which is located on chromosome 15 at 15q26.1. Nonsense or frameshift mutations leading to a premature termination codon, and missense mutations have been found in BLM gene from BS patients. One particular BLM gene mutation corresponding to a 6-bp deletion and a 7-bp insertion at nucleotide position 2207, referred to as the blm Ash mutation, is homozygous in nearly all BS patients with Ashkenazi Jewish ancestry and is due to a founder effect. Screening for BLM gene mutations can be done by analyzing the 21 coding exons (4,437 bp total length).

### Frequency

BS affects all human populations, and its reported frequency is 1 in 10,836,000 in Japan, 1 in 3,331,000 in the United States, 1 in 5,590,000 in West Germany, and 1 in 2,395,000 in the Netherlands. In the Ashkenazi Jewish population, the frequency of BS is

exchange is seen in normal cell metaphase (*left panel*), whereas most of the chromosomes in a Bloom syndrome cell metaphase (*right panel*) show chromatid exchange

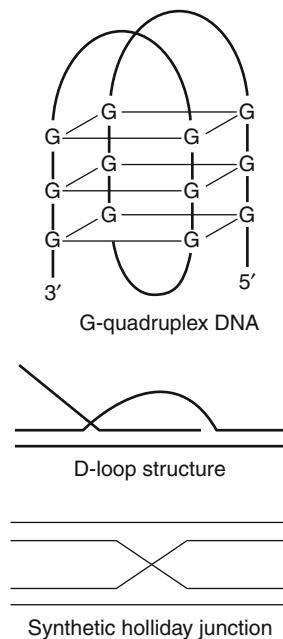
~ 1 in 48,000. This is due to a founder effect, and ~ 1% of the Ashkenazi Jewish population are heterozygous carriers for the blm<sup>Ash</sup> mutation.

### BLM Protein

The BLM gene codes for the BLM protein, which is 1,417 amino acids in length with a predicted molecular mass of 159 kDa, and it belongs to the DExH box-containing RecQ helicase subfamily. BLM displays an ATP- and Mg<sup>2+</sup> dependent 3'-5'-DNA helicase activity that separates the complementary strands of DNA in a 3'-5' direction. However, the exact function of BLM is still unclear. BLM protein accumulates in S and ► [G2/M](#) phases of the cell cycle, and localizes in two distinct nuclear structures, PML nuclear bodies (also called ND10) and the nucleolus. The preferred substrates for BLM are ► [G1-S transition](#), D-loop structures, and X-junctions (Fig. 2). BLM promotes branch migration of RecA-generated Holliday junctions and effects, with topoisomerase III $\alpha$ , the resolution of a recombination intermediate containing a double Holliday junction with no flanking sequence exchanges (► [Rho GTPases](#)). BLM also catalyzes the annealing of complementary single-stranded DNA molecules (DNA strand annealing activity). BLM interacts with several proteins involved in the maintenance of genome integrity. It participates in a super complex of ► [BRCA1](#)-associated proteins called BASC (BRCA1-associated genome surveillance complex) which includes BRCA1 (mutated in some familial breast cancers) ► [ATM](#) defective in Ataxia Telangiectasia, AT, NBS1(defective in ► [Nijmegen Breakage syndrome](#)), and MRE11 (defective in ataxia-telangiectasia-like disorder), MLH1,

### Bloom Syndrome.

**Fig. 2** Preferred substrates of the BLM helicase. The recombinant BLM protein efficiently unwinds DNA structures such as G-quadruplex, D-loop, and synthetic Holliday junctions



MSH2, and MSH6 (involved in Human Non-Polyposis Colorectal Cancer, HNPCC syndrome or ► [Lynch syndrome](#)), and several other proteins known to be involved in replicational and/or post-replicational repair processes. BLM also participates in a complex called BRAFT (BLM, RPA, FA, Topoisomerase III $\alpha$ ), which contains five of the ► [Fanconi anemia](#) (FA) complementation group proteins (FANCA, FANCG, FANCC, FANCE, and FANCF), RPA and topoisomerase III $\alpha$  (which are also known to interact independently with BLM), and a newly identified factor called BLAP75. Among the other proteins known to co-localize and/or to interact physically and/or functionally with BLM in undamaged cells and/or in cells submitted to genotoxic stresses, are the tumor suppressor protein ► [p53](#), WRN protein (a RecQ helicase defective in the Werner syndrome), RAD51 (a key protein in ► [homologous recombination](#)), RAD51L3 (a RAD51 paralog), ATR (ataxia telangiectasia and rad<sup>3+</sup> related kinase), TRF2 (a double-stranded telomeric DNA binding protein), Mus81 (a DNA-structure specific endonuclease),  $\gamma$ -H2AX (► [Ganglioside](#); histone H2AX phosphorylated on Ser 139 in response to DNA double-strand breaks), hp150 (the largest subunit of chromatin assembly factor 1, CAF1), FEN1, (flap endonuclease 1, involved in the removal of RNA primers of Okazaki fragments), FANCD2 (Fanconi anemia complementation group D2 protein), and the Chk1 kinase

(a serine/threonine protein kinase that is a key mediator in DNA damage-induced cell cycle checkpoints).

Altogether, these data support a major role for BLM in maintaining genomic stability during DNA replication, homologous recombination and repair. Several models for the role of BLM have been proposed and suggest that BLM acts as a “roadblock” remover during DNA replication by disrupting complex structures such as G-quadruplexes or DNA hairpins. BLM may also restart replication after the fork stalls and/or resolve recombination intermediates during DNA double-strand break repair through its reverse branch migration and DNA strand annealing activities.

### Mouse Models

Among the five BS knockout alleles that have been generated, four led to embryonic lethality when the targeted allele was homozygous, and only one resulted in viable “BS” mice through a complex rearrangement of the targeted region. By 20 months of age, 29% of these Blm-deficient mice had developed a wide spectrum of cancer, similar to human BS patients.

### Genetic Counseling

Due to the autosomal recessive transmission of BS, sibs of two heterozygous carriers are at 25% risk of having BS and at 50% risk of being carriers. When the risk of BS transmission has been well evaluated, prenatal diagnosis can be proposed (SCE analysis of fetal cells or detection of a specific BLM gene mutation when causative mutations are identified).

### Therapy

There is no curative treatment for BS. However, a physician should carefully follow BS patients to ensure early cancer diagnosis.

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## Bloom-Torre-Mackacek Syndrome

- Bloom Syndrome

## Blue Dye

### Definition

A blue substance that visualizes lymph vessels and (sentinel) lymph nodes, which helps identifying tumor sites.

- Sentinel Lymph Nodes

## BM-40

- Secreted Protein Acidic and Rich in Cysteine

## BMP

### Definition

Bone morphogenetic protein; Group of growth factors able to induce the formation of bone and cartilage.

## BMS-247550

- Epothilone B Analogue

## Body-Mass Index

### Definition

The body mass index (BMI) is a ratio between weight and height, a mathematical formula that correlates with body fat. BMI is determined by calculating your weight in kilograms divided by your height in meters squared ( $\text{BMI} = \text{kg}/\text{m}^2$ ).

- Obesity and Cancer Risk

## Bombesin

### Synonyms

BBS

### Definition

Neuropeptide hormone from the fire-bellied toad *Bombina bombina* analogous to the mammalian gastrin-releasing peptide (GRP).

- Gut Peptides

## Bone Loss Cancer Mediated

Andrew C. W. Zannettino

Bone and Cancer Laboratories, IMVS, Adelaide, SA, Australia

### Synonyms

Cancer-mediated bone loss; Osteolysis; Osteolytic bone disease; Osteoblastic bone disease; Osteolytic lesions of bone; Osteoblastic lesions of bone; Skeletal complications (skeletal-related events)

### Definition

Metastasis describes the spread of cancer from its site of origin (the “primary site”) to another location in the body (the “secondary site”). Bone is the most frequent site affected by metastatic cancer. While any type of cancer can spread to bone, metastatic bone disease is most commonly associated with cancers whose primary origin is the breast, prostate, or lung. Although less common, bone metastases are also associated with cancers arising in the thyroid, kidney, stomach, uterus, colon, bladder, and rectum (Table 1). Metastasis of cancer to bone often results in significant skeletal morbidity which manifests as severe bone pain, pathologic fractures, spinal cord compression, and life-threatening ► hypercalcemia.

**Bone Loss Cancer Mediated. Table 1** Incidence of primary tumors which metastasis to bone as a percentage of all bone metastases

Primary tumor	As a percentage of all bone metastases
Breast	35
Prostate	30
Lung	10
Kidney	5
Thyroid	2
Others	18

## Characteristics

### Types of Bone Metastasis

Bone metastases are classified as osteolytic, osteoblastic, or mixed, based on their radiographic appearance. Patients can present with either osteolytic or ► **osteoblastic metastasis**, or mixed lesions containing both elements. Bone metastases from prostate cancer are predominantly osteoblastic, whereas bone lesions from breast cancer can be osteoblastic, osteolytic, or mixed. Only in multiple myeloma do purely lytic bone lesions develop. Regardless of the mechanisms involved in the formation of osteolytic or osteoblastic metastases, the end result is a change to the bone architecture which predisposes the patient to a variety of skeletal comorbidities.

### Bone Physiology: Control of Normal Bone Remodeling

Healthy bone is a dynamic organ which is constantly rejuvenated by the coordinated activity of two cell types, namely, the bone-resorbing ► **osteoclasts** and the bone-forming osteoblasts. Osteoblasts are responsible for the synthesis of collagen and non-collagenous bone proteins which are involved in the mineralization of the bone matrix. Normal bone remodeling (or turnover) is initiated by osteoclasts which degrade the bone matrix, creating cavities or lacunae. Once resorption is complete, osteoblasts synthesize a new osteoid matrix which is then mineralized. Under normal physiological conditions, the amount of newly formed calcified matrix is equal to the amount of bone resorbed by the osteoclast, thereby maintaining bone mass and skeletal integrity.

The bone-resorbing osteoclasts are derived from hematopoietic stem cells. Osteoclast formation and

activity is regulated by systemic hormones and growth factors which are synthesized in the bone microenvironment, including macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor  $\kappa$ B (RANK) ligand (RANKL). RANKL, a member of the tumor necrosis factor family, is expressed on the surface of stromal cells and osteoblasts. RANKL binds the RANK receptor on the osteoclast precursor cell surface and induces the formation of osteoclasts by signaling through the nuclear factor  $\kappa$ B and Jun N-terminal kinase pathways. A number of osteotropic factors, such as parathyroid hormone, 1,25-dihydroxyvitamin D3, and prostaglandins, induce the formation of osteoclasts by increasing the expression of RANKL on marrow stromal cells and osteoblasts rather than by acting directly on osteoclast precursors. The activities of RANKL are further regulated by the decoy receptor, ► **osteoprotegerin** (OPG) which is synthesized by numerous cells including marrow stromal cells and osteoblasts. The binding of OPG to RANKL precludes RANKL from binding to RANK, thereby inhibiting the differentiation and activity of osteoclasts. The ratio of RANKL to OPG will ultimately determine whether osteoclast formation will occur. Osteoclasts resorb bone by forming a tight seal between the ruffled border of the plasma membrane and the bone surface and secreting proteases and acid which dissolves the bone matrix.

Bone-forming osteoblasts are derived from mesenchymal stem cells. The transcription factor, Runx-2, (also termed core-binding factor  $\alpha$  1; CBFA1), is critical for the differentiation of osteoblasts by activating the expression of numerous genes which “drive” osteoblast differentiation. Many factors can enhance the growth and differentiation of osteoblasts, including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF)- $\beta$ , and the bone morphogenetic proteins (BMPs).

### Bone is a Unique Environment for Metastasis

In 1889, Stephan Paget proffered the “seed-and-soil hypothesis” to explain why primary cancer cells selectively colonize distant organs. Paget suggested that cancer metastasis is reliant upon multiple interactions (“cross talk”) between cancer cells (the “seeds”) and certain organ environments (“the soil”) which provide a favorable environment for tumor localization and growth. To this day, this hypothesis still holds true.

Metastasis to bone is a complex multistep event which involves a bidirectional interaction of the tumor cells with cellular elements in three different microenvironments: (1) the site of primary tumor, (2) the circulation, and (3) the bone microenvironment. The metastatic tumor cells must first break away from the primary tumor and move into the circulation and reach various skeletal sites, where they colonize, proliferate, and induce metastatic lesions. The preferential skeletal localization of tumor cells is attributed to the biologic and molecular characteristics of tumor cells as well as that of the bone microenvironment. For example, while unable to confer a bone metastatic potential individually, overexpression of an array of proteins, including the chemokine receptor, (CXCR)-4, interleukin (IL)-11, connective tissue growth factor (CTGF), and matrix metalloproteinase, (MMP)-1, along with the osteopontin, enhance the metastatic potential of breast cancer cells to bone. These proteins participate in one or more of the steps involved in the homing, invasion, angiogenesis, and proliferation of tumor cells in the bone microenvironment.

The multifocal nature and predilection of tumor cells for the hematopoietic marrow sites in the proximal long bones and axial skeleton (vertebrae, pelvis, ribs, and cranium) can be attributed to the continuous and dynamic turnover of the bone matrix and bone marrow which provides a fertile ground for tumor cells to utilize resources (cells, growth factors, cytokines, and receptors) for their homing and growth. The anatomical and molecular characteristics of bone also make it a favorable site for metastasis, with the slow-moving but copious metaphyseal blood flow assisting intimate interactions between endothelium and tumor cells, a process necessary for the initial colonization of tumor cells within the bone marrow. In addition, various growth factors and cytokines in the bone marrow such as endothelin (ET)-1, basic fibroblast growth factor (bFGF), TGF- $\beta$ , IL-6, and IL-8 serve as paracrine regulators of the initial growth of metastatic tumor cells. The interaction of chemotactic extracellular matrix and stromal cell expressed proteins (stromal cell-derived factor-1, vascular cell adhesion molecule-1, fibronectin, type I collagen, type IV collagen, vitronectin, osteopontin, osteocalcin, bone sialoprotein, and osteonectin) with ligands that are over-expressed on tumor cells (integrins  $\alpha$ 4 $\beta$ 1 and  $\alpha$ 5 $\beta$ 1, CTGF and CXCR4) promote tumor colonization within the bone marrow. Moreover, the bone matrix

is a large repository of latent growth factors such as insulin-like growth factor (IGF), TGF- $\beta$ , BMPs, PDGF, and vascular endothelial growth factor (VEGF), which are released during the formation of both osteolytic and osteoblastic lesions and serve to stimulate the “vicious cycle” of tumor growth and progression of bone lesions.

### **The Vicious Cycle of Osteolytic Metastasis**

The most common manifestation of bone metastasis is osteolysis (osteolytic metastasis). Osteolytic bone metastases are common in solid tumor metastases of lung, renal, breast cancer and the hematological malignancy, multiple myeloma. In osteolytic metastases, skeletal destruction is mediated primarily by osteoclasts rather than the tumor cells. However, the factors responsible for the activation of osteoclasts vary depending on the tumor type. The pathogenesis and progression of osteolytic metastases are often the result of a complex “vicious cycle” which involves the interactions between tumor cells, bone cells (osteoclasts and osteoblasts), and the bone matrix. The tumor cells secrete various soluble factors including IL-6, IL-8, IL-11, TNF- $\alpha$ , M-CSF, prostaglandin E2 (PGE2), and parathyroid-related protein (PTHRP) that directly or indirectly promote osteoclast differentiation, proliferation, and activation leading to increased osteolysis. Furthermore, the process of bone resorption itself results in the release of growth factors, including TGF- $\beta$ , IGF, bFGF, and BMP, from the bone matrix which support the growth and survival of the tumor cells. In turn, the growing tumor secretes more pro-osteolytic factors, which results in further osteolysis and perpetuation of the vicious cycle.

### **The Vicious Cycle of Osteoblastic Metastases**

Osteoblastic bone metastases are characterized by an increase in woven bone formation which radiographically appear as sclerotic lesions, and are most commonly seen in patients with prostate cancer. Tumor cells forming osteoblastic metastases secrete numerous pro-osteoblastic factors that drive bone remodeling toward a predominant bone-forming state. Furthermore, activated osteoblasts secrete numerous growth factors during woven bone formation, including TGF- $\beta$ , BMP, and VEGF, which further stimulate tumor survival and growth and perpetuate the vicious cycle. In addition, the Wnt (wingless int) pathway, the ET axis, and the BMP pathway have emerged as key

regulators in the establishment of osteoblastic skeletal metastasis. Wnt proteins are soluble glycoproteins that promote embryonic and postnatal bone formation by binding to a membrane receptor complex comprised of frizzled (FZD) G-protein-coupled receptor and a low-density lipoprotein receptor-related protein. The formation of this ligand–receptor complex initiates a number of intracellular signaling cascades that modulate differentiation, survival, and activity of the osteoblasts. ET-1 promotes osteogenic differentiation, stimulates bone matrix formation, and inhibits osteoclast formation and motility. BMPs (BMP-2, BMP-6, and BMP-7) play a central role in skeletal development and postnatal bone repair, and are implicated in metastatic bone formation due to their osteoinductive properties.

### Osteolytic Multiple Myeloma

Multiple myeloma is a hematological malignancy characterized by the “homing” of a clonal population of neoplastic plasma cells from secondary lymphoid organs to sites within the bone marrow, close to the bone surface. Here, multiple myeloma plasma cells recruit and activate the bone-resorbing activities of osteoclasts. Lytic lesions are observed in more than 80% of patients afflicted with multiple myeloma. Several osteoclastogenic factors have been implicated in the increased activity of osteoclasts in myeloma, including macrophage inflammatory protein (MIP)-1 $\alpha$ , SDF-1, and RANKL. RANKL is produced by both the myeloma cells and marrow stromal cells in response to factors secreted by the myeloma cells themselves. In the bone microenvironment of myeloma patients, RANKL production is increased and  $\blacktriangleright$  [osteoprotegerin](#) is markedly decreased. MIP-1 $\alpha$  is a potent inducer of osteoclast formation and enhances both RANKL- and IL-6-stimulated osteoclast formations. MIP-1 $\alpha$  also stimulates integrin-mediated adhesion of myeloma cells to stromal cells resulting in an increased production of IL-6, RANKL, and MIP-1 $\alpha$ , which in turn further stimulates bone destruction. MIP-1 $\alpha$  levels in myeloma patient serum correlate strongly with the presence of osteolytic lesions. Similarly, levels of the CXC chemokine, SDF-1, are elevated in myeloma patients which exhibit radiographically detectable bone lesions. While incapable of stimulating osteoclast formation in the absence of RANKL, myeloma cell-derived SDF-1 serves to hyper-activate the resorptive activity of osteoclasts leading to bone loss.

Bone lesions in myeloma are purely lytic and are not accompanied by an osteoblastic response. While the basis for the lack of an osteoblastic response in myeloma remains to be determined, recent studies suggest roles for the soluble Wnt-signaling antagonists, dickkopf 1 (DKK1) and soluble frizzled-related protein, sFRP-2 ([Fig. 1](#)). High serum levels of soluble DKK1 and sFRP2 have been correlated with myeloma-induced focal bone lesions.

Myeloma tumor cells secrete numerous factors (RANKL, MIP-1a, SDF-1, and PTHrP) which directly or indirectly (via the osteoblast) stimulate osteoclast formation and activity. Myeloma tumor cells also express factors which suppress normal osteoblast function, including the Wnt signaling pathway antagonists DKK-1 and sFRP-1.

### Osteolytic Metastasis from Breast Cancer

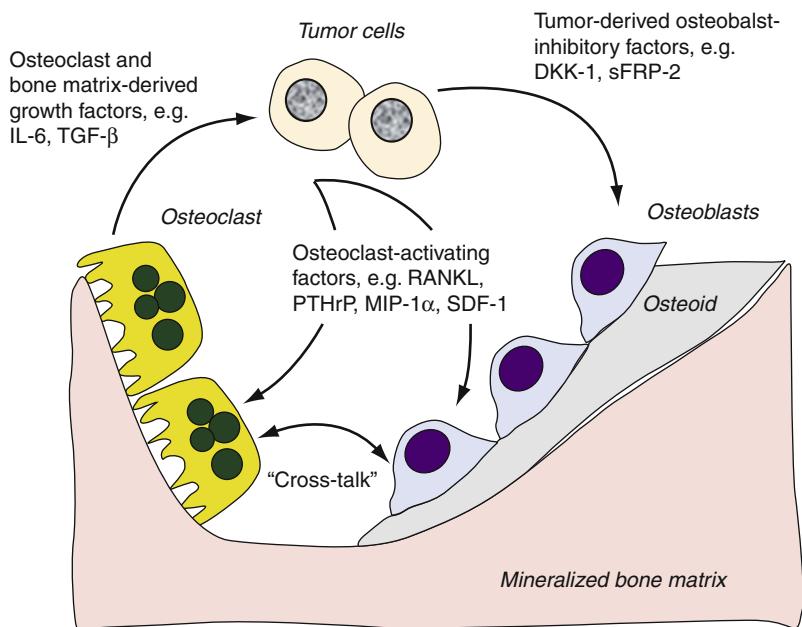
Breast cancer commonly metastasizes to and destroys bone, causing pain and fracture, and the median survival of patients with bone metastases is only 19–25 months. Almost one third of patients present with radiographically detectable mixed osteoblastic and osteolytic lesions. Tumor cells in breast cancer produce factors that directly or indirectly induce the formation of osteoclasts, including parathyroid hormone-related protein (PTHrP), interleukin (IL)-6, IL-8, IL-11, M-CSF, PGE-2, TNF- $\alpha$ , and RANKL. The resultant osteoclastic bone resorption releases growth factors from the bone matrix which further stimulate tumor growth and bone destruction. In particular, PTHrP functions by inducing the expression of RANKL on marrow stromal cells which further stimulates osteoclast formation and activity. The resultant osteoclastic resorption releases growth factors such as TGF- $\beta$  from the bone matrix, with TGF- $\beta$  in turn stimulating PTHrP production by tumor cells, and perpetuating the vicious cycle of breast cancer metastases.

### Osteoblastic Metastasis in Prostate Cancer

Prostate cancer is the most prevalent non-dermatologic cancer in males, and the clinical course of patients with metastatic prostate cancer can be relatively long with a median survival measured in years not months. At presentation, 10% of patients have bone metastases, and almost all patients who die of prostate cancer have skeletal involvement. Based on radiographic appearance, prostate metastases are classified as osteoblastic; however, studies have shown that both bone resorption and bone formation are dysregulated in this disease.

**Bone Loss Cancer**

**Mediated. Fig. 1** The formation of lytic bone disease in myeloma



Although the mechanisms and factors involved in osteoblastic metastasis are unknown, a number of factors have been implicated, including ET-1, PDGF, urokinase-type plasminogen activator (u-PA), and prostate-specific antigen (PSA). ET-1 stimulates the formation of bone and the proliferation of osteoblasts in bone organ cultures, and serum endothelin-1 levels are increased in patients with osteoblastic prostate metastases. Overproduction of u-PA by prostate cancer cells imparts an enhanced capacity to initiate osteoblastic metastases. Prostate cancer cells also release PSA, a kallikrein serine protease, which can cleave and inactivate parathyroid hormone-related peptide which can block tumor-induced bone resorption. It may also activate osteoblastic growth factors released in the bone microenvironment during the development of bone metastases, such as IGF-I and -II or TGF- $\beta$ . These data suggest that a vicious cycle may also be responsible for osteoblastic metastasis. While markers of bone resorption are also increased in prostate cancer patients with metastasis, there is usually no histologic evidence of increased numbers of osteoclasts. Studies show that blocking osteoclastic bone resorption in patients with prostate cancer decreases the number of skeletal-related events suggesting that bone resorption may precede bone formation in the development of osteoblastic metastases.

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**Bone Marrow****Definition**

The bone marrow is the site of hematopoiesis, the generation of the cellular elements of blood, including red blood cells, monocytes, polymorphonuclear leukocytes, and platelets. It is also the site of B-cell development in mammals and the source of stem cells that give rise to T cells upon migration to the thymus. Thus, bone marrow transplantation can restore all the cellular elements of the blood, including the cells required for adaptive immunity.

## Bone Marrow-Derived Mesenchymal Stem Cells

### Synonyms

Marrow stromal cells; MSC

### Definition

These bone marrow fibroblasts act as a supportive framework within bone marrow and support hematopoiesis (i.e., blood cell development). Mesenchymal Stem Cells are also an adult stem cell population. They have the ability to self-renew, and they can give rise to osteoblasts (bone), adipocytes (fat), and cartilage.

► Desmoplasia

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## Bone Morphogenetic Protein

► BMP

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## Bone Neoplasms

► Bone Tumors

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## Bone Resorption

### Definition

Is a complex biological mechanism leading to the degradation of bone organic and mineral extracellular matrix and is closely related to osteoclast activity. Bone resorption corresponds to an extracellular degradation mechanism associated with the release of protons and proteases by osteoclasts.

► Bisphosphonates  
► Zoledronic Acid

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## Bone Sarcomas

► Bone Tumors

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## Bone Sialoprotein

► Osteopontin

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## Bone Tropism

Alessandro Fatatis

Department of Pharmacology and Physiology,  
Drexel University College of Medicine, Philadelphia,  
PA, USA

### Synonyms

Bone-seeking malignant phenotypes; Skeletal secondary tumors

### Definition

Bone tropism is the propensity of certain tumors to spread from the organ(s) in which they initially originated and target preferentially the skeleton, in which they may eventually grow into secondary tumors or

► metastasis.

### Characteristics

Solid tumors at their initial stage present unique challenges for the selection of the appropriate treatment. Several considerations come into play, including the feasibility of their surgical ablation, the age of the patient, and the histological grade of the neoplasia, among others. However, the risk of metastatic spread is indeed one of the most critical factors in deciding the therapeutic strategy to adopt. It is also widely recognized that the detection of secondary tumors at the time of the initial diagnosis poses additional and most often prohibitive hurdles to a positive clinical

outcome. This is because after secondary tumors become clinically evident there is no turning back and, despite slight individual differences in disease progression, both the prognosis and the quality of life dramatically worsen.

Although primitive tumors may be rapidly lethal or are defeated before a significant metastatic disease delineates itself, a defined group of solid tumors, including prostate and breast adenocarcinomas, consistently spread to the skeleton. Major complications caused by the growth of cancer cells in the skeleton include pathological fractures, spinal cord compression, and an overall organ impairment affecting both mielogenic and immunological properties of the bone marrow. For several forms of neoplasia, skeletal metastases are the sole site of spread in  $\geq 80\%$  of patients; their distribution overlaps that of bone marrow in the adult and they represent the major cause of death. In fact, patients may succumb to the metastatic disease years after their primitive tumors were surgically removed.

Bone secondary tumors are extremely difficult to treat; since surgical procedures are commonly limited to mere ► **palliative therapy**, the treatments of choice are either ► **chemoradiotherapy** or immunotherapy. However, cancer cells located in the bone marrow benefit from the “sanctuary” characteristics of this tissue. Small foci of cancer cells can grow almost undisturbed because of the restricted access to the site combined to the high availability of nutrients and growth factors, which are constantly produced locally or delivered by the blood circulation.

There are several postulated mechanisms that could explain the bone tropism of cancer cells:

### Blood Circulation Patterns

The anatomical description of the Paravertebral Venous Plexus, made by Batson in 1940, led to postulate that retrograde blood flow would deliver cells from tumors such as prostate and colon carcinomas preferentially to the spine, thereby determining the occurrence of secondary tumors at the vertebral level. Based on this paradigm, migrating cancer cells would not specifically arrest to the vertebrae but rather be passively forced by blood-flow patterns to settle in the first capillary bed they encounter. Their lodging into the bone would then occur by size restriction arrest, with capillaries progressively smaller in diameter trapping the traveling cancer cells.

The role of blood flow and circulatory patterns in hematogenous skeletal metastases is obviously fundamental; however, a large body of evidence discounts mechanical entrapment as the exclusive factor and points toward additional mechanisms underlining the bone tropism of tumors. For example, certain tumors, such as prostate cancer, affect the skeleton at both the pelvic and upper torso regions, an unlikely scenario for mechanical trapping by simple blood-flow distribution; in addition, several studies have shown that cancer cells can bypass more than one capillary bed and lodge in selected distant organs.

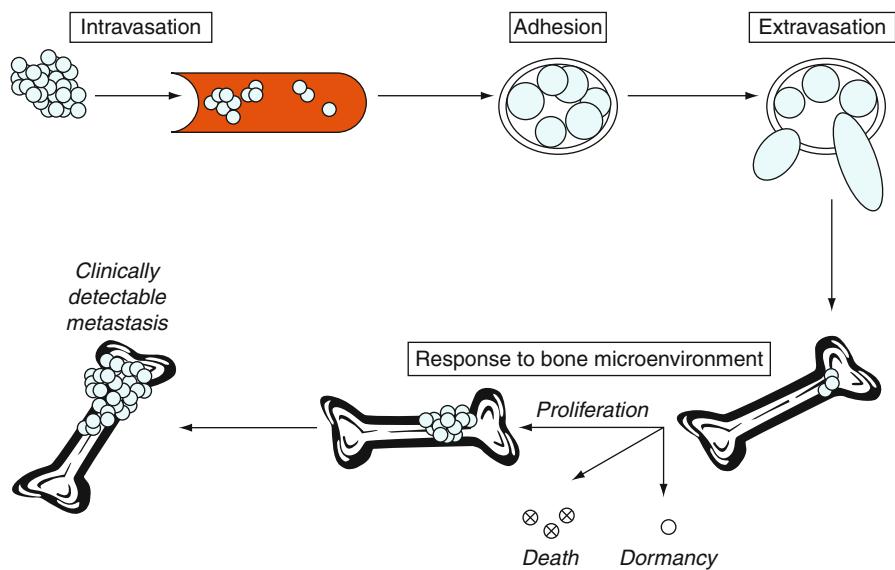
### Cellular Adhesive Interactions

When cancer cells reach the marrow of different bones through blood circulation, they encounter the vascular wall of the bone sinusoids and interact with the endothelial cells which are lining it. The times required for adhesion of cancer cells to the endothelium by simple vicinity of cellular surfaces are in fact considerably in excess of their luminal transit times, suggesting the formation of adhesive bridges. Therefore, unique characteristics of bone marrow endothelial cells could promote the specific adhesion of cancer cells (Fig. 1).

This scenario is compatible with the trafficking of immunocompetent cells and their adhesion to different types of endothelia, including that of bone marrow sinusoids. For instance, the recruitment of leukocytes into sites of inflammation requires the sequential execution of events such as *capture* and subsequent firm *adhesion* to endothelial cells. The capture of leukocytes from the lumen of blood vessels involves the intervention of specific ► **cell adhesion molecules** (CAMs) exposed on the surface of leukocytes and endothelial cells. These molecules – including ► **integrins** and ► **selectins** among others – need to establish firm interactions with their respective substrates, such as ► **fibronectin**, to resist the shear force exerted by the blood flow while arresting the cells traveling through the lumen of the vessel. Similar mechanisms are in place to ensure the trafficking of hematopoietic progenitor cells from the bone marrow to the peripheral blood and vice versa.

It has been proposed that adhesive mechanisms involving CAMs could be usurped by cancer cells, allowing them to colonize the skeleton, among other organs. The activation of selected CAMs can be induced by molecules involved in the inflammatory

**Bone Tropism. Fig. 1** Bone tropism. Schematic representation of the sequential steps of skeletal metastasis that can be determinant in conferring bone tropism to cancer cells (Artwork by Whitney Jamieson)



response, named chemotactic cytokines or ► **chemokines**, through their interaction with specific receptors located on the surface of leukocytes and other cell types. During the last few years, a large number of studies have shown that different types of cancer cells express receptors for chemokines that are produced in organs which are common sites of metastasis. For example, breast cancer cells express the CXCR4 receptor for the chemokine CXCL12/SDF-1, which is detected in the bone marrow. Similarly, prostate cancer cells express the ► **CX3CR1** receptor for the chemokine ► **CX3CL1/fractalkine**, which is constitutively anchored to the surface of the bone marrow endothelium, whereas other endothelia need inflammatory conditions to express it. Comparable chemokine/receptor interactions have been reported for different types of cancer targeting the skeleton, suggesting that chemokine-induced adhesive interactions might promote the preferential, albeit not exclusive, arrest of cancer cells into a specific tissue and indeed represent a crucial determinant of bone tropism.

### Chemoattraction of Cancer Cells

Cancer cells can proliferate and grow within the blood vessels at their primary site of attachment to the endothelium, as shown by studies conducted by Dr. Muschel and coworkers, among others. However, particularly for skeletal metastases, simply adhering to the bone marrow endothelial wall may not suffice to invade the bone. Similarly to leukocytes, cancer cells

need to migrate from the luminal side of the endothelial cells into the surrounding bone marrow stroma. This process, named ► **extravasation**, is comparable to the diapedesis observed for leukocytes and can be similarly regulated by chemokines. Thus, the mutual interactions between chemokines present in the bone tissue and their receptors expressed by bone-seeking cancer cells could exert an attractive force and induce migratory events involved in the skeletal secondary location of specific tumors. In support of this hypothesis, numerous studies have found CXCL12/SDF-1, CX3CL1/fractalkine, and other chemokines being produced at high levels by the cells of the bone marrow.

However, bone marrow-produced chemokines are also abundant in other organs – for example, the optic nerve and cardiac muscle for CXCL12/SDF-1 – which are seldom sites of metastasis from breast cancer cells, which express the compatible receptor CXCR4. Therefore, bone tropism likely depends on multiple factors; the idea of favorable conditions for cancer cell growth offered only by selected organs is epitomized by the ► **seed and soil hypothesis**.

### Tissue Conditions Supporting the Growth of Cancer Cells

Once cancer cells have migrated into a foreign tissue, they will need to find favorable conditions to survive and proliferate. The seed and soil hypothesis – originally conceived by the English surgeon Stephen

Paget in 1889 – emphasizes the importance of appropriate local trophic factors (the soil) in determining the growth of disseminated cancer cells (the seeds) into secondary tumors. Thus, cancer cells targeting distinct organs would differ in their responsiveness to molecules produced by different tissues; this idea could also explain the discrepancy between the relatively high blood supply of certain organs and their relatively low frequency of metastatic growth.

The seed and soil hypothesis might imply that tumor cells leave the blood and lymphatic circulation to the same degree at all organs, but survive and proliferate only in those organs producing congenial growth factors. However, it is highly plausible that selective adhesion/extravasation as well as trophic interactions between cancer cells and skeletal tissue are not mutually exclusive phenomena and they all play a crucial role in determining the bone tropism of tumors.

The bone is composed of different cell types and is characterized by sustained levels of metabolic activity due to bone remodeling or repair, inflammation and reactive hematopoiesis, throughout the entire life of the individual. Thus, bone tissue homeostasis is orchestrated by a plethora of trophic molecules to which cancer cells might also be sensitive. In fact, an important role for bone turnover in the preferential location of prostate cancer cells to the skeleton has been recently reported.

The quest to identify factors produced by the bone microenvironment that could support the growth of cancer cells has led to implicate several molecules, including ► [transforming growth factor](#), ► [osteopontin](#), ► [platelet derived growth factor](#), among others.

The disruption of the trophic interactions between cancer cells and bone, for example by using inhibitors of growth factor receptors such as ► [imatinib](#), has been recently attempted. In general, the identification of crucial molecules supporting the survival of cancer cells in the bone will provide novel targets for the treatment or prevention of skeletal tumors by pharmaceutical, immunological, or gene therapy approaches.

Cancer cells may leave the primitive tumors and enter the blood circulation in large numbers, a process called ► [intravasation](#). However, the metastatic process is highly inefficient and it has been shown that the vast majority of disseminated cancer cells fail to produce secondary tumors. Using animal models, only a very small fraction of cells delivered into the blood

stream were seen reaching peripheral organs; of those that located to secondary organs some would proliferate while others would remain dormant. Thus, growth factors produced by the bone tissue can be determinant for supporting the small foci of cancer cells immediately after their arrival and their successful lodging in the skeleton.

### Alteration of Bone Microenvironment by Cancer Cells

The small number of cancer cells reaching the skeleton are probably unable to immediately affect the surrounding cells and stroma and will depend on growth factors already present in the microenvironment to sustain their survival and growth. However, it is conceivable that once the initially small foci of malignant cells have reached significant size, functional cross-talking with the bone resident cells is established. This phenomenon has been shown to regulate phenotype and genotype of tumor as well as bone stromal cells and ultimately modify the microenvironment in a fashion that can potentially benefit metastatic growth.

An intriguing hypothesis is that cancer cells, in order to survive in the bone, need to acquire bone-like properties. This phenomenon – defined ► [osteomimicry](#) – is characterized by the expression of bone cell-related genes and is regulated by the interaction with bone resident cells, which can occur through either physical contacts or released factors. Some bone cell-related genes are those for osteopontin, osteocalcin, and bone sialoprotein. A soluble factor, ► [osteoprotegerin](#), a member of the ► tumor necrosis factor superfamily, can be detected in high levels in the serum of patients affected by advanced prostate cancer, which is almost invariably associated with skeletal metastasis. Osteoprotegerin acts as a ► [decoy receptor](#) and blocks TRAIL/Apo 2L, a cytokine that can trigger cancer cell death by interacting with death-receptors. Interestingly, osteoprotegerin is overexpressed by cancer cells as a consequence of osteomimicry. However, as bone marrow stromal cells also produce this protein during bone remodeling, the bone microenvironment could independently protect cancer cells from the TRAIL-induced death. Thus, the production of osteoprotegerin by cancer cells upon osteomimicry would essentially amplify this phenomenon.

Because of the obvious problems posed by bone secondary tumors for prognosis and quality of life, therapies to successfully counteract malignant cells

growing at the skeletal level are of utmost importance for the management of cancer.

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## Bone Tumors

Michael J. Klein

Center for Metabolic Bone Disease, The University of Alabama at Birmingham, Birmingham, AL, USA

## Synonyms

[Bone neoplasms](#); [Bone sarcomas](#)

## Definition

Bone tumors are space-occupying lesions arising in bone that are usually derived from primitive connective tissue elements.

They constitute a diverse group of neoplasms that are collectively less common than those of almost all other body sites. Malignant primary bone tumors have an approximate incidence of one new malignant bone tumor per 100,000 individuals per year. Since the incidence of benign bone tumors is about half that of malignant tumors, the average aggregate incidence of all bone tumors is about one in 67,000 persons per year.

With a few notable exceptions, bone tumors have a predilection for individuals in the second and third decade; there is a second, smaller peak of incidence in the sixth and seventh decade. In most large series of bone tumors there is a slight male predominance. Each tumor type has a characteristic range age, skeletal distribution, and sometimes sex and racial predilection.

## Characteristics

### Etiology

While the link between bone tumors and a predisposing cause is not demonstrable in most cases, there are conditions in which the incidence of bone tumors is increased. The presence of preexisting ► [Paget disease of bone](#), ► [idiopathic bone infarctions](#), and ► [fibrous dysplasia](#) are all associated with increased frequency of bone sarcomas. Ionizing radiation, whether given for therapeutic purposes or accidentally acquired through external exposure or ingestion also predisposes to the development of bone sarcomas. Ollier’s disease, a congenital but nonhereditary disorder characterized by multiple ► [enchondromas](#) predisposes to malignant cartilage tumors in 10–20% of cases. About half of all patients with Maffucci syndrome, which is characterized by multiple soft tissue ► [hemangiomas](#) in patients with Ollier’s disease, develop malignant cartilage tumors. Multiple hereditary exostoses, an autosomal dominant disorder, are associated with a specific genetic abnormality, usually at the EXT-1 or EXT-2 chromosomal loci. In this disorder, which is characterized by the development of multiple ► [osteochondromas](#), resulting in bone modeling abnormalities, the incidence of malignant cartilage tumors is in about 10% of patients.

Other familial disorders having an increased incidence of other malignant neoplasms also have an increased predisposition for ► [osteosarcomas](#). Chief among these are the Li–Fraumeni Syndrome, in which mutations in the p53 gene cause an increased incidence of many tumors (► [p53 gene family](#)). Patients with familial retinoblastoma gene mutations (Rb) have a high risk for the development of osteosarcoma. Two other autosomally recessive disorders that usually present with skin abnormalities, the ► [Rothmund–Thomson syndrome](#) and ► [Bloom syndrome](#), have associated specific chromosomal mutations that result in an increased association with osteosarcomas. A specific and reproducible chromosomal abnormality, namely, a fixed reciprocal translocation between chromosomes 11 and 22 and, less frequently, between chromosomes 7 or 21 and 22 results in a chimeric gene product that encodes for the proliferation protein promoter found in the cells of more than 90% of patients with ► [Ewing sarcoma](#).

**Bone Tumors. Table 1** Bone tumor classification

Matrix	Benign	Malignant
Bone	Osteoma	Osteosarcoma surface
	Osteoid osteoma	
Osteoblastoma	Osteoblastoma	
Cartilage	Enchondroma	Central chondrosarcoma
	Periosteal chondroma	Peripheral chondrosarcoma
	Osteochondroma	
Chondroid	Chondroblastoma	
	Chondromyxoid fibroma	
Fibrous	Non-ossifying fibroma	Fibrosarcoma
	Desmoplastic fibroma	Malignant fibrous histiocytoma
	Benign fibrous histiocytoma	
Fat	Lipoma	Liposarcoma
Muscle	Leiomyoma	Leiomyosarcoma
		Rhabdomyosarcoma
Notochord		Chordoma
Neural	Schwannoma	Ewing's sarcoma/primitive neuroectodermal
Tumor	Neurofibroma	
Vascular	Hemangioma	Angiosarcoma
	Hemangioendothelioma	
Epithelial		Adamantinoma
Lymphoid		Lymphoma
Myeloid		Myeloma
Histiocytic	Giant cell tumor	

## Diagnosis

Bone tumors present with fairly nonspecific symptoms, the most common of which is pain that is noticed at rest or is severe enough to wake a patient from sleep. Interference with normal limb function or the presence of a mass may also be present, but these usually occur later in disease evolution. A specific diagnosis is made by biopsy, which may be as simple as a fine needle aspiration or as extensive as a complete excision. Since the overlying soft tissues and skin cover the bones, bone tumors are essentially invisible without clinical imaging studies. The most important of these are routine radiographic studies done using multiple views. Imaging studies should always be reviewed in tandem with the histology prior to rendering a diagnosis, because not only do they provide information regarding the location, extent, and aggressiveness of the disease, but also correlative information on the likelihood that the biopsy is representative of the process.

Primary bone tumors are classified into their various histologic subtypes on the basis of their histologic grade, their anatomic location, and especially by the

kinds of extracellular connective tissue (e.g., bone, cartilage, fibrous tissue) produced by each or whether there is differentiation into any other types of soft tissue elements (Table 1). For example, osteosarcoma is a malignant tumor in which the cells have the capacity to produce bone. While the diagnosis is usually made from the histologic features, some bone tumors that look deceptively benign histologically may actually be diagnosed as malignant tumors when the imaging and histologic features are correlated. In addition, a very few varieties of tumors are characteristically unpredictable in biologic potential. A few primary bone tumors may behave in a locally destructive fashion but have an unpredictable propensity for metastatic behavior. The most important examples include giant cell tumors of bone and ► *hemangioendotheliomas*.

Because they often produce so large a quantity of extracellular matrix, the volume of primary bone tumors tend to be comprised more of extracellular connective tissue elements rather than by tumor cells. This relationship is the exact opposite of what is observed in carcinomas. With the notable exception

of chordoma, adamantinoma, and neural tumors, it is believed that almost all primary bone tumors are derived from embryonic ► mesoderm. This is again in contrast to adenomas and carcinomas, which are derived from ► ectodermal or ► endodermal precursors.

### Staging and Prognosis

Benign tumors of bone are almost always of a limited growth potential and their morbidity is confined to interference with local anatomy or function. They are usually controlled or cured by limited surgery such as curettage or simple excision. Locally aggressive bone tumors may require more aggressive surgery depending upon their size, location, and whether there has been previous treatment. Most malignant bone tumors are high-grade lesions and are usually treated as systemic diseases even if they seem localized at the time of diagnosis. With the exception of ► chondrosarcoma, malignant tumors are now treated with systemic chemotherapy (► neoadjuvant Therapy). A few tumors, notably lymphoma and myeloma, may also be treated with local radiation therapy, but most other bone tumors are no longer irradiated. Most malignant bone tumors spread systemically to the lungs though other bones and sometimes other parenchymal organs may be affected. In general, regional lymph nodes are not affected, so the most meaningful staging schemas of bone tumors do not necessarily include the presence of nodal disease. The musculoskeletal tumor society staging system first proposed by Enneking takes into account only whether a malignant tumor is high grade or low grade, confined to one or more compartments, or is metastatic. This system both summarizes tumor biology and helps to clarify the treatment regimen required for a particular patient.

In general, patients with low-grade malignant bone tumors have a good prognosis so long as their initial surgery is adequate. If surgery is not adequate, malignant tumors recur locally. The longer a low-grade tumor persists locally or the more times it recurs, the greater is the likelihood for systemic spread by the tumor. Certain low-grade tumors such as central chondrosarcoma and low-grade osteosarcomas are notorious for undergoing ► dedifferentiation, with greatly increased likelihood for metastasis. The prognosis of patients with high-grade bone sarcomas has improved with the advent of present-day chemotherapy. For example, the 5-year survival for patients with

osteosarcomas was in the range of 20% but is now better than 70% in some series.

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### Bone-seeking Malignant Phenotypes

#### ► Bone Tropism

### Borderline Appendiceal Mucinous Tumor

#### ► Appendiceal Epithelial Neoplasms

### BORIS

Dmitri Loukinov<sup>1</sup>, Victor Lobanenkov<sup>1</sup> and Elena Klenova<sup>2</sup>

<sup>1</sup>Section of Molecular Pathology, Laboratory of Immunopathology, NIAID, National Institutes of Health, Bethesda, MD, USA

<sup>2</sup>Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex, UK

### Synonyms

Brother of the regulator of imprinted sites; CTCFL (stands for CTCF-like); CTCF-T (stands for CTCF-testis-specific)

## Definition

BORIS (acronym for *Brother of the Regulator of Imprinted Sites*) is a member of Cancer–Testis Antigen (CTA) family and a mammalian parologue of ► CTCF, with the suggested role in the regulation of epigenetic reprogramming.

## Characteristics

Studies on a transcription factor CTCF, a candidate ► tumor suppressor gene (TSG) involved in transcriptional regulation, control of imprinted genes, and insulators, led to a discovery of its parologue, termed BORIS. The rationale behind the discovery of BORIS was to explain the process of resetting of ► imprinting marks in testis; in other words, how methylation-sensitive CTCF binding sites can be established in germ cells de novo. The existence of a protein with certain characteristics in these cells was therefore postulated. On one hand, such a hypothetic protein needed to recognize the same DNA sequences as CTCF to modify CTCF binding sites. On the other hand, this protein should be associated with a different biochemical machinery to erase and/or establish the methylation marks in germ cells that will later be read by CTCF in somatic cells. This hypothesis of the existence of germ cell–specific molecule was later confirmed by the identification of BORIS, which was the ideal candidate for the role.

Firstly, in contrast to the ubiquitous CTCF, BORIS was only expressed in germ cells (spermatocytes) in testis; other testicular cells (Sertoli, Leydig, and the somatic cells) were BORIS negative. It is worth noting that, contrary to BORIS, CTCF was significantly downregulated in the spermatocytes. Secondly, BORIS had the DNA-binding domain identical to CTCF (74% identity), whereas the flanking N- and C-terminal domains were dissimilar (Fig. 1a). These structural features indicated that BORIS could recognize the same set of DNA targets as CTCF, while the dissimilar flanking domains could bring different interacting partners to the sequences to perform epigenetic modifications. The ability of BORIS to bind to a variety of DNA targets of CTCF was tested experimentally. In this study, all CTCF DNA targets inspected formed complexes with BORIS. This implies that evolutionary

pressure maintained the same specificity of the DNA binding domain in CTCF and BORIS, thus suggesting important functions for both proteins; these functions are discussed in this and CTCF entries in the encyclopedia.

BORIS is a 85 kDa protein; it can be detected both in the nucleus and in the cytoplasm, which is not common for transcription factors. This is in contrast to CTCF, which has strictly nuclear localization. The cytoplasmic form of BORIS may represent the inactive, sequestered protein, although the precise function(s) of the cytoplasmic BORIS remains to be investigated.

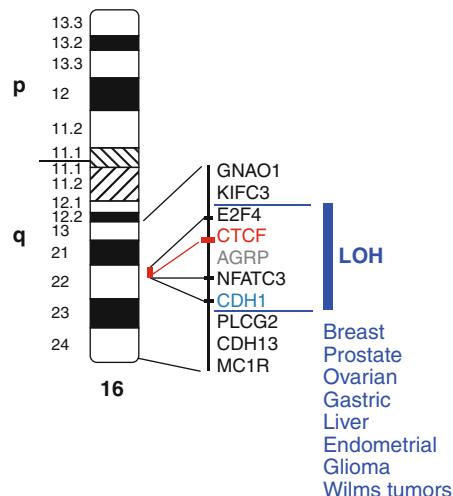
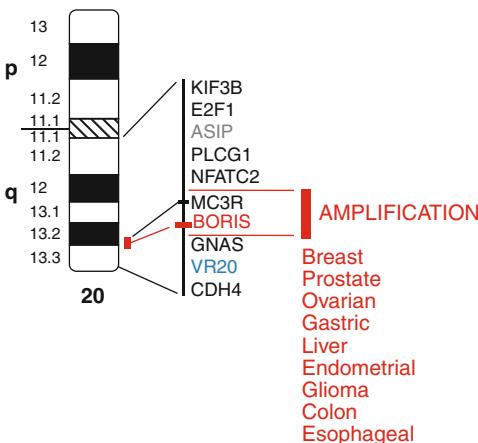
The human BORIS gene is mapped to chromosome 20q13 (Fig. 1b), whereas its mouse counterpart is mapped to chromosome 2, bands H3-4. Of note, the mouse chromosome 2 has significant homology to the human chromosome 20q. Surprisingly, BORIS not only possesses the DNA-binding domain similar to CTCF, but its gene also preserved similar exon/intron structure, with identical splice sites for each exon–exon junctions in the ZF domain as the mammalian CTCF (Fig. 1c). It is worth noting that the exon/intron structure of chicken CTCF is very different from that of the mammalian CTCF suggesting that BORIS gene most likely appeared in the evolution after the separation of mammals and birds possibly due to the duplication and translocation of the CTCF DNA-binding domain (Fig. 1c).

So far, the human and mouse BORIS genes have been cloned, while the rat, dog, and chimpanzee homologues of BORIS can be easily found using bioinformatics approaches, such as a homology search of the GenBank.

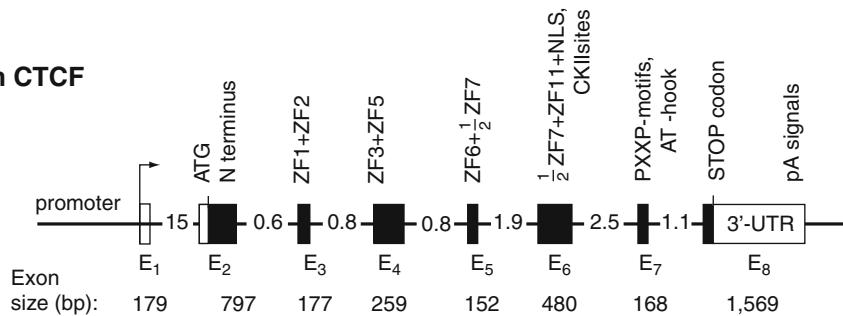
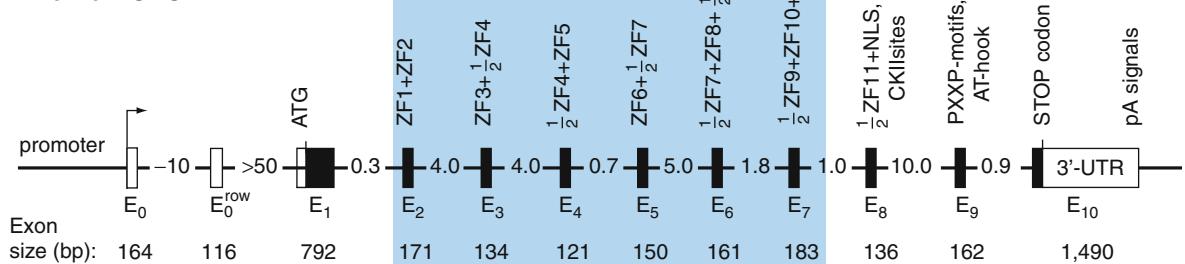
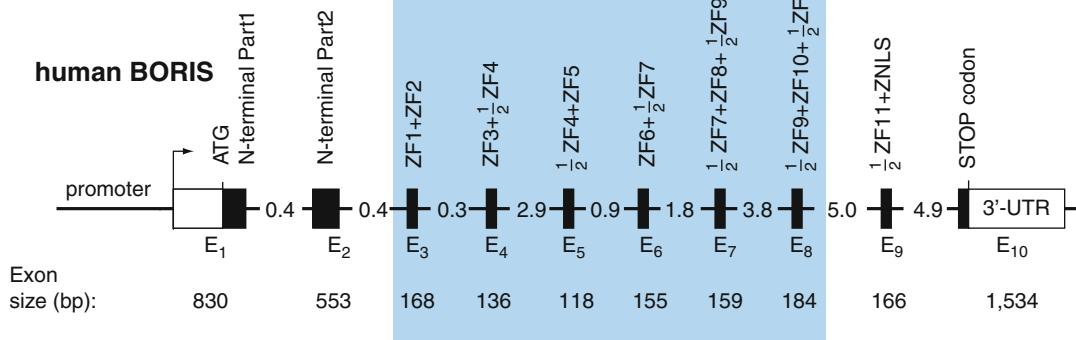
## BORIS Functions

Although indirect evidence indicates that BORIS plays important functional roles in the cells where it is expressed, there are only a few published reports investigating BORIS functions. The fact that CTCF and BORIS are expressed in a mutually exclusive manner during male germ-line development led to the hypothesis that BORIS may be important for epigenetic reprogramming occurring in these cells during development. Indeed, BORIS has been implicated into the initiation of a series of methylation events at the imprinting control regions (ICR), in the vicinity of the CTCF/BORIS binding sites. In this model, BORIS interaction with a protein arginine methyltransferase

<b>a</b>	MAATEIS-VLSEQFTKIKELELMPEKGLKEEKDGVCREKDHRSPSELEAERTSG MEGDAVEAIVEESETFIKGKERKTYQRRREGGQEEDACHLPQ-----NQTDG	54 47	
	-----AFQDSVLEE-----EV-ELVLAPSEESE---KYIILTLQTVHFT GEVVQDVNSSVQMVMMEQLDPTLLQMKTEVMEGTVAPEAAVDDTQIITLQVVNME	127 104	
	SEAV---ELQDMSLLS1QQQEGVQVQQPQPGPLLWLEEGPRQSLQQCVAISIQQELYSPQ EQPINIGELQ-----LVQVPVPVTVP-VATTSVEE-----LGAYENEVSKEGLAES	145 150	Distinct
	EMEVLQFHALEE--NVMVASEDSKLAVSLAETAGLIKLEEEQEKN---QLLAERTKEQLFFVE --EPMICHTLPLPEGFQVVKGANGEVETLEQGELPPQEDPSWQKDPDYQPPAKTKTKKSKL	163 212	
	TMSGDERSDEIVLTVSNNSNVEEQQEDQPTAGQADAeka----KSTKNQRKTGAKGT RYTEEGKD---VDVSVYDFEEEQQEGLLSEVNAEKVVGNMKPPKPTKIKKKGVKKT	256 265	
	<b>FH</b> CDVCMFTSSRMSSFNR <b>HMKTH</b> TSEKPHL <b>CHLC</b> LKTFR <sup>T</sup> VTLRN <b>HVNTH</b> TGTRP <b>FQ</b> CELC <b>SYTC</b> PRRSNLDR <b>HMKSH</b> TDERPHK <b>CHLC</b> GRAFRTVTLRN <b>HLNTH</b> TGTRP	312 321	
ZF1	ZF2		
<b>YK</b> CND <b>C</b> NMAFVTSGELVR <b>HRYK</b> THEKPKFK <b>CSM</b> CKYASVEASKLKR <b>HVRSH</b> TGERP <b>HK</b> CPDCDMAFVTSGELVR <b>HRYK</b> THEKPKFK <b>CSM</b> CDYASVEVSKLKR <b>HIRS</b> TGERP	369 378		
ZF3	ZF4		
<b>FQ</b> CCQC <b>SYASRDTYKLKR</b> <b>HMRTH</b> SGEKPYE <b>CHICHTRFT</b> QSGTMK <b>HILQK</b> GENVPK <b>FQ</b> CSLC <b>SYASRDTYKLKR</b> <b>HMRTH</b> SGEKPYE <b>CYICHARFT</b> QSGTMK <b>HILQK</b> TENVAK	427 436	Identical ZF Domain	
ZF5	ZF6		
<b>YQ</b> CPHCATIIARKSDLRV <b>HMRNL</b> AYSAEEL <b>KCRY</b> CSAVFH <sup>E</sup> RYALIQ <b>HQKT</b> KNEKR <b>FH</b> CPHC <b>D</b> TVIARKSDLGV <b>HLRKQH</b> SYIEQGKK <b>CRY</b> DAVFH <sup>E</sup> RYALIQ <b>HQKS</b> KNEKR	485 494		
ZF7	ZF8		
<b>FK</b> CKH <b>C</b> YACKQERHMTA <b>HIRTH</b> TGEKPFT <b>CLSC</b> NKCFRQKQLNA <b>HFRKY</b> DANFIP <sup>T</sup> V <b>FK</b> CDQ <b>C</b> DYACRQERHIM <b>HKRTH</b> TGEKPYAC <b>SHCD</b> KTFRQKQLDM <b>HFKRY</b> DPNFVPA <sup>A</sup>	545 554		
ZF9	ZF10		
<b>YK</b> CSKC <b>GKGF</b> SRWINLHR <b>HSE</b> K <b>CGS</b> ---GEAKSAASGKGRTRKRQ <sup>T</sup> ILKEATKGQKE <b>FV</b> CSKC <b>GKTF</b> TRRNTMAR <b>HADNC</b> AGPDGV <sup>E</sup> GENGETKSKRGKRMRSKKEDSSDSEN	601 614		
ZF11			
AAKGWKEAANGDEAAAAE EASTTKGEQFPGEMFPVACRETTAR----- AEPDL---DDNEDEEPAVEIEPEPEPEPQPVTPAPPPAKRRGPPGRTNQPQKQNQP	643 667	Distinct	
	VKEEVDEGVTC <sup>E</sup> MLNTMDK* TAIIQVEDQNTGAIENIIVEVKKEPDAEPAEGEEEEAQPAATDAPNGD <sup>L</sup> TPEMILSMSMDR*	663 727	

**b**

BORIS, Fig. 1 (continued)

**c****Chicken CTCF****human CTCF****human BORIS**

**BORIS. Fig. 1** Comparative amino acid alignment of human CTCF and BORIS. Identical and homologous residues in the ZF domain are shown in red, homologous in magenta, nonhomologous in black. Residues responsible to form DNA contacts in Zn-fingers are shown in blue. Residues chelating Zn ion are shown in larger font sizes. (b) Both BORIS and CTCF map to cancer-associated “hot spots”: on 20q13 and 16q22, respectively. According to the modified “two hit” hypothesis, two hits occur on different chromosomes – first in the region of

frequent LOH at 16q22 where CTCF is localized and second in a region of 20q13 frequently amplified in multiple human cancers, where BORIS is localized. BORIS in this context can be regarded as a dominant-negative mutant of CTCF. (c) Comparison of the overall exon/intron structures of CTCF and BORIS genes. The region of homology over the exons encoding the ZF domain is highlighted. BORIS contains duplicated ZF-coding exons of mammalian, but not chicken, CTCF

PRMT7 leads to methylation of histones, followed by methylation of DNA by the de novo methyltransferase Dnmt3b and the establishment of a heterochromatic (silent) configuration of chromatin in this locus. BORIS was also found to be important for regulation of genes essential for spermatogenesis. However, the exact role that BORIS plays during spermatogenesis needs to be further investigated using a wide range of knockout and/or knockin cell and animal models.

When BORIS is introduced into cells that do not normally express this molecule, for example, into somatic cells such as normal human dermal fibroblasts, it activates a group of members of CTA family (MAGE-A1, MAGE-A2, MAGE-B1, MAGE-B4, GAGE-3-8, RAGE-2, NY-ESO-1 (CTAG1B), LAGE-1 (CTAG2)) and also transcription factors playing a role in maintaining germ/stem cells phenotype (Oct-3/4, or POU5F1). The molecular events underlying this function of BORIS involve demethylation of the promoters of the genes in question, followed by activation of these genes. This implies the involvement of different biochemical machinery recruited by BORIS to the DNA targets in these cells. Interaction between BORIS and a transcription factor, SP1, facilitates derepression of *NY-ESO-1* gene in lung cancer cells. These findings further signify the importance of protein partners interacting with BORIS; the identification of such proteins will be instrumental in the understanding of the exact molecular mechanisms of BORIS functions in the processes of DNA methylation and demethylation.

Some of the CTAs (for instance, MAGE-A1 and NY-ESO) are considered to be potential clinical targets for cancer immunotherapy. Therefore the investigations into how these genes may be regulated by BORIS are very important as they may provide the means to increase the expression from the relevant genes and enhance the response from the patients' immune system.

BORIS is capable to compete with CTCF on different targets both in vitro and in vivo. Taking into account the same DNA binding specificity, it is conceivable that the aberrantly expressed BORIS can act as an interfering mutation to CTCF. As CTCF regulates several genes implicated in cancer development (► *c-Myc*, hTERT, ► *BRCA1*, IGF2, ► *p53*, *p27*, ► *p21*, ► *ARF*, etc. – see the entry on CTCF in this encyclopedia), aberrantly expressed BORIS can compete with CTCF for binding thus ultimately leading to deregulation of those genes.

## BORIS in Cancers

Some of BORIS features indicate that BORIS can be classified as an ► oncogene. It is located at the 20q13, together with Aurora kinase; this small 20q amplicon is a hotspot for chromosomal amplification in many human cancers (Fig. 1b). BORIS proximity to Aurora and their frequent coamplification raises an interesting possibility of potential cooperation of those two potential oncogenes in the process of cell transformation.

Normal BORIS expression is restricted to adult testis while abnormal expression is detected in a wide variety of cancers including breast, prostate, colon, melanomas, testicular, endometrial, and many others. This expression pattern and the ability to induce immune response in patients, both antibody and cellular, place BORIS into a category of CTAs. The CTAs include around 14 families of tumor antigens. The function of the majority of the CTAs is still unknown, although some CTAs are thought to be implicated in the regulation of gene expression and others may control gametogenesis. As a member of the CTA family, BORIS is now seen as an attractive target for both diagnostics and therapy of many human tumors (see below).

Although the mechanisms of BORIS activation in cancers are not yet known, the consequences of such an event can be dramatic. For example, BORIS can reverse the function of CTCF as a TSG by binding to CTCF targets and deregulating them. The possibility of such a "two chromosome hit" scenario, when a TSG is inactivated by the events occurring on two different chromosomes, creates a necessity to revise Knudsen's "two hit" theory, suggesting that while one copy of a TSG can be eliminated by loss of heterozygosity (LOH), the other copy can be either inactivated by epigenetic means or by somatic mutations. For the CTCF/BORIS pair, the second hit can occur at a different chromosome (20q13) by activation and subsequent chromosomal amplification of a gene with the same DNA-binding specificity but different regulatory domains. This activation of a different gene on a different chromosome but capable of interfering with a tumor suppressor can be considered as analogous to the action of a dominant negative mutant (Fig. 1b). The aberrantly expressed BORIS is likely to interfere with the CTCF regulatory pathways that include a number of cancer-related genes, thus leading to deregulation of these genes and contributing to transformed phenotype.

BORIS interaction with the protein arginine methyl transferase PRMT7 that can result in DNA methylation and formation of heterochromatin may be responsible for silencing of some tumor suppressor genes that are inactivated in cancers due to aberrant methylation of the promoter regions. Finally, BORIS appears to be capable of reversing the epigenetically silenced multiple CTAs, which results in activation of these genes and may contribute to tumor development.

### Clinical Aspects

**BORIS as a Cancer Biomarker** The identification of new markers to discriminate tumorigenic from normal cells, as well as the different stages of tumor pathology, has now become of critical importance for cancer diagnosis, prognosis, and monitoring. All currently available tumor markers are not ideal as in most cases they lack of sensitivity for early cancer and specificity for malignancy. Therefore, the quest to identify additional “cancer genes” implicated in breast tumorigenesis, along with delineation of prognostic biomarkers, has now become the most important step toward developing better diagnostic tools and a possibility of curing the disease. The finding that, similar to other CTAs, BORIS is aberrantly expressed in a wide variety of cancers points to important practical applications, namely, using BORIS as a molecular

► **Biomarker** of cancer, especially for early diagnostics of the disease.

BORIS has the potential to be an early circulating marker, the detection of which may indicate the existence of a cancerous condition in the patient or of a predisposition of the patient to such a condition. In these investigations, BORIS was found to be present in the white blood cells (or leukocytes) in patients with breast cancer, but not in healthy donors. The type of the leukocytes was determined as the neutrophil polymorphonuclear granulocytes (PMNs). These findings place BORIS in a new category of cancer biomarkers, different from those currently used in medical practice.

The molecular mechanisms of BORIS activation and the functions in PMNs of breast cancer patients remain to be established. However, it is acknowledged that this is a tumor-related occurrence because BORIS was not detected in PMNs in donors with injuries and immune and inflammatory diseases. This opens the perspective to utilize BORIS as a valuable blood marker for early detection of breast cancer (and may

be other types of tumors) as well as the attractive target for early intervention and prevention of the disease.

This discovery is of great importance in the context of an ongoing quest to identify accurate circulating markers as far there are no established circulating tumor markers available for clinical use in the determination of cancer susceptibility, screening, diagnosis, and prognosis. The presence in such markers in blood makes them particularly useful since clinical analysis will involve relatively noninvasive procedures.

**BORIS as a Target for Cancer Immunotherapy** Multiple CTAs are promising candidates for immunotherapeutic approach to treat cancer, although they have limitations. One of the main disadvantages for using these targets is their relatively narrow expression patterns. Another problem lies in the fact that sometimes during the treatment of the cancerous condition, the expression of the target antigen ceases without affecting the tumor growth, thus allowing tumor cells to escape from the immune response directed against them.

The fact that BORIS belongs to cancer–testis gene family as well as its wide expression in multiple cancers suggests that BORIS can be a good candidate for cancer immunotherapy. Using a mouse model, it was recently demonstrated that immune response to BORIS can be developed in the organism and, furthermore, such immune response has protective effects against several mouse tumors of different origin. Importantly, this immune reaction seems to be of a MHC class I-restricted response of cytotoxic T lymphocytes (CTLs) against histologically diverse tumor cells expressing only endogenous BORIS.

As BORIS seems to reverse tumor suppressor functions of CTCF in cancer, it is likely that BORIS is important to sustain the transformed phenotype. Therefore, if some tumor cells escape the anti-BORIS immune cells following the cessation of BORIS production in these cells, such BORIS-negative cells are expected to have much weaker growth/tumorigenic potential than BORIS-positive cells. This means that even if complete elimination of tumor cannot be achieved, the patient still might benefit from anti-BORIS therapy.

In summary, BORIS has an excellent potential for immunotherapy and even preventive vaccination against cancers, although more research is required to advance BORIS to clinical trials.

► **CCCTC-Binding Factor (CTCF)**

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## Bortezomib

Jochen Lorch  
Dana Farbur Cancer Institute, Boston, MA, USA

### Synonyms

PS 341; Velcade®

### Definition

An antineoplastic agent targeting the proteasome.

### Characteristics

#### Mechanism of Action

Bortezomib is a proteasome inhibitor and the first drug to emerge from a new class of therapeutic agents targeting the ubiquitin–proteasome pathway. This pathway mediates the degradation of polyubiquitinated proteins and accounts for 80% of the protein degradation in eukaryotic cells. Proteins that are marked for degradation undergo conjugation of polyubiquitin chains to lysine residues in a process called ubiquitination. The proteasome is an enzymatic complex that recognizes

ubiquitin-tagged proteins and catalyzes their proteolytic breakdown in an ATP-dependent fashion. Bortezomib is a reversible inhibitor of the chymotrypsin-like activity of the 26 S proteasome in mammalian cells. It has been approved in the United States and Europe for the treatment of ► multiple myeloma and has recently been approved in the United States for the treatment of relapsed ► mantle cell lymphoma. In addition, it has also shown significant antitumor activity in many other types of cancer. Due to the wide range of proteins that are subject to ubiquitination and subsequent proteasomal degradation, bortezomib interferes with multiple pathways ultimately leading to ► apoptosis. Proposed mechanism of action includes NF-κB inhibition through reduced Iκα degradation, leading to reduced NF-κB-dependent synthesis of antiapoptotic factors such as c-Flip. Other mechanisms include inhibition of apoptosis (IAP)1/2 and ► BCL-2, stabilization of ► p53, deregulation of cyclin turnover, and subsequently ► cyclin-dependent kinases activity as well as effects on stability of cdc25 family proteins, KIP1, and WAF1 during cell cycle. In addition, it has been shown to influence the balance between pro- and antiapoptotic Bcl-2-family proteins, stabilizes JNK, as well as increases c-Jun phosphorylation and AP-1 DNA-binding activity with subsequent Fas upregulation. Other pathways include deregulated proapoptotic signaling via ► tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and disruption of the unfolded protein response with endoplasmic reticulum (ER) stress induction. Increased intracellular reactive oxygen species and oxidative stress may also contribute to its antitumor activity. Preclinical data suggests that bortezomib reverses the antiapoptotic effects of ► interleukin (IL)-6 and ► insulin growth factor (IGF)-1. It also reduces tumor cell migration in multiple myeloma cells and squamous cell cancer cells due to its effects on ► VEGF and dysregulation of focal ► adhesion assembly. Furthermore, it may affect the tumor ► microenvironment, thus exerting an antiangiogenic effect. As a result, bortezomib ultimately triggers an apoptotic cascade mediated by ► caspases. The number of proposed mechanisms is likely to grow in the future as none of these pathways can fully explain the clinical effectiveness of bortezomib in malignancy, and research is under way to fill these gaps. Depending on the tumor cell type, one or a combination of several pathways may be responsible for the clinical effects

and different cell types may therefore be more or less sensitive to the effects of bortezomib. For example, terminally differentiated immunoglobulin-secreting plasma cells have impaired proteasome activity, which could explain bortezomib's activity against malignant plasma cells in multiple myeloma.

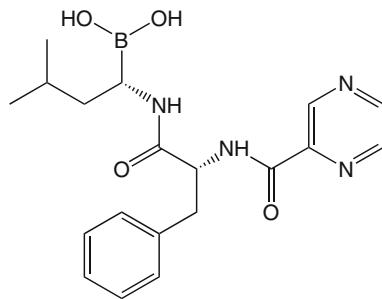
### Pharmacology

The chemical formula for bortezomib is [3-methyl-1-(3-phenyl-2-pyrazin-2-ylcarbonylamino-propanoyl)amino-butyl] boronic acid. The chemical structure is shown in Fig. 1. Bortezomib is administered intravenously and weekly once and twice dosing schedules have been tested. The mean elimination half-life of bortezomib after first dose ranges from 9 to 15 h at doses ranging from 1.45 to 2.00 mg/m<sup>2</sup> in patients with advanced malignancies. In vitro studies with human liver microsomes and human cDNA-expressed cytochrome P450 isozymes indicate that bortezomib is oxidatively metabolized primarily via cytochrome P450 enzymes 3A4, 2 C19, and 1A2, while bortezomib metabolism by CYP 2D6 and 2 C9 enzymes is minor. The major metabolic pathway is deboronation to form two deboronated metabolites that subsequently undergo hydroxylation to several metabolites, which are inactive as 26 S proteasome inhibitors. The most common side effects include asthenia, nausea, diarrhea, anorrhexia, constipation, ▶ **thrombocytopenia**, peripheral neuropathy, and pyrexia.

### Clinical Aspects

#### In Cancer

The effectiveness of bortezomib is based on response rates which led to the accelerated approval of bortezomib by the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). The approval of bortezomib was based on an open-label, single-arm, multicenter study of 202 subjects with multiple myeloma who had received at least two prior therapies. An IV bolus injection of bortezomib (1.3 mg/m<sup>2</sup>/dose) was administered twice a week for 2 weeks, followed by a 10-day rest period (21-day treatment cycle) for a maximum of eight treatment cycles. Subjects who experienced a response to bortezomib treatment were allowed to continue treatment in an extension study. Results showed 52 (27.7%) subjects achieved an overall response rate, 5 (2.7%) achieved a complete response, 47 (25%) achieved a partial response, and 33 (17.6%) demonstrated a clinical



**Bortezomib. Fig. 1** Chemical structure of bortezomib

remission. The ▶ **Kaplan–Meier** estimated median duration of response was 1 year. In another trial, bortezomib was compared with high-dose dexamethasone in 669 patients with relapsed multiple myeloma, which confirmed superior activity of bortezomib over dexamethasone resulting in a higher response rate, a longer time to progression (the primary end point), and a longer survival than patients treated with dexamethasone. The combined complete and partial response rates were significantly longer for the group receiving bortezomib (38% vs 18%), and the complete response rates were 6% and less than 1%, respectively. Median times of progression in the bortezomib and dexamethasone groups were 6.22 months (189 days) and 3.49 months (106 days), respectively. The 1-year survival rate was 80% among patients treated with bortezomib and 66% among patients treated with dexamethasone, and the hazard ratio for overall survival with bortezomib was 0.57. Since then, more data from phase 2 and 3 trials have emerged and have confirmed the activity of bortezomib in relapsed and newly diagnosed multiple myeloma. Trials evaluating the combination of bortezomib and other drugs such as ▶ **thalidomide** and its analog lenalidomide are ongoing, and the results will be available in the near future. Bortezomib has recently been approved for the treatment of relapsed and refractory mantle cell lymphoma. The approval was based on data from an open-label, single-group, multicenter phase 2 clinical trial of 155 patients with relapsed or refractory mantle cell lymphoma who had received at least one prior therapy. Participants received single-agent bortezomib (1.3 mg/m<sup>2</sup> twice a week for 2 weeks every 21 days) for up to a year. Results showed that 31% of patients achieved overall response to bortezomib, with 8% demonstrating a complete response, and 23% a partial response, as determined by computed tomography scan reviews. The median number of cycles in

responding patients was eight, and the median time to response was 40 days. The median duration of response was 9.3 months overall and longer for those achieving complete response compared with those with partial response to bortezomib. The median time to progression of disease was 6.2 months.

Trials are also investigating thalidomide in combination with bortezomib in patients with ► **myelodysplastic syndromes**. Phase 2 studies on bortezomib in other types of lymphoma are ongoing, and interesting results have been presented as abstracts which indicate that bortezomib may be useful in the treatment of these diseases with high response rates and substantial number of complete remissions in pretreated patients.

Early data is also emerging showing that bortezomib has activity in various solid tumors such as lung, head, and neck, prostate cancer. Preclinical and early clinical data suggests that bortezomib may enhance the activity of EGF-R inhibitors through the upregulation of the EGF-receptor. As trials are under way to evaluate the use of bortezomib alone and in combination with other agents, other indications for bortezomib in solid tumors are likely to emerge.

#### In Graft Versus Host Disease and Immune Disorders

The use of bortezomib in graft versus host disease (GVHD) is based on bortezomib's effect on antigen processing, apoptosis, cell cycle onco-stimulation, and chemotaxis. The ability of proteasome inhibitors to prevent NF- $\kappa$ B activation has made proteasome inhibitors attractive candidates for the treatment of immune-mediated disorders. Preclinical and early clinical data suggests that proteasome inhibitors may be effective in the treatment of osteoarthritis, psoriasis, and a spectrum of other autoimmune conditions. In preclinical models, GVHD was effectively prevented without affecting the beneficial graft versus tumor effects. At this point, it is unknown whether this will be reproducible in humans. It appears that bortezomib has direct proapoptotic effects on human T-lymphocytes which have been shown in vitro which may explain the clinical observation of lymphopenia in some patients undergoing treatment with bortezomib.

#### Other Uses

Preclinical models suggest that bortezomib may also be useful in the treatment of cardiovascular disease and ischemic strokes. It is unclear whether these effects

may be related to its effects on the NF- $\kappa$ B inactivation; cytokine secretion and modulation of cell adhesion may also play an important role.

#### Future Directions

Other compounds that target the proteasome are in preclinical and clinical development. NPI-0052, a compound derived from the marine actinomycete *Salinospora tropica*, an inhibitor of the 20 S subunit of the proteasome, is currently undergoing early phase clinical testing. It has shown significant activity in multiple myeloma.

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#### Bosniak Classification

##### Definition

Classification of renal cysts; ► **renal cancer diagnosis**.

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#### Bovine Papillomavirus

Giuseppe Borzacchiello

Department of Pathology and Animal health,  
University of Naples Federico II, Naples, Italy

#### Definition

Oncogenic DNA viruses causing both benign and malignant epithelial and mesenchymal tumors in cows and equids.

## Characteristics

Bovine papillomaviruses (BPV) belong to the Papillomaviruses (PV) family. BPV are small oncogenic DNA viruses strictly species-specific and, even in experimental conditions, do not infect any other host than the natural one. The only known case of cross-species infection is the infection of horses and other equids by BPV type 1 (BPV-1) or BPV type-2 (BPV-2). Papillomavirus infections usually regress, but occasionally they develop to cancer.

Ten BPV types (BPV 1–10) have been characterized associated with different histopathological lesions. The different genotypes have been classified into three genera. Two novel BPV were characterized further and the phylogenetic analysis showed that both viruses were new BPV types: one was designated as BPV-7 and classified as a member of a new PV genus, and the other was designated as BPV-8 and classified as a member of the *Epsilonpapillomavirus* genus.

Recently, two new BPV types belonging to the genus *Xipapillomavirus* have been characterized and designated as BPV-9 and BPV-10.

Xi-papillomaviruses encompassing the pure epitheliotropic BPV-3; BPV-4 and BPV-6; Delta-papillomaviruses encompassing BPV-1 and BPV-2 associated to fibro-►**Papillomas** (i.e., benign tumors of both epithelium and underlying derma) and Epsilon papillomavirus comprising the BPV-5 whose genome seems to share similarities with the former two BPV groups.

The BPV virion is a nonenveloped structure of 55–60 nm diameter containing a double-stranded covalently closed circular DNA. Three different regions compose the genome: the long control region (LCR) and two regions encoding for early and late genes.

The LCR is the genome region containing signals for both viral DNA replication and transcription. E2 regulates BPV transcription at LCR level; the LCR of BPV-4 contains different E2 binding sites; depending on the sites involved the transcription may be repressed or activated. The E2 sites are also bound by different cellular transcription factors and the E2 can also bind to mitotic chromosomes resulting in efficient distribution of the BPV genome into daughter cells.

## BPV Gene Products

### E5

The papillomavirus ►E5 proteins are short hydrophobic polypeptides [from 83 amino acid residues in ►Human Papillomavirus type 16 (HPV-16) to 42 residues in BPV-4], many of which have transforming activity. *BPV-1 E5* oncogene encodes for a 44 amino acid protein that is the major BPV transforming oncoprotein. It is a type II transmembrane protein which is expressed in the deep layers of the infected epithelia and is largely localized to the membranes of the endoplasmic reticulum (ER) and Golgi apparatus (GA) of the host cells. BPV E5 is expressed in the cytoplasm of both basal and suprabasal transformed epithelial cells with a typical juxtanuclear pattern due to its localization in the GA. It may also be expressed in neoplastic cells of mesenchymal origin such those of endothelial origin.

Due to its relative small size BPV E5 has no intrinsic enzymatic activity, and its transformation activity is related to the activation of several kinases from growth factor receptor to cdk cyclins. E5 interacts with the 16-K subunit c protein, a component of the vacuolar H<sup>+</sup>-ATPase pump. This proton pump acidifies the lumen of intracellular compartments, (endosomes, lysosomes, and GA) that process growth factors so that E5 binding may result in alteration of this processing. Another consequence of E5-mediated impaired acidification is the downregulation (both *in vivo* and *in vitro*) of the major histocompatibility complex class I (►MHC-I) expression, representing one of the mechanisms by which the BPV evade the immunoresponse by the host.

The mechanism by which BPV-1 E5 induces cell transformation is its binding to and activation of the cellular β receptor for the ►platelet-derived growth factor (PDGFβ). The activation of endogenous PDGFβ receptors is characterized by the formation of stable E5-receptor complexes, persistent tyrosine phosphorylation of the receptor, its dimerization and cellular transformation. This interaction takes place also in naturally occurring BPV-2 associated bovine urinary bladder cancer.

### E6

The *BPV-1 E6* gene of Xi BPV encodes an oncoprotein of 137 amino acids. It binds to paxillin blocking its interaction with vinculin and the focal adhesion kinase.

It also binds to several others cellular proteins such as ERC-55, the E3 ubiquitin ligase E6AP, and with the AP-1. Finally, it has been demonstrated that E6 interacts with the CBP/p300 inhibiting the p53.

### E7

The *BPV E7* gene encodes a 127 amino acids zinc binding protein which cooperates with E5 and E6 in inducing cell transformation. Once ► *E7* is coexpressed with E5 and E6, its transformation capacity increases many folds, and such coexpression may also occur in tumors of mesenchymal origin. BPV-1 E7 transformation function correlates with its binding to a cellular target p-600, which is a shared transformation pathway of HPV-16 E7.

The BPV-4 E7 can also cooperate with E8 in inducing cellular transformation, and the activation of the ras oncogene is responsible for morphological changes of primary bovine fibroblasts (PalF). Like other E7 PV, BPV-4 E7 has a p105Rb-binding domain whose mutation may reduce or abolish its transforming activity.

BPV E7 localizes in the cytoplasm and nucleoli of basal and lower-spinous epithelial cells. It may also be found in mesenchymal neoplastic cells.

### L1 and L2

The BPV late proteins L1 and L2 are expressed into the more differentiated epithelial cells. The former mediates virus interaction with cellular receptor, the latter induces virion assembly by binding to viral DNA.

Infection by delta-PVs leads to transformation of subepithelial fibroblasts followed by epithelial acanthosis and then papillomatosis, while infection by Xi-PVs induces transformation only of the epithelial component. Virus replication can take place only in keratinocytes undergoing terminal differentiation to squamous epithelium, so it is seen only in the epithelial component of the tumors and only at certain stages of its development. Virus replication has never been found in fibroblasts where the BPV genome is present in a nonintegrated episomal form, although *BPV* viral gene expression has been recently found in tumors of mesenchymal origin such those arising from blood vessels (hemangioma and ► *Hemangiosarcoma*), suggesting a role of the virus even in neoplastic transformation other than epithelial.

### BPV and Papillomas

Papillomas and fibropapillomas may occur in different organs in cattle, and different BPV genotypes are found. BPV-1, BPV-5, and BPV-6 are associated to papillomas of the teats and udders in cows. This can become a great economic problem once the papillomas spread around the primary tumors and the cows cannot be milked, veals are unable to suckle properly, and the site may become infected inducing mastitis. Occasionally, the herds should be culled if the papillomatosis progress.

Epithelia of both prepuce and penis may be infected by BPV-1, resulting in fibropapillomas. The tumors can spread along the perineum and even up toward the back; they can become necrotic and cause loss of reproductive functions.

BPV-4 induces fibropapillomas of the upper gastrointestinal (GI) tract. All sites from the tongue to stomach can be affected. Healthy cattles normally recover from papillomatosis in approximately 1 year time, but if the animals are not able to reject the tumors, they are at high risk to develop cancer such as squamous cell carcinoma.

Normally, these benign lesions (papillomas and fibropapillomas) regress, but some animals may even die due to widespread cutaneous or mucosal papillomatosis if they are not able to reject the infection. Progression of benign persistent lesions to cancer occur once cofactors synergize with the virus.

### BPV and Cancer

One of the major environmental cofactor involved in BPV-associated carcinogenesis is the ► *bracken fern* (genus *Pteridium*), the only higher plant proven to cause cancer naturally in animals.

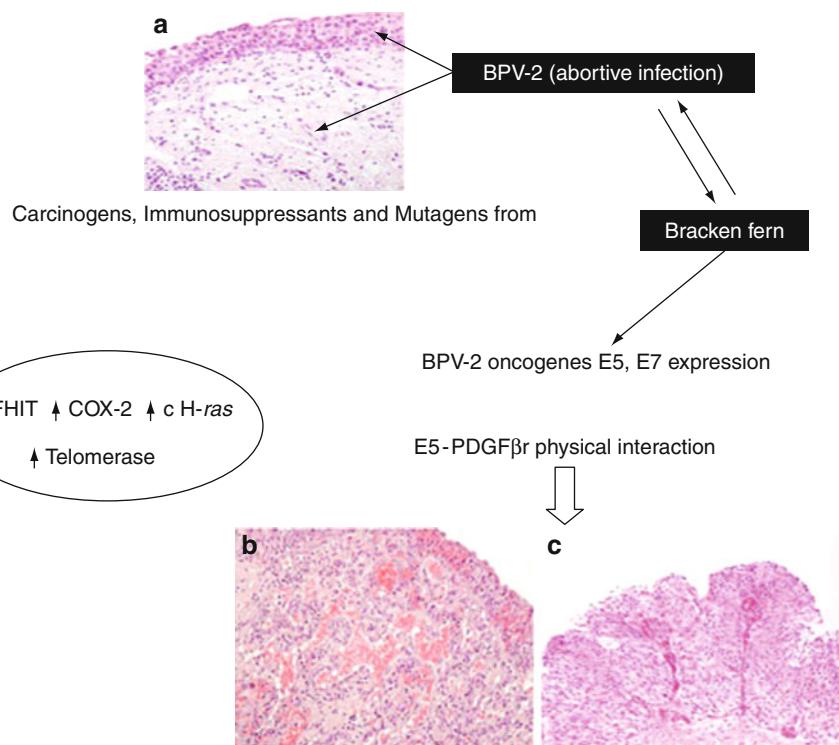
Bracken-eating animals may develop cancer since the plant contains immunosuppressants as well as a number of mutagens and oncogenic principles such as ► *ptaquiloside*. Bracken-fed cows become chronically immunosuppressed and the latent BPV is activated. Full malignant transformation depends on others mutagens that are believed to trigger *BPV* gene expression leading to initiation and development of cancer.

Additionally, bracken-eating animals develop a clinical syndrome known as chronic enzootic hematuria and chromosomal abnormalities.

Field cases of urinary bladder and GI cancers in cattle occur wherever the plant is spread. The disease

**Bovine Papillomavirus.**

**Fig. 1** Schematic representation of the multistep carcinogenesis of bovine urinary bladder tumors. Histological sections of (a) normal bladder mucosa; (b) hemangiosarcoma; (c) papillary urothelial carcinoma



is known to occur in continental Europe, Azores Islands, in some regions of Kenya, Brasil, New Zealand, India, and in China. Human exposure to bracken fern directly or indirectly through milk from bracken-eating cattle has been linked to human GI cancer.

Cows affected by BPV-4-associated papillomas of the upper GI tract and naturally exposed to bracken fern are at high risk of developing carcinoma. The fern induces immunosuppression and the fibropapillomas spread, additionally the mutagens from the plant such as ► **quercetin** and ptaquiloside act synergistically with the virus in the carcinogenic process. The BPV-4 E7 oncprotein cooperates with quercetin for neoplastic transformation, in so doing the ras oncogene is activated, the p53 is mutated, and the number of the cellular receptors for epidermal growth factors is increased. From a comparative point of view, it is worth noting that some human GI cancer may have the same etiology: papillomavirus and bracken suggesting that similar molecular mechanisms underlying bovine cancer may even occur in humans.

At the same time, cows suffering from chronic enzootic hematuria may develop urinary ► **bladder**

**cancer**. The cancer are of both epithelial and mesenchymal origin with ► **hemangiosarcomas** being the most frequent histotype. In both cases, the BPV-2 is involved testifying that the virus is not a pure epitheliotropic agent in its natural host. The BPV-2 infects the urinary bladder mucosa inducing an abortive and latent infection with no production of virions. The exposure to immunosuppressants, mutagenic, and carcinogenic principles from bracken triggers viral gene expression leading to cell transformation. In both cancers of epithelial and mesenchymal origin, the BPV-2 E5 oncprotein is expressed and is in complex with the activated form of the PDGF $\beta$  receptor. Additionally, in ► **urothelial cancers**, the ► **telomerase** activity is upregulated, expression of ras and ► **cyclooxygenase-2 (COX-2)** is increased and, as already observed in HPV-associated ► **Cervical cancer**, the ► **fragile sites** are disrupted and the expression of the tumor suppressor fragile histidine tetrads (► **FHIT**) is downregulated (Fig. 1).

**BPV and Equine Sarcoïds**

The ► **sarcoïds** are benign tumors of fibroblastic skin origin affecting horses, mules, and donkeys. They are

locally invasive often occurring at sites of previous injury or scarring. Tumors can exist as single or multiple lesions in different forms. Clinically, five different types of sarcoids can be distinguished: Occult sarcoid is an hairless circular area of the skin; Verrucous: tumors with wart-like appearance; Fibroblastic sarcoids present as a fleshy mass; Nodular sarcoids consist of firm masses lying under the skin and mixed sarcoid show a combination of features of verrucous, fibroblastic, and nodular types.

It is the most common dermatological neoplasm reported in horses. The most common sites of appearance is the skin of the head, ventral abdomen, legs, and the paragenital region.

Despite the failure to isolate any papillomavirus from the sarcoids, a large body of evidence strongly supports the hypothesis that BPV is the etiological agent of this tumor.

Both BPV-1 and BPV-2 have been detected in sarcoid tumors with the BPV-1 being the predominant type. The BPV exists as episomally, and its major oncoprotein E5 is expressed, thus suggesting the viral genes are expressed.

Equine sarcoids is a biologically attractive tumor since it is the only known case of natural cross-species PV infection. Moreover, while BPV infection in cattle produces benign lesions that may regress, the sarcoids are nonpermissive for virus production, locally aggressive and nonregressing.

Cell cycle regulatory proteins are involved in the pathogenesis of equine sarcoids. P53 is stabilized in sarcoid cells being expressed in the nuclei as well as in perinuclear region; however, its transactivation function is abrogated. Low levels of cell proliferation are characteristic of sarcoids with no overexpression neither of cyclin A, p27<sup>kip1</sup> nor of CDK-2.

The loss of p53 function and the low levels of cell proliferation indicate that sarcoid cellular and molecular pathology may not be associated with abnormal cell cycle control mechanisms.

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## Bowen Disease

### Definition

A red patch on the mucosa that is not attributable to any obvious cause. Generally, these lesions have a well-defined border and a soft, velvet-like appearance. Their atrophic nature contributes to the red coloration, as underlying vasculature is more prominent. Around 90% show signs of severe dysplasia or carcinoma-in-situ and may progress to invasive squamous cell carcinoma.

► [Squamous Cell Carcinoma](#)

## Bowman-Birk Inhibitor

### Synonyms

### BBI

### Definition

Serine protease inhibitor consisting of a single chain of 71 amino acid residues cross-linked by seven pairs of disulfide bonds and a well-characterized ability to inhibit trypsin and chymotrypsin. It has been shown to be capable of preventing or suppressing carcinogenic processes in a wide variety of in vitro and in vivo animal model systems.

► [Lunasin](#)

## Boyden Chambers

### Definition

A hollow plastic chamber sealed at one end with a porous membrane and suspended in a well containing chemoattractants. Cells are placed inside the chamber and allowed to migrate through the pores to the other side of the membrane.

## 53BP

### Definition

p53 binding protein 1, a checkpoint molecule that acts as a mediator for transducing DNA damage signals, especially following detection of DNA double-stranded breaks.

- ▶ [BRIT1 Gene](#)
- ▶ [p53 family](#)

## BPH

### Definition

- ▶ [Benign Prostate Hyperplasia](#).

## BR 27-29

- ▶ [Serum Biomarkers](#)

## Brachytherapy

Caroline L. Holloway<sup>1</sup> and Akila N. Viswanathan<sup>2</sup>

<sup>1</sup>BC Cancer Agency, Vancouver Island Centre, Victoria, BC, Canada

<sup>2</sup>Brigham and Women's/Dana-Farber Cancer Center, Boston, MA, USA

### Synonyms

[Endocurietherapy](#); [Radioactive seed therapy](#)

### Definition

Brachytherapy treatments deliver radiation dose using radioactive isotopes placed via applicator devices or catheters directly into tumors or into cavities in close approximation to the tumor.

### Characteristics

Radiation therapy is the treatment of cancer with radiation. Radiation targets the DNA in cells and causes DNA strand breaks. Normal cells have the ability to repair the DNA damage, whereas cancer cells lack such repair mechanisms.

Brachytherapy is one method of delivering radiation. The word “brachy” is derived from Greek meaning “short.” The radiation from the radioactive isotopes penetrates a short distance, allowing for conformity to a target volume or tumor while sparing the normal structures in the vicinity. The ▶ [dose fall-off](#) for brachytherapy sources follows the inverse square law, in that the distance traveled by the radiation is inversely proportional to the square of the radius of distance ( $d = 1/r^2$ ).

Historically, the isotopes used for brachytherapy were radium, radon and its derivatives, and gold. In modern times, isotopes must be nongaseous, have effective energies for treatments, be able to be encapsulated in a size that is clinically useful, and have a ▶ [half-life](#) either suitable for permanent implants or temporary implants ([Table 1](#)).

### Dose Rate

Brachytherapy treatments can utilize different ▶ [dose rates](#) to treat cancer. The definitions for dose rates were defined by the International Commission on Radiation Units and Measurements Report #38. Low dose rate (LDR) is defined as a range of 0.4–2 Gy edges/h. Medium dose rate (MDR) is a range of 2–12 Gy/h, and high dose rate (HDR) is defined as >12 Gy/h. VLDR (very low dose rate) radiation is used in permanent radioactive seed implants, at a dose rate of less than 40 cGy/h. Temporary implants are placed into the tumor/adjacent tissues in order to deliver LDR, MDR, or HDR treatments. VLDR implants typically reside permanently in the tissue implanted, but decay over the course of a few months. In the delivery of LDR or MDR radiation, the temporary implant stays in

**Brachytherapy. Table 1** Characteristics of some commonly used radioisotopes in the United States

Isotope	Half-life	Energy (MeV)
<sup>137</sup> Cesium	30 years	0.66
<sup>192</sup> Iridium	74 days	0.29–0.6
<sup>125</sup> Iodine	60 days	0.028
<sup>103</sup> Palladium	17 days	0.023

place over several hours, whereas HDR treatments usually last only a few minutes.

LDR techniques involve the static placement of radiation isotopes within the applicators for a period of time. <sup>137</sup>Cesium (<sup>137</sup>Cs) for gynecologic brachytherapy or <sup>192</sup>Iridium (<sup>192</sup>Ir) for gynecologic cancers or sarcomas are most commonly used. The radiation is either manually afterloaded by a physician or can be remotely afterloaded if a cesium selectron afterloader (for gynecologic brachytherapy) is available. HDR treatments involve a single <sup>192</sup>Ir source fixed to a wire that is guided remotely by a computer. The HDR afterloader attaches to individual applicators by transfer tubes. Computer programming determines the position of the radiation isotope within the applicator and calculates a radiation ► **isodose** curve that may be manipulated by altering the dwell times. ► **Dwell positions** are defined along the applicators every 2.5–10 mm, and the isotope remains at designated dwell positions for a preset time as determined by the optimized plan. LDR radiation may have a ► **radiobiological** advantage over HDR radiation, as the normal tissue is more likely to be able to repair sublethal damage. Additionally, the continuous dose may prevent repopulation of the tumor cells, and the longer period of time that the cells are exposed to radiation allows the cell cycle to move through radioresistant and radiosensitive phases. HDR radiation may lead to an increase in normal tissue toxicity if the total dose delivered compared to LDR is not decreased. It is important to fractionate the HDR radiation sufficiently and deliver as small a fraction size as feasible depending on the tissue treated, the indications for treatments, and the amount of normal tissue in proximity to the source. In ► **Cervical cancer** brachytherapy, packing the vagina can reduce the amount of normal tissue exposed to radiation.

Pulsed dose rate (PDR) brachytherapy uses an HDR afterloader and source but attempts to mimic the

radiobiologic effect of LDR by giving a large number of very small fractions over a longer period of time than HDR.

### Dose Calculations

Historically, the dose delivered to a treatment volume was hand-calculated, based on one of three methods of implantation. The Paris and Quimby systems place parallel sources with uniform spacing and source activity to give a higher central dose compared with the periphery. The Paterson–Parker method utilizes higher peripheral radioactivity compared with the centers resulting in increased ► **dose homogeneity** throughout the implant. These methods have been replaced in several radiation oncology clinics by computer programs that utilize information gathered from imaging techniques such as CT scans to define the target volume and identify the implant geometry within that volume to calculate the dose.

### Implantation Techniques

The placement of the radiation source in relation to the treatment volume is the most important determinant in the effectiveness of brachytherapy. Therefore, the techniques used depend on the location of the tissue being targeted.

Surface applicators involve sculpting a radiotherapy delivery system on or around the target surface area. A superficial dose may be delivered to lesions of the skin or intraoperatively to exposed tumor beds.

Intracavitary radiation utilizes orifices within the human body to introduce applicators in close proximity to the tumor. Common examples of intracavitary radiation include gynecologic malignancies, in which the vagina, cervical os, and uterine cavity allow for the relatively easy placement of applicators. Other intracavitary treatments include the bronchus, esophagus, and rectum.

Interstitial radiation entails passing catheters through normal tissue to reach the target volume or placing tubes within a surgical bed at the time of operation.

### Clinical Applications of Brachytherapy

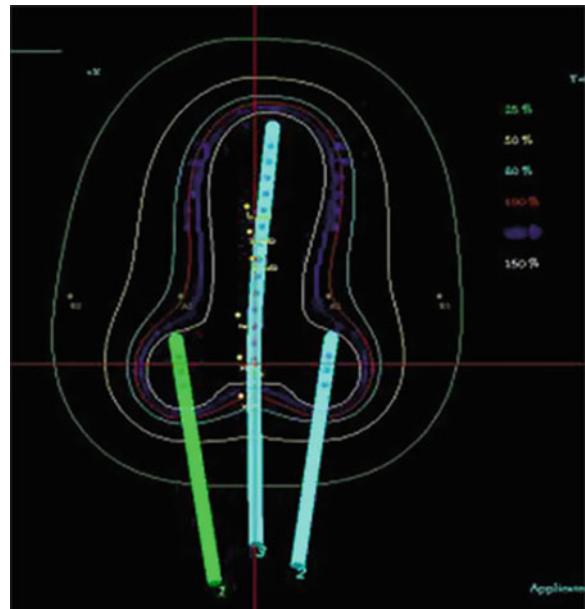
Brachytherapy may be administered alone or in combination with external beam radiation, chemotherapy, or surgery to provide either cure or palliation for the patient. The most common uses of brachytherapy are discussed below.



**Brachytherapy.** **Fig. 1** A high dose rate vaginal cylinder is inserted into the vagina to treat the vaginal surface for patients who have had a hysterectomy for uterine or cervical cancer. The applicator is attached to a brachytherapy board for stabilization



**Brachytherapy.** **Fig. 2** Low dose rate Fletcher–Suit–Delclos tandem and ovoid applicator will be loaded with  $^{137}\text{Cs}$ . The central tandem is inserted into the uterus. The ovoids may have plastic caps placed over them in order to fill the vaginal fornices. A flange rests outside the external os of the cervix. The apparatus is held in place by vaginal packing



**Brachytherapy.** **Fig. 3** High dose rate tandem and ovoid isodose curve demonstrates the 100% isodose line optimized to point A, a point 2 cm above and lateral to the cervical os

### Gynecologic Malignancies

The most common gynecologic malignancy treated with brachytherapy in the United States is ► [endometrial cancer](#). Intracavitary radiation targets the vaginal vault in women thought to be at high risk of local recurrence to the vagina following definitive surgery. This treatment involves the insertion of a cylinder into the vagina (Fig. 1). LDR or HDR radiation may be used. The dose and fractionation of the radiation depend on both the dose rate and the patient's history of prior external beam radiation therapy. The dose may be prescribed at either the surface of the applicator or at a depth, typically 5 mm, from the applicator.

Cervical cancer is treated using a combination of external beam radiation with or without chemotherapy and brachytherapy, commonly referred to as a tandem and ovoid application. A central uterine tandem is placed through the cervical os into the uterine cavity. Vaginal ovoids or a vaginal ring or cylinder are secured to the central tandem (Fig. 2). Historically, cervical cancer brachytherapy was administered using LDR radiation, most commonly using tandem and ovoids placed twice with 1 week between treatments. In most centers, plain films assess the location of normal tissue structures; however, several radiation oncology clinics have acquired CT imaging capability,

allowing for 3D imaging of the normal tissues and more accurate dose calculation. In more recent times, several centers have incorporated HDR radiation into the management of cervical cancer. HDR tandem and ovoid dose is delivered in minutes, and most commonly requires four or five separate insertions, with each treatment lasting several minutes. The HDR isodose curve approximates a standard LDR loading (Fig. 3).

Vulvar and vaginal cancers are rare, but their treatment may involve interstitial or intracavitary radiation after external beam radiation.

### Prostate Cancer

Brachytherapy in ► [prostate cancer](#) may be the sole treatment for low-risk disease or in combination with external beam radiation as a form of dose escalation. VLDR brachytherapy places permanent radioactive seeds of either  $^{125}\text{I}$  or  $^{103}\text{Pd}$  into the prostate through the perineal skin, under image guidance and using catheters. The seeds remain permanently within the prostate and deliver a low dose of radiation continuously until they have decayed. HDR brachytherapy for prostate cancer is currently being investigated in research protocols.

### Other

Other cancers that can be treated with brachytherapy as part of combined care include head and neck cancers, including nasopharynx and tongue, breast cancers, sarcomas, thoracics, and some gastrointestinal cancers.

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## Bracken Fern

### Definition

A worldwide diffuse plant belonging to *Pteridium* genus known to cause cancer naturally in animals. Bracken fern eating is also related to human cancer.

## Bradykinin

### Definition

Bradykinin is an active peptide of the kinin protein group. It consists of nine amino acid residues and is a potent vasodilator.

- [Kallikreins](#)

## BRAF

### Synonyms

[BRAF1](#)

## B-RAF

- [B-Raf Signaling](#)

## B-Raf Signaling

Tilmann Brummer

Cancer Research Program, Garvan Institute of Medical Research, Sydney, NSW, Australia

### Synonyms

[BRAF1](#); [B-raf-1](#); [c-Rmil](#); [EC 2.7.11.1](#); [MGC126806](#); [MGC138284](#); [p94](#); [RAFB1](#); [v-raf murine sarcoma viral oncogene homolog B1](#)

### Definition

B-Raf signaling comprises the activation of the proto-oncogene product B-Raf and its downstream effectors and represents a key regulatory step in the activation of the canonical ► [MAP kinase](#) pathway by various extracellular stimuli and oncogene products such as ► [RAS](#) and activated receptor tyrosine kinases like NTRK and

► **RET**. Aberrant B-Raf activity as a result of somatic mutations is observed in 8% of human cancers.

## Characteristics

### Physiological Aspects of B-Raf Signaling

B-Raf is a member of the ► **Raf kinase** family and represents an important component of the Ras/Raf/MEK/ERK MAP kinase signal transduction pathway, which plays a pivotal role in growth control and differentiation. Dysregulation of this pathway is observed in about 30% of human tumors and represents an established mechanism for tumorigenesis. In their role as gatekeepers of this pathway, Raf-kinases appear as attractive targets for therapeutic intervention. The Raf-family contains three genes in vertebrates, A-Raf, B-Raf, and Raf-1, as well as D-Raf and LIN-45 in *Drosophila* und *Caenorhabditis*, respectively. While the *RAF1* gene displays a ubiquitous and prominent expression pattern, B-Raf is predominantly expressed in neuro-ectoderm-derived tissues, placenta, the hematopoietic system, and the testis. However, gene-targeting experiments in mice and ► **DT40 B cells** revealed that B-Raf represents the major ERK activator, even if it is expressed at barely detectable levels, whereas Raf-1 serves as an accessory ERK activator. Among the three mammalian isoforms, B-Raf displays the highest affinity toward its substrate MEK and has the highest activities in biological and ► **in vitro kinase assays**. In many cell types, B-Raf plays a nonredundant role in the maintenance of ERK signaling induced by various extracellular signals and thereby regulates directly, or in concert with other signaling pathways, the expression of important target gene products such as growth factors and cytokines. The importance of B-Raf for the efficient expression of ERK-regulated target gene products is most likely explained by the fact that ERK activation is not only required for the induction of ► **immediate early genes** transcription, but also for the stabilization of the resulting proteins by phosphorylation through sustained ERK signaling. The correlation between B-Raf expression and sustained ERK signaling has been implicated in various physiological processes such as lymphocyte activation, myelopoiesis, angiogenesis, development of extra-embryonic tissues, as well as for the growth-factor-mediated survival of neurons and their effector

functions. The discovery of germ-line mutations with mostly slight to moderate gain-of-function character in the SOS, KRAS, HRAS, *SHP2/PTPN11*, *BRAF*, and *MEK1/2* genes in patients suffering from the various ► **neuro-cardio-facial-cutaneous syndromes** illustrates that tight control of this pathway upstream or at the level of the B-Raf/MEK interface is key to the normal development and homeostasis of many organs.

### B-Raf Signaling and Tumor Development

The high biological relevance of B-Raf is also reflected in the discovery that somatic alterations of the *BRAF* gene occur in about 8% of all human tumors with particular high frequencies in ► **melanoma** (70%), ovarian (30%), thyroid (27%), colorectal, and biliary tract carcinoma (both 15%). Many of the resulting mutant B-Raf proteins cause chronic ERK activation and transform a variety of cell types in vitro. Furthermore, the B-Raf<sup>V600E</sup> oncoprotein, which is the most frequently found mutant and occurs in 7% of human tumors, induces neoplasms in transgenic mice and zebrafish. Apart from their established role as ERK activators, B-Raf<sup>V600E</sup> and other oncogenic mutants have been shown to activate the ► **NF-κB** pathway, although the exact mechanism for this oncologically relevant aspect of B-Raf signaling remains elusive.

Dysregulated B-Raf signaling in the absence of any *BRAF* mutations has been also implicated in various neoplastic diseases. For example, hyperactivation of wild-type B-Raf has been observed in ► **Polycystic Kidney Disease**. Similarly, overexpression and deregulation of B-Raf have been implicated in ► **Kaposi Sarcoma**. Likewise, amplification and/or overexpression of the *BRAF* gene were described as alternative events to *BRAF* mutations in melanoma. Furthermore, B-Raf serves as an important signal transducer of upstream oncogene products such as RAS or activated receptor tyrosine kinases (RTKs) such as ► **RET**, NTRK, ► **Epidermal Growth Factor Receptor** family members or the ► **Kit/Stem cell factor receptor**. In many cell types where the chronic activation of the RAF/MEK/ERK effector arm by these oncoproteins represents a major mechanism of cellular transformation, a mutual exclusivity is observed between mutations in *BRAF* or genes encoding its upstream activators. For example, gain-of-function mutations in either the ► **RET**, NTRK, ► **RAS**, or *BRAF* proto-oncogenes account for 70% of papillary

thyroid carcinoma and provoke similar transformed phenotypes indicating that the activation of B-Raf effectors such as ERK and NF- $\kappa$ B is a major driving force in thyrocyte transformation. Similar constellations have been described for *RAS* and *BRAF* in melanoma, colorectal, and ovarian carcinoma. However, Ras and B-Raf transformed cells differ in their responsiveness to MEK-inhibitors showing that both oncoproteins, while having a large group of effectors in common, also trigger ► **oncogene addiction** through distinct mechanisms. Oncogenic B-Raf not only mimics growth factor signaling, but also induces a variety of auto- and paracrine acting growth factors itself, e.g., Heparin-Binding Epidermal Growth factor (EGF)-Like Growth Factor, chemokines, and pro-inflammatory and angiogenic cytokines like ► **Vascular Endothelial Growth Factor A**. Apart from tumor initiation, tissue culture experiments suggest that oncogenic B-Raf also contributes to tumor progression by inducing two additional key events in metastasis: the ► **epithelial to mesenchymal transition** of the oncogene-bearing cell and the ► **angiogenic switch** in its environment through the aforementioned growth factors and cytokines.

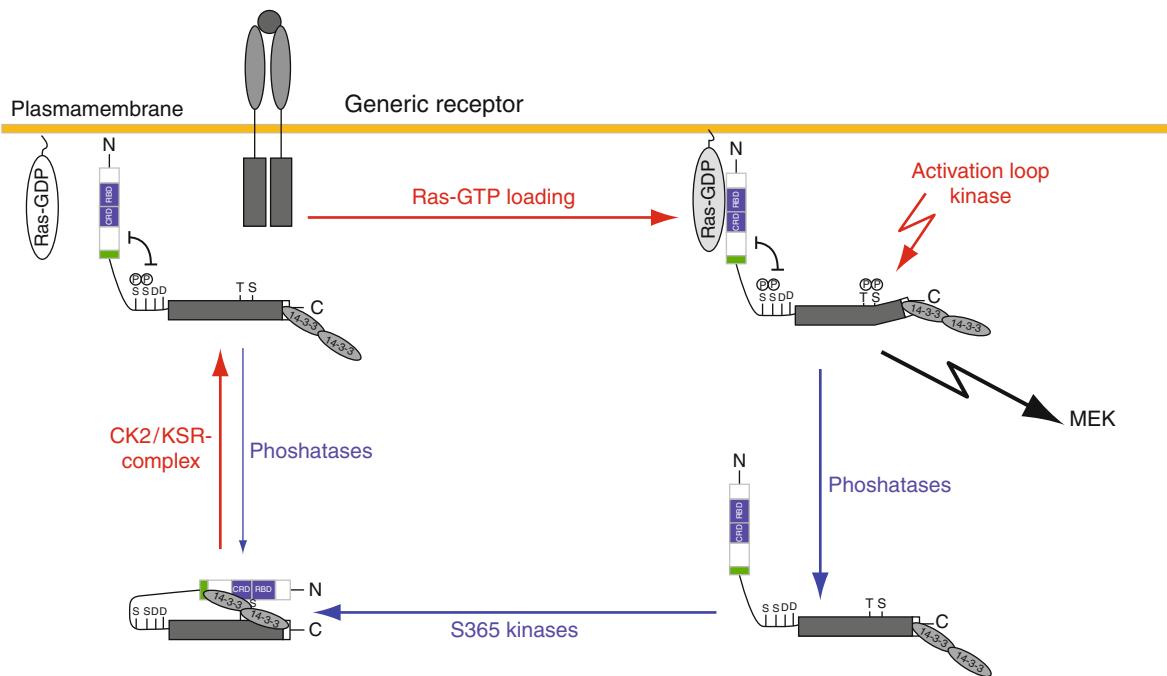
Aberrant B-Raf activity does not necessarily result in tumorigenesis unless profound changes in the regulatory network underlying cell cycle control have occurred. Through the ERK and NF- $\kappa$ B pathways, oncogenic B-Raf stimulates not only the production of positive cell cycle regulators such as cyclin D1, but also induces negative regulators such as cyclin-dependent kinase inhibitors like p16<sup>INK4A</sup>. Consequently, chronic B-Raf/ERK signaling ultimately results in cell cycle arrest and cellular ► **senescence**. For example, melanocytes with an intact cell cycle control program become growth arrested by chronic B-Raf signaling and develop only into benign nevi. However, if important negative cell cycle regulators and tumor suppressor genes like ► **INK4A** or ► **p53** are lost, oncogenic B-Raf signaling will trigger cell cycle progression and drive tumor development.

### B-Raf Structure and Regulation

Like many other protein kinases, B-Raf is part of a large multi-protein complex or ► **signalosome** in which the individual components regulate B-Raf conformation and activity through various protein–protein interactions in a dynamic spatiotemporal manner. Key to the understanding of the (dys-)regulation of B-Raf is

the knowledge of its modular structure. B-Raf shares three highly conserved regions (CR) with the other members of the Raf-family (Fig. 1): the N-terminal CR1 contains the Ras-GTP binding domain, which initiates the interaction with activated Ras, and the cysteine-rich domain involved in the stabilization of Ras/Raf interaction. The CR2 contains a negative regulatory serine residue (S365) that serves as a binding site for ► **14-3-3 proteins** upon phosphorylation by ► **Akt** and other kinases. The catalytic domain (CR3) harbors phosphorylation sites for Raf-regulating enzymes within two segments, the N-region and the ► **activation loop**. B-Raf carries a second 14-3-3 binding motif around S729 at the C-terminal end of the CR3 domain, which is essential to couple B-Raf to its downstream effector MEK.

Similar to the better-characterized Raf-1 isoform, B-Raf is activated by its interaction with small GTPases of the RAS family. Although no crystal structure for any of the full-length Raf-proteins is available, various experimental approaches imply that Raf activation is accompanied by a transition from a closed, auto-inhibited into an open, active conformation in which the N-terminal lobe consisting of the CR1 and CR2 domains is displaced from the C-terminal lobe encompassing the CR3 (Fig. 1). The degree of auto-inhibition of B-Raf is influenced by the inclusion/exclusion of amino acid sequences within the linker region between N- and C-terminal lobe, which are encoded by alternatively spliced, tissue-specific exons and various phosphorylation events. Among the latter, two phosphorylation sites within the CR3, the N-region and the activation loop, are of particular importance (Fig. 1). The introduction of negative charges into the N-region, which is located at the N-terminal end of the CR3 domain, plays a critical, multifaceted role in Raf activation. While the N-region of Raf-1 is charged through phosphorylation of its S<sup>338</sup>SYY<sup>341</sup>-sequence in a RAS-dependent manner by Ser/Thr- and Tyr-kinases, the equivalent serine residues within the N-region of B-Raf (S<sup>446</sup>SDD<sup>449</sup>-motif) are phosphorylated in a constitutive and RAS-independent manner (Fig. 1). Although structural data are still missing, several lines of evidence propose that N-region phosphorylation primes B-Raf for activation at the membrane by reducing the affinity between N-terminal and C-terminal lobe. The significance of the aspartate residues, which are the functional equivalents of the phospho-tyrosine residues in the SSYY-sequence of



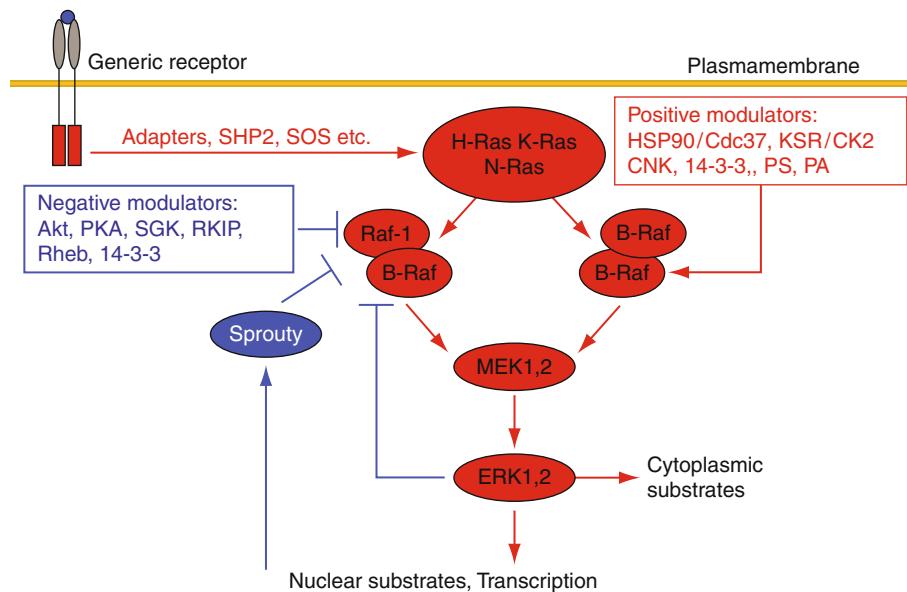
**B-Raf Signaling. Fig. 1** Model of the B-Raf activation cycle. B-Raf contains three conserved regions: CR1 (blue) consisting of the Ras-binding domain (RBD) and the cysteine-rich domain (CRD), CR2 (green), and the kinase domain CR3 (blue). Inactive B-Raf resides in the cytoplasm in a closed, inactive conformation stabilized by 14-3-3. Interaction of B-Raf with a complex consisting of CK2 and the scaffold protein KSR results in phosphorylation of S446 (and perhaps S447) in the N-region thereby transferring B-Raf into a more open

Raf-1, is twofold: firstly the negative charge of the aspartate residues is supposed to prime B-Raf for N-region phosphorylation by casein kinase 2 (CK2). Secondly, the D448 residue stabilizes the conformation of activated B-Raf through the formation of a salt-bridge with R506 within the  $\alpha$ C-helix of the CR3. The important role of the SSDD-sequence is highlighted by the fact that mutation of the serine and/or aspartate residues results in drastic reduction of the basal in vitro kinase and biological activities. Furthermore, it has been suggested that the different mechanisms that supply the N-region of B-Raf and Raf-1 with negative charges account not only for the aforementioned isoform-specific differences in the enzymatic, biological and transforming activities, but also predispose the *BRAF* gene for oncogenic hits. However, while tissue culture experiments demonstrated that the rare B-Raf<sup>E586K</sup> mutant indeed requires an intact SSDD sequence to induce MEK/ERK activation and oncogenic transformation, the biological activity of the most

conformation. The constitutive basal phosphorylation of B-Raf at S446 suggests that a large fraction of B-Raf resides in this primed state. Interaction with activated Ras (Ras-GTP) leads to phosphorylation of T599 and S602 within the activation loop, which induces a conformational change within the CR3 and renders B-Raf active. B-Raf is supposedly inactivated by phosphatases, re-phosphorylation of the inhibitory residue S365 and transition into the closed conformation

frequently found mutant, B-Raf<sup>V600E</sup>, is not affected by N-region neutralization, at least not in experimental approaches involving the ectopic expression of this oncoprotein.

The interaction with Ras recruits B-Raf to the plasma membrane followed by the phosphorylation of the activation loop residues T599 and S602 (Fig. 2). This phosphorylation event presumably leads to the dislocation of the activation loop relative to the overall catalytic domain thereby resulting in full B-Raf activity. The importance of the activation segment phosphorylation is established by the fact that mutation of these phosphorylation sites to alanine residues renders B-Raf resistant to extracellular signals and even to strong activators like oncogenic Ras<sup>G12V</sup>. Conversely, mutations that mimic the phosphorylation-induced dislocation of the activation segment, such as B-Raf<sup>V600E</sup>, lock B-Raf in an active conformation and confer high constitutive enzymatic and transforming activities to B-Raf independent of RAS.



**B-Raf Signaling.** **Fig. 2** Modulation of B-Raf signaling. Extracellular signals received by various receptor classes trigger the activation of Ras-GTPases by stimulating their loading with GTP. Activated Ras not only recruits B-Raf and promotes its phosphorylation by unknown activation loop kinases, but also stimulates its homo- and heterodimerization. The activity

of B-Raf (and Raf-1) is fine-tuned by a multitude of positive and negative modulators. The longevity of B-Raf/Raf-1 heterodimers is determined by a rapid negative feedback loop from ERK. In a delayed negative feedback loop, sustained B-Raf/ERK signaling also induces the transcription of Sprouty-2, a negative regulator of B-Raf

Consequently, these activation loop mutations are frequently found as somatic alterations of the *BRAF* gene in human tumors.

Intracellular B-Raf activity is also regulated by the phosphorylation-dependent recruitment of ► **14-3-3 proteins** in an opposing manner (Fig. 1). Binding of 14-3-3 proteins to phospho-S729 at the C-Terminus of B-Raf is essential to couple B-Raf to the MEK/ERK pathway. In contrast, phosphorylation of S365 within the CR2 by Protein kinases A, Akt, or serum-and-glucocorticoid-induced kinase (SGK) generates a second binding site for 14-3-3 proteins, which negatively regulates B-Raf activity, most likely through the stabilization of the auto-inhibited conformation through the simultaneous binding of the 14-3-3 dimer to S365 and S729 (Figs. 1 and 2). 14-3-3 proteins are also involved in the RAS-stimulated formation of homodimers of B-Raf and its heterodimerization with Raf-1 (Fig. 2). Indeed, B-Raf/Raf-1 heterodimers represent the most potent form of Raf activity within the cell. Activated ERK limits the longevity of these dimers by targeting an evolutionary conserved phosphorylation motif at the C-terminus of B-Raf (Fig. 2). In addition, B-Raf activity is modulated by other

components of the signalosome such as the ► **HSP90/Cdc37** chaperone complex and ► **scaffold proteins** like kinase-suppressor-of-Ras (KSR) and connector-and-enhancer-of-KSR (CNK). Membrane phospholipids such as phosphatidylserine (PS) and phosphatidic acid (PA) are also discussed as important regulators of Raf activation. B-Raf is also negatively regulated by Sprouty-2 and Raf-kinase-inhibitory protein (RKIP), two proteins, which are both often downregulated in human cancer raising the possibility that their epigenetic silencing represents an alternative mechanism to gain-of-function mutations in genes linked to the Ras/Raf/MEK/ERK pathway in human cancer. Similarly, B-Raf<sup>V600E</sup> and other activation loop mutants are incapable of interacting with Sprouty demonstrating that the V600E mutation not only uncouples B-Raf from positive (interaction with Ras, N-region, and activation loop phosphorylation) but also negative regulatory mechanisms.

### B-Raf as a Therapeutic Target

The growing importance of B-Raf in tumor biology has fostered the development of therapeutic strategies aiming at either reducing the expression or activity of

B-Raf or its downstream effector MEK. Various MEK inhibitors are currently in clinical trials and experiments in tissue culture and xenograft models indicate that tumor cells harboring the *BRAF*<sup>V600E</sup> mutation, but not those with RAS mutations, are highly “addicted” to ERK activity and are consequently particularly sensitive toward MEK inhibition. Similar results have been obtained in experiments in which the expression of B-Raf<sup>V600E</sup> but not of wild-type B-Raf was specifically abolished by allele-specific ► RNA interference illustrating the importance of this oncoprotein for the maintenance of the tumor phenotype. Recent strategies also target B-Raf directly. The orally available multi-kinase inhibitor BAY 43–9006 (also known as Sorafenib or Nexavar), which was originally designed to block Raf-1, inhibits B-Raf as well as several receptor tyrosine kinases (RTKs) involved in neo-angiogenesis and tumor progression. However, it is assumed that the inhibition of the latter kinase class or the simultaneous inhibition of several kinases, rather than the inhibition of Raf itself, is responsible for the antitumor activity of BAY 43–9006, in particular in renal cell carcinoma. A third approach employs the requirement of the HSP90/Cdc37 chaperone complex for the stability of B-Raf. In this regard, the HSP90 inhibitor ► Geldanamycin was shown to trigger the degradation of B-Raf by disrupting its association with the HSP90/Cdc37 chaperone complex. The stability of most activated B-Raf mutants, including B-Raf<sup>V600E</sup>, appears to be more reliant on the chaperone complex than those of wild-type B-Raf suggesting that tumor cells driven by *BRAF* mutations will be particularly sensitive to Geldanamycin.

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## B-Raf Somatic Alterations

Tilman Brummer

Cancer Research Program, Garvan Institute of Medical Research, Sydney, NSW, Australia

B

### Definition

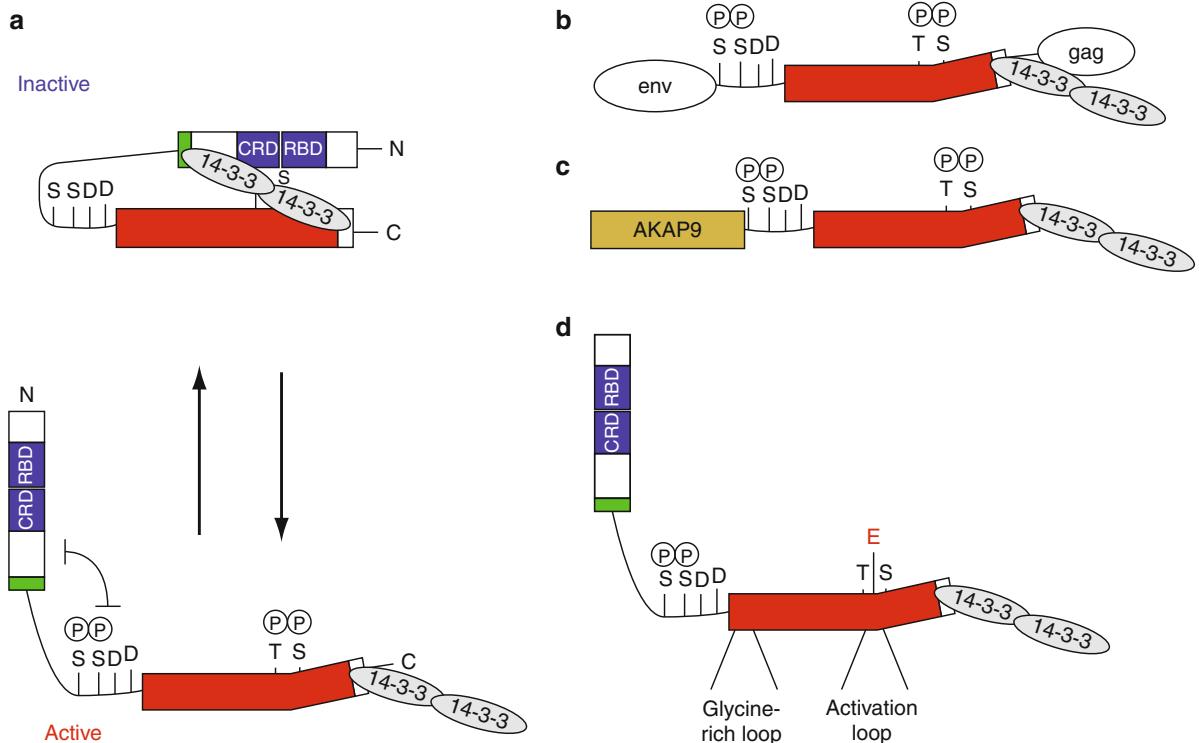
Somatic alterations of the *BRAF* gene in cancer, either caused by point mutation or genomic rearrangement of the *BRAF* proto-oncogene.

### Characteristics

The Ser/Thr-kinase B-Raf, a product of the human *BRAF* proto-oncogene, plays a pivotal role in the activation of the classical ► ERK/MAP kinase pathway that is involved in the control of proliferation and differentiation of various tissues. Consequently, alterations of the expression level or the activity of B-Raf are associated with malignancies like ► polycystic kidney disease and various cancers. Proto-oncogenes can be converted into oncogenes by point mutations, amplifications, genomic rearrangement, e.g., translocation or inversion, or by retroviral transduction. Interestingly, all four mechanisms of oncogene activation have been documented for the *BRAF* genes in human and/or animal tumors.

### History of the *BRAF* Proto-oncogene

The discovery of the *raf*-oncogenes originates back to the isolation of the chicken Mill Hill 2 (MH2) retrovirus by Begg in 1927. Genetic studies in the 1980s demonstrated that MH2 contains two unrelated retroviral oncogenes that were designated as *v-myc* and *v-mil*. Subsequent analysis of *v-mil* revealed a high sequence homology to the *v-raf* oncogene of the murine sarcoma retrovirus 3611. Further analyses showed that both *v-mil* and *v-raf* arose independently by retroviral transduction from the chicken *c-mil* and mammalian *raf-1* genes, respectively. In 1988, a *v-mil* related oncogene was discovered in ► transforming retroviruses that were generated by passaging the non-oncogenic Rous-associated virus type 1 (RAV-1) on embryonic chicken neuroretina cells. Due to its



**B-Raf Somatic Alterations. Fig. 1** B-Raf oncoproteins. (a) Situation for wildtype B-Raf. In its inactive state, the *BRAF* proto-oncogene product resides in a closed conformation stabilized by 14-3-3 proteins. Activation of B-Raf by activated RAS results in a displacement of the N-terminal autoinhibitory region (Conserved region (CR) 1 in blue, CR2 in green) from the CR3 or kinase domain (red) allowing access of the activation loop kinase to the TVKS-motif. Phosphorylation of T599 and S602 within this motif renders B-Raf active. (► **B-Raf Signaling**).

(b) Schematic representation of v-Rmil. Due to the retroviral transduction event, the genome of RAV encodes for a fusion protein flanking the B-Raf (CR3) kinase domain with an N-terminal portion encoded by the *env* gene and a C-terminal moiety encoded by a portion of the *gag* gene. Both the *env* and *gag* genes are integral components of retroviral genomes. (c) Schematic representation of AKAP9-B-Raf. (d) Schematic representation of B-Raf proteins with point mutations as exemplified for the activation loop mutant B-Raf<sup>V600E</sup>.

origin in retinal cultures, this relative of v-mil was designated as v-Rmil. Subsequent studies showed that v-Rmil was generated by retroviral transduction from the proto-oncogene c-Rmil, which is related but distinct to the c-mil/raf-1 proto-oncogenes and represents the avian ► **orthologue** of *BRAF*. Similar to the avian c-Rmil/B-raf gene, the *BRAF* genes of other vertebrates display a conserved exon/intron structure with 18–20 coding exons, in which the first eight exons encode the N-terminal autoinhibitory region (Fig. 1a).

At the same time as v-Rmil was discovered, the human *BRAF* oncogene was identified in a ► **NIH3T3 transformation assay** using ► **Ewing sarcoma** DNA. Importantly and in striking analogy to v-Mil and v-Raf, both the v-Rmil and the B-Raf oncprotein from the Ewing sarcoma isolate represent N-terminally truncated B-Raf proteins (Fig. 1b), which have lost the

N-terminal regulatory lobe and consequently the ability for autoinhibition (► **B-Raf Signaling**). Therefore, all these Raf-oncoproteins display constitutive activity and induce chronic activation of the ERK pathway. Thus, loss of exons encoding for the autoinhibitory N-terminal moiety is a common mechanism of oncogenic activation of *raf* proto-oncogenes. This notion is further supported by recent experiments showing that the murine *Braf* gene represents a frequent integration point for the *Sleeping Beauty* transposon. All transposon integrations were observed between exons 9 and 10 resulting in a disruption of the coding sequence of full-length B-Raf and expression of an N-terminally truncated B-Raf protein with an intact kinase domain and structural similarity to v-Rmil and v-Raf. However, it should be mentioned that neither retroviral B-Raf oncogenes nor transposon-mediated oncogenic

activation of the *BRAF* gene have been observed in human beings. Likewise N-terminal truncations of B-Raf like those found in the original publication on the human B-Raf gene, which most likely represents a transfection artifact, have not been found in human tumors until recently. Nevertheless, the human *BRAF* proto-oncogene is affected by somatic alteration in about 7% of human tumors. The following alterations are observed in human tumors:

#### Chromosomal Aberrations

A recent study has identified an oncogenic *BRAF* allele in about 11% of papillary thyroid carcinomas (PTC) in children and adolescents that had been exposed to radiation following the Chernobyl nuclear power plant station accident in 1986. This oncogene was generated *via* a paracentric inversion of the *BRAF* locus on chromosome 7q34 resulting in an in-frame fusion with exons 1–8 of the *A-kinase anchor protein 9* (*AKAP9*) gene on 7q21-22. The resulting *AKAP9-B-Raf* fusion protein is made up by exons 1–8 of *AKAP9* and exons 9–18 of *BRAF*. Thus, this *AKAP9-B-Raf* protein contains an intact kinase domain, but the autoinhibitory N-terminal regulatory domain of B-Raf is replaced by the *AKAP9* moiety, which cannot confer autoinhibition (Fig. 1c). Consequently, the activity of this fusion protein is, similar to the situation in v-Rmil, unrestrained and able to transform NIH3T3 cells. Interestingly these mutations were only found in tumors that had developed within a short latency period suggesting that this chromosomal aberration is a driver of radiation induced PTC rather than being a secondary event.

Another recent study has reported the occurrence of chromosomal translocations involving the human *BRAF* gene in two cases of large congenital melanocytic nevi, which can progress into malignant melanoma. In both cases and similar to the situation of the *AKAP9-B-Raf* fusion protein, these translocations give rise to fusion proteins, which lack the exons encoding the autoinhibitory N-terminal regulatory domain, but again contain an intact B-Raf kinase domain.

#### Somatic and Germ-Line Point Mutations

Although Raf proteins were implicated early on as important effectors of human oncoproteins, e.g., Ras, they were not considered as frequent mutational targets in cancer. In 2002, however, the ► *cancer genome*

*project* (CGP) reported a high frequency of somatic point mutations in the human *BRAF* gene in malignant melanoma (27–70%). Subsequent studies also revealed high point mutation frequencies in thyroid (36–53%), ovarian (30%), biliary (14%), and colorectal cancer (5–22%) and lower frequencies in a wide range of other human tumors. It is estimated that the human *BRAF* gene bears somatic mutations in about 7% of all human cancers. In contrast to the aforementioned alterations of the *BRAF* gene, these point mutations do not affect the overall primary structure of B-Raf (Fig. 1d), but mostly bypass critical regulatory events required for the activation of wildtype B-Raf (► *B-Raf Signaling*).

While mutations in the human *CRAF* gene are still considered as a very rare event, over 40 different somatic mutations, involving 24 different codons, have been identified in *BRAF* since 2002. Most alterations represent point mutations, however, codon deletions or in-frame insertions have been occasionally identified as well. A detailed overview on these mutations can be found on the CGP homepage ([http://www.sanger.ac.uk/perl/genetics/CGP/cgp\\_viewer?action= gene& In=BRAF](http://www.sanger.ac.uk/perl/genetics/CGP/cgp_viewer?action= gene& In=BRAF)). Most mutations cluster within the activation loop codons, and to a lesser extent, within the nucleotide sequence encoding the glycine-rich loop (also known as P-loop; Fig. 1d). Among the activation segment mutations, the thymidine to adenine transversion at nucleotide 1799, which results in the substitution of valine 600 within the T<sup>599</sup>V<sup>600</sup>K<sup>602</sup>-motif in the activation segment by glutamate, represents the most common mutation and is found in 6% of human cancers. Structural analysis of the B-Raf kinase domain suggests that the inactive conformation of B-Raf is stabilized by a hydrophobic interaction between the activation loop residues with the glycine rich loop, with V600 and F467 playing key roles in this process. Upon activation of wildtype B-Raf by activated Ras, T599, and S602 in the activation loop become phosphorylated by an unknown kinase resulting in the disruption of the inhibitory hydrophobic interaction between the activation and glycine rich loop and consequently full activation of B-Raf (Fig. 1a). In a similar way, any mutation in either the activation or glycine-rich loop mutation that disrupts this hydrophobic interaction, e.g., replacement of V600 by bulky and/or charged amino acids like glutamate, mimics the activated state and confers constitutive activity to B-Raf. As described in ► *B-Raf signaling*, the current model of

B-Raf activation proposes a sequence of positive regulatory events leading to a relief of autoinhibition by the N-terminal lobe followed by activation loop phosphorylation and full B-Raf activation. According to this sequential model of B-Raf activation, the V600E mutation not only bypasses these events, but is also able to counteract autoinhibition, which would explain why this mutation is so frequently found in tumors driven by chronic ► **B-Raf signaling**. However, why the V600E mutation occurs more frequently than any other activation loop or glycine-rich loop mutations that would also disrupt the inactive conformation, remains controversial. The V600E codon might represent a mutational “hotspot” or, due to still unknown details of B-Raf activation, might be an extremely efficient oncogene that subjects B-Raf<sup>V600E</sup> expressing cells to a particularly strong positive selection. It should be also mentioned that the occurrence of the *BRAF*<sup>V600E</sup> allele in colorectal cancer is correlated with ► **microsatellite instability** (MSI) and widespread methylation of CpG islands in a highly statistically significant manner. However, it remains to be clarified as to whether the MSI phenotype that is caused by absence or hypoactivity of DNA mismatch repair genes and is characterized by a widespread methylation of CpG islands, reflects a cause or a consequence of dysregulated B-Raf signaling.

In 2006, germ-line mutations in the human *BRAF* gene were found in patients suffering from the ► **cardio-facial-cutaneous (CFC) syndrome**. Some of these mostly gain-of-function mutations in CFC patients are also found in cancer, however, mutations conferring high activity to B-Raf such as *BRAF*<sup>V600E</sup> have not been found. Indeed, *knock-in* experiments in mice have shown that ubiquitous expression of B-Raf<sup>V600E</sup> confers early embryonic lethality suggesting that high levels of chronic B-Raf activity would not be tolerated during human development as well.

However, it should be noted that not all of the point mutations found in cancer or CFC patients represent obvious gain-of-function mutations as some of them actually display impaired in vitro kinase activity. Nevertheless, these impaired activity mutants still appear to activate the ERK pathway within the cell, either through stimulating the activity of Raf-1 in Raf-1/B-Raf heterodimers or, potentially, by acting as a buffer against negative regulators, e.g., RKIP, or negative feedback loops controlling ► **B-Raf signaling**.

## Amplification

Amplification of the *BRAF* locus is another mechanism contributing to elevated B-Raf protein expression and activity. Studies in malignant melanoma have described the amplification of *BRAF* alleles with point mutations such as V600E at the expense of the wildtype *BRAF* allele.

Genetic experiments have identified B-Raf as an important factor for ERK activation under basal and steady state conditions (► **B-Raf Signaling**). Experiments in various cell types have shown that increasing levels of wildtype B-Raf enhanced basal and steady state ERK signaling suggesting that overexpression of endogenous wildtype B-Raf might contribute to tumorigenesis. Indeed, amplification of the *BRAF* locus in the absence of any mutations in exon 11 (Gly-rich loop) and exon 15 (activation loop) was described as an important contributor to the proliferation of malignant melanoma cell lines.

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## BRAF1

### ► **B-Raf Signaling**

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## Bragg (Curve) Peak

### **Definition**

A characteristic dose distribution of a single-energy charged particle beam (e.g., protons) with a sharp peak

close to the end of the range. The range is a distance that particles travel inside the medium.

#### ► Radiation Oncology

## Brain Capillaries

#### ► Blood–Brain Barrier

## Brain Microvascular Endothelial Cells

#### ► Blood–Brain Barrier

## Brain Tumors

Yasuyuki Hitoshi, Mark A. Israel and Paula M. Kuzontkoski

Departments of Pediatrics and of Genetics, Norris Cotton Cancer Center, Dartmouth Medical School, Hanover, NH, USA

### Definition

Primary brain tumors present most commonly as ► meningioma or various grades of ► astrocytoma. ► Gliomas constitute 78% of all malignant brain and central nervous system tumors. It is estimated that approximately 20,500 individuals will be diagnosed with cancer of the brain and nervous system in 2007, or about 1.4% of all newly occurring malignancies. Of those diagnosed, there will be 10% more men than women. Primary brain and nervous system cancers will account for 2.3% of the estimated 560,000 cancer deaths in 2007. Based on the most recent report of the Central Brain Tumor Registry of the United States, benign tumors of the CNS arise in numbers comparable to malignant brain tumors. In children and young adults, brain tumors are responsible for 25% of all cancer-related deaths, second only to leukemia in this age group. The estimated 5-year relative survival rate for malignant brain tumors is 29%, but there is much variation in survival, depending on tumor histology.

The 5-year survival rate exceeds 91% for pilocytic astrocytomas, but is less than 4% for glioblastomas. Generally, survival decreases with increasing age at diagnosis.

## Characteristics

### Classification and Pathology

The cell of origin of commonly occurring brain tumors is not known, although recent evidence suggests that these tumors arise either from ► neural stem cells or from other cells that take on many characteristics of neural stem cells as a result of malignant transformation caused by the activation of oncogenes and the inactivation of ► tumor suppressor genes within the cells. Pathologically, these tumors are classified according to the World Health Organization (WHO) nomenclature and grading criteria. Tumors that share cytologic and histologic evidence of astrocytic differentiation are known as ► astrocytoma and are the most frequent primary intracranial neoplasms. Their neuro-pathological appearance is highly variable. Tumors with evidence of oligodendroglial differentiation are known as ► oligodendrogioma. Some tumors that have cells reminiscent of both lineages are known as mixed oligoastrocytomas. Each of these tumor types can be graded histologically according to a four-tiered system of increasing malignancy from Grades I through IV. Grade I, for example, has an excellent prognosis following surgical excision, and Grade IV, ► glioblastoma multiforme, has multiple features of clinical aggressiveness and is typically incurable. Hypercellularity with evidence of high mitotic activity, nuclear and cytoplasmic atypia, endothelial proliferation, and necrosis correspond closely to tumor virulence and are most characteristically present in Grade IV tumors. The overwhelming majority of gliomas arising in adults are high grade and arise in a supratentorial location. High-grade tumors do not have a clear margin separating neoplastic and normal tissue. This finding is consistent with the observation that tumor cells usually have infiltrated adjacent normal brain by the time of diagnosis, when complete resection is oftentimes not possible. Tumor cells capable of initiating new tumor foci can now be recognized as tumor stem cells.

Cytogenetic examination of chromosomes within the cells of a brain tumor has revealed characteristic

**Brain Tumors. Table 1** Cytogenetic and genetic alterations in brain tumors

Tumor type	Chromosomal alteration	Genetic changes	
		Oncogene	Tumor suppressor gene
Astrocytoma	1p <sup>-</sup> , 7 <sup>+</sup> , 9p <sup>-</sup> , de110, 11p <sup>-</sup> , 12q <sup>-</sup> , 12q <sup>+</sup> , 13q <sup>-</sup> , 13q <sup>+</sup> , 18p <sup>+</sup> , 17p <sup>-</sup> , 17q <sup>-</sup> , 19p <sup>-</sup> , 19q <sup>-</sup> , 22q <sup>-</sup> , DMs <sup>a</sup>	<i>EGFR, PDGFRA, KIT, CROS, MET, CDK4, NEU, RAS, MDM2, GLI, CMYC</i>	<i>P53, RB1, NF1, PTEN, DMBT, CDKN2A, CDKN2D, RASSF1A</i>
Oligodendrogioma	1p <sup>-</sup> , 19q <sup>-</sup> , 7 <sup>+</sup> , 10 <sup>-</sup>	► <i>EGFR</i>	<i>TP53, PTEN, CDKN2A, CDKN2D, PIK3CA</i>
Medulloblastoma	5p <sup>+</sup> , 5p <sup>-</sup> , 5q <sup>-</sup> , del6, 8p <sup>-</sup> , 8q <sup>+</sup> , 9q <sup>-</sup> , 10q <sup>-</sup> , 17p <sup>-</sup> , 17q <sup>-</sup> , 17q <sup>+</sup> , 21q <sup>+</sup>	<i>GLI, CMYC, CTNNB1</i>	<i>TP53, PTCH, SUFU, APC, RASSF1A</i>

<sup>a</sup>DMs double minute chromosomes

regions that tend to be altered in specific tumor types (Table 1). Frequent sites for chromosomal DNA loss in astrocytic tumors include chromosomes 17p, 13q, and 9. In oligodendrogloma, DNA from 1p and 19q is frequently lost, and in ► meningiomas, 22q is often lost. Molecular genetic analysis can also reveal evidence of tumor-specific genetic alterations at sites where chromosomes appear normal upon cytogenetic analysis. Using a variety of molecular technologies, it has been possible to document the alteration of many different genes in brain tumors, particularly astrocytic tumors (Table 1).

While the particular constellation of genetic alterations that activate oncogenes and inactivate tumor suppressor genes varies among individual brain tumors that appear to be histologically indistinguishable, an accumulation of mutations is typically associated with increasingly aggressive malignant behavior. Glioblastoma multiforme (► GBM) typically presents without evidence of a precursor lesion, referred to as de novo or primary GBM. These tumors typically have evidence for chromosome 10 deletions in the region where the tumor suppressor ► *PTEN* is known to be located, and activation of the ► *epidermal growth factor receptor (EGFR)* gene either by amplification or deletion of ~275 amino acids from the extracellular domain of the receptor. This and closely related mutations occur in ~60% of GBM. *EGFR* is the gene most frequently activated in malignant astrocytomas. *EGFR* amplification and activation by mutation occurs in ~5% of low-grade astrocytomas and about 30% of GBM, indicating that this molecular change is principally associated with the progression from low- or intermediate-grade neoplasia to high-grade astrocytic neoplasia. In fewer than 20% of cases, GBM arises in association with progressive genetic alterations after the diagnosis of a lower-grade astrocytoma. These tumors are referred

to as secondary GBMs. The most widely described alterations are amplification or overexpression of the ► *PDGF* receptor, mutations of ► *p53* or ► *MDM2* and ► *INK4a*, and loss of *PTEN* (Table 1). Deletion of the ► *CDKN2* gene, which encodes the cyclin-dependent kinase inhibitor *p16*, has been reported to occur in ~40–70% of glioblastoma. The ► *RBI* tumor suppressor gene is homozygously deleted or mutated in about 30% of high-grade gliomas.

The protein products of tumor suppressor genes are proteins that act to regulate or suppress cell growth or promote cell death. These genes are inactivated during tumorigenesis, and several such genes have been implicated in the development of astrocytoma. Occasionally, inactivation of one of these alleles in the germ line can occur without disturbing development, and patients who carry germ line mutations of some tumor suppressor genes can be predisposed to the development of cancer. Several inherited cancer-predisposition syndromes are known to be associated with the development of different brain tumors. Patients with ► *Li–Fraumeni syndrome*, caused by an inherited constitutional *p53* mutation, have a predisposition for the development of brain tumors. The *p53* gene, located on chromosome 17p, has been found to influence multiple cellular functions thought to be important in tumorigenesis. *p53* mutations have been reported in sporadically arising astrocytic tumors of all grades, occurring in ~40% of astrocytomas, in 30% of ► *anaplastic astrocytomas*, and in a slightly smaller fraction of GBM. Other brain tumor predisposition syndromes associated with the inactivation of one copy of a particular gene in the germ line include ► *neurofibromatosis type 1 (NF1 gene)*, which is associated with meningioma and optic glioma; ► *neurofibromatosis type 2 (NF2 gene)*, which is associated with acoustic neuroma and glioma; familial

► **retinoblastoma** (► *Rb* Gene), which is associated with retinoblastoma and pinealoblastoma; ► **von Hippel-Lindau syndrome** (VHL gene), which is associated with cerebellar hemangioblastoma; ► **tuberous sclerosis** (TSC1 and TSC2 genes), which is associated with subependymal giant cell astrocytoma; ► **Turcot syndrome** (► *APC* gene), which is associated with astrocytoma and ► **medulloblastoma**; and Gorlin's syndrome (PTCH gene), which is associated with desmoplastic medulloblastoma.

The second most common primary brain tumor is oligodendrogloma, which has a more benign course than astrocytoma. Many ► **gliomas** have mixtures of cells with astrocytic and oligodendroglial features. If this mixed histology is prominent, the tumor is termed a mixed glioma or an ► **oligoastrocytoma**. Many investigators believe that the greater the oligodendroglial component, the more benign the clinical course. The presence of such histologic characteristics as mitosis, necrosis, and nuclear atypia generally is associated with a more aggressive clinical course. If these features are prominent, the tumor is termed a malignant oligodendrogloma. The highest grade oligodendrogloma are indistinguishable from glioblastoma multiforme.

Other malignant primary brain tumors include ► **primitive neuroectodermal tumors** (► **PNET**) such as ► **medulloblastoma**, ► **ependymoma**, and ► **atypical teratoid/rhabdoid tumors**; ► **germinomas**; and CNS ► **lymphoma**. Cerebral PNETs and medulloblastoma, a PNET that arises in the posterior fossa, are highly cellular malignant tumors thought to arise in neural precursor cells. These tumors are difficult to distinguish from one another and typically appear histologically as sheets of small round malignant cells. Germ line mutation of *PTCH* and *SUFU* in rare patients has called attention to the importance of sonic hedgehog signaling in medulloblastoma. Similarly, *APC* germ line mutations in rare patients implicate *WNT* signaling as well. These tumors most commonly occur in children. Ependymomas are rare tumors, and when these occur in children, they typically are within the fourth ventricle, where they are thought to arise from cells lining the fourth ventricle. In adults, they arise more frequently in the spinal cord. Patients with neurofibromatosis type 2 are at increased risk of developing ependymoma, and 30% of sporadically occurring tumors exhibit deletion of Ch22q where the *NF2* gene is located. Histologically, these

tumors exhibit diagnostic ependymal rosettes. Atypical teratoid/rhabdoid tumors histologically appear as fields of undifferentiated malignant neuroectodermal cells that are indistinguishable from PNET, except for infrequent cells that exhibit evidence of rhabdoid differentiation and the presence of mesenchymal and epithelial elements. Germinomas arise most commonly during the second decade of life at midline locations. Both malignant and benign variants occur frequently. These tumors present with hypothalamic-pituitary dysfunction and visual field deficits.

Primary CNS lymphomas are most commonly seen in immunocompromised patients, and have a clinical presentation similar to other primary brain tumors with signs and symptoms referable to cerebral and cranial nerve involvement. Imaging studies typically demonstrate a uniformly enhancing mass lesion. Secondary CNS lymphoma almost always occurs in association with the progression of systemic disease. Several kinds of tumors that are most often benign also occur in the nervous system. ► **Meningiomas** are derived from cells of the arachnoid membranes. They are more frequent in women than in men, with a peak incidence in middle age. Meningiomas rarely have histological evidence of malignancy. Other tumors that have a benign clinical course include giant cell astrocytomas, pleomorphic xanthroastrocytomas, neurocytomas, and gangliogliomas. Colloid cysts, dermoid cysts, and epidermoid cysts also occur in the brain.

### Clinical Presentation of Brain Tumor Patients

The most common symptoms that bring patients with a tumor arising in the brain to their physician include a slow progressive focal neurological disability, or a nonfocal neurological syndrome such as headache, dementia, gait disorder, or seizure. Other systemic symptoms suggest a tumor from some other location that may have metastasized to the brain, since patients with primary brain tumors typically do not exhibit systemic symptoms. Patients with primary brain tumors rarely have any biochemical abnormalities; thus CT (computerized tomography) and MR (magnetic resonance) imaging are key diagnostic modalities for the identification of brain tumors. The characteristic imaging features of brain tumors are mass effect, edema, and contrast media enhancement. Positron emission tomography (PET) scanning and single photon emission computed tomography (SPECT) have

ancillary roles in the imaging of brain tumors. Meningiomas and other slow-growing tumors may be found incidentally on a CT or MRI scan or they may present with a focal seizure, a slow progressive focal deficit, or symptoms of increased intracranial pressure. Brain tumors are also recognizable in many inherited syndromes including ► von Recklinghausen syndrome (► Neurofibromatosis Type 1), ► neurofibromatosis type 2, ► Li-Fraumeni syndrome, ► Multiple endocrine neoplasia type 1, ► tuberous sclerosis syndrome, ► Turcot syndrome, and ► Gorlin syndrome.

### Clinical Management of Brain Tumor Patients and Prognosis

Stereotactic needle biopsy may establish the histological diagnosis of primary brain tumor, although open biopsy is also often utilized to establish the diagnosis. The primary modality of treatment for most primary brain tumors is surgery. The goals of surgery are to obtain tissue for pathological examination, to remove tumor, and to control mass effect. In the case of low-grade and benign tumors, the removal of tumor tissue can be curative or contribute substantially to extending the time to symptomatic progression. In higher-grade tumors, the role of surgery in contributing to curative therapy is less clearly defined, but in younger patients most surgeons aggressively pursue the removal of as much tumor as possible. Following total excision of an ependymoma, the prognosis is excellent. However, many ependymomas cannot be totally excised. Following surgery, radiation therapy has been shown to prolong survival and improve the quality of life of patients with high-grade glioma, PNET, ependymoma, or meningioma when malignant histologic elements can be pathologically identified within the tumor.

The medical management of most brain tumors is symptomatic, although a role for chemotherapy is clearly defined in oligodendrogloma and medulloblastoma. In patients with oligodendrogloma, a combination of procarbazine, lomustine, and ► vincristine has been shown to be most effective in patients with a deletion of chromosome 1p. Various combination therapies have been shown to contribute to the treatment of medulloblastoma, which has a propensity to spread throughout the neuroaxis. If medulloblastoma is limited to the posterior fossa and completely resected, this tumor has a good prognosis. Temozolamide given during radiation therapy for glioblastoma has been shown to contribute to longer overall survival time.

► Chemotherapy and ► radiation therapy typically play a central role in the treatment of ► germinoma, although there is a role for surgery as well. Patients whose brain tumors are associated with surrounding ► cerebral edema benefit symptomatically from the administration of high doses of ► glucocorticoids. Anticonvulsants are useful in the control of seizures. Some glioma patients receive anticoagulation therapy to avoid complications of venous thrombosis that occurs in these patients.

The prognosis for patients with primary brain tumors varies greatly as a function of the histology and location of the tumor. Benign tumors are often cured by surgery alone. ► Germinomas and medulloblastomas are more sensitive to cytotoxic therapies than are other brain tumors, and the prognosis for patients with these tumors is generally better than for patients with high-grade glioma. In modern studies, the median survival of patients with high-grade glioma is ~1–2 years.

### Complications of Therapy

Neurological damage associated with surgical intervention presents a key challenge in the management of brain tumors. Furthermore, the nervous system is vulnerable to injury by therapeutic radiation, and this is frequently manifested by neuropsychological compromise and disability, particularly in very young children who have been treated with high doses of radiation. Pathologically, there is demyelination, hyaline degeneration of small arterioles, and eventually brain infarction and necrosis. Endocrine dysfunction is also commonly seen when the hypothalamus or pituitary gland has been exposed to therapeutic radiation. Depending on the radiated field, secondary tumors such as glioma, meningioma, sarcoma, and thyroid cancer occur following radiation therapy. Toxicities associated with chemotherapy can be significant, but they are not usually different from the toxicities associated with comparable treatments for tumors arising elsewhere in the body.

### ► Neuro-oncology: Primary CNS Tumors

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## Brain-Derived Neurotrophic Factor

### Definition

BDNF; is a neurotrophin in the central nervous system (CNS), predominantly in the brain and the periphery. This is in contrast to ► **NGF** acting predominantly in the peripheral nervous system. It acts on certain neurons of the CNS and the peripheral nervous system that helps to support the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses. In the brain, it is active in the hippocampus, cortex, and basal forebrain – areas vital to learning, memory, and higher thinking. BDNF was the second neurotrophic factor to be characterized after NGF.

## Branching Morphogenesis

### Definition

Branching morphogenesis refers to the formation of tree-like networks of epithelial tubes through reiterated cycles of branch initiation, branch outgrowth, and branch arrest. This process relies on the precise spatio-temporal control of gene expression, cell proliferation, and migration and is essential for the physiological function of many organs including the lung, the vascular system, and the kidney.

► **Sprouty**

## BRCA1

### Definition

Breast cancer susceptibility gene 1.

► **Breast Cancer Genes BRCA1 and BRCA2**

B

## BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk

Peter Devilee

Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

### Definition

Mutations in the ► **breast cancer genes BRCA1 and BRCA2** cause elevated risks to ► **breast cancer** and ► **ovarian cancer**. BRCA1 maps to chromosome 17 (band q21), and BRCA2 maps to chromosome 13 (band q12).

At the genetic level there are interesting analogies between the two genes, even though they are not detectably related by sequence. Both genes are large (coding regions of 5.6 and 10.2 kb, respectively), complex (22 and 26 coding exons, respectively), and span about 80 kb of genomic DNA. Both have extremely large central exons encoding >50% of the protein. The majority of the mutations in both genes detected to date lead to premature termination of protein translation, presumably resulting in an inactive truncated protein. Gene changes are distributed nearly ubiquitously over the coding exons and immediate flanking introns. Even though more than half of all mutations are found only once, many mutations have been detected repeatedly in certain populations. For most of these, this has been shown to be the result of a ► **founder effect**: These mutations arose a long time ago, and have since spread in the population. Typical founder mutations are the 1185delAG and 15382insC in BRCA1 and 26174delT in BRCA2 that have a joint frequency of about 2.5% among individuals of Ashkenazi Jewish descent.

## Characteristics

### Clinical Characteristics

Female carriers of a deleterious BRCA1 mutation were estimated by the Breast Cancer Linkage Consortium (BCLC) to have an 87% cumulative risk to develop breast cancer before the age of 70, and 40–63% risk to develop ovarian cancer before that age (Fig. 1). The gene frequency of BRCA1 was estimated at 1 in 833 women, implying that 1.7% of all breast cancer patients diagnosed between the ages of 20 and 70 are carriers of such a mutation. The estimated cumulative risk of breast cancer conferred by BRCA2 reached 84% by age 70 years. The corresponding ovarian cancer risk was 27% (Fig. 1). These estimates imply that BRCA2 mutations are about as prevalent as BRCA1 mutations. It has been suggested that the ovarian cancer risks are dependent on the position of the mutation in the gene, for BRCA1 as well as BRCA2 mutations. There is also some evidence that cancer risks can be modified by other factors. For example, a strong variability in phenotype can be seen among families segregating the same mutation. This can range from early-onset breast cancer and ovarian cancer, to late-onset breast cancer without ovarian cancer. Even within a single pedigree, ages of onset of cancer can vary substantially. It seems likely that environmental and hormonally related factors (smoking, oral contraceptives) importantly co-determine disease outcome in carriers.

### Molecular and Cellular Characteristics

#### Tumor Suppressor Genes

The first clues to the roles of BRCA1 and BRCA2 in tumorigenesis were genetic. The fact that most germline mutations are predicted to inactivate the protein, and the observed loss of the wild-type allele in almost all breast and ovarian cancers arising in mutation carriers, are strong indicators that BRCA1 and BRCA2 proteins act as ► tumor suppressor genes. This is supported by the finding that induced overexpression of wild type but not mutant BRCA1 in MCF-7 breast cancer cells leads to growth inhibition and inhibited tumor growth in nude mice.

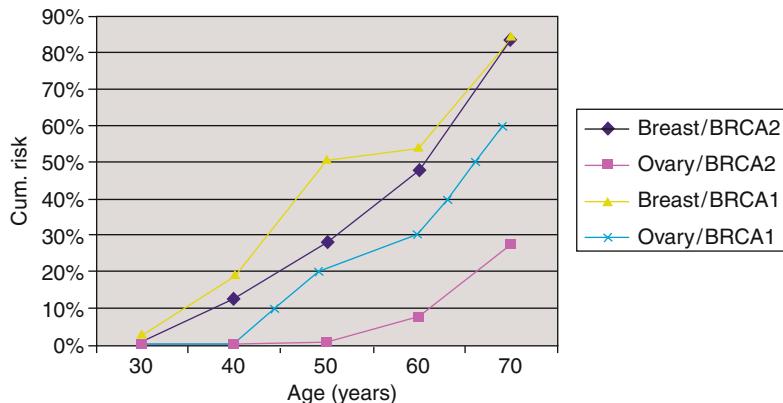
#### Expression of BRCA1 and BRCA2

In normal cells, BRCA1 and BRCA2 encode nuclear proteins, preferentially expressed during the late-G1/early-S phase of the ► cell cycle, but downregulated

in quiescent cells. While apparently at odds with the observations that BRCA1 expression inhibits cellular proliferation, the proliferation-induced expression could represent a negative feedback loop tending to decrease breast cancer risk. However, BRCA1 expression can also be upregulated in a proliferation-independent way in mammary ► epithelial cells induced to differentiate into lactating cells by ► glucocorticoids. Hence, BRCA1 might also play a role in controlling mammary gland development. In mice, expression of BRCA1 and BRCA2 is coordinately upregulated with proliferation of breast epithelial cells during puberty, pregnancy, and lactation. Intriguingly, BRCA1 might suppress estrogen-dependent mammary epithelial proliferation by inhibiting ► estrogen receptor-alpha (ER- $\alpha$ )-mediated transcriptional pathways related to cell proliferation. Whatever the cellular function of BRCA1, it appears to be regulated by phosphorylation: It becomes hyperphosphorylated at G1/S with dephosphorylation occurring at M phase. BRCA1 might regulate the G1/S ► checkpoint by binding hypophosphorylated ► retinoblastoma protein. BRCA1 and BRCA2 have also been suggested to regulate the G2/M checkpoint by controlling the assembly of the ► mitotic spindle and the appropriate segregation of chromosomes to daughter cells.

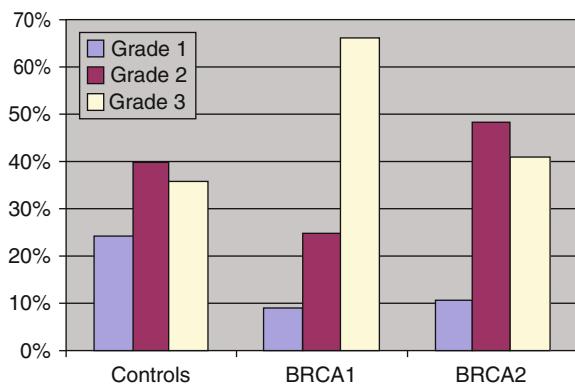
**BRCA1- and BRCA2-Related Breast Cancer** A close examination of the ► pathology of BRCA1- and BRCA2-related breast cancers has defined a typical pathology for each category, differing from that in sporadic cases. In general, cancers in carriers are of higher grade than age-matched controls (Fig. 2), and the BRCA1 cancers more frequently display a “medullary”-like appearance. This is due to a higher mitotic count and lymphocytic infiltrate. BRCA2-related breast cancers generally show fewer mitoses and less tubule formation. For both BRCA1- and BRCA2-related cancers, greater proportions of the tumor show continuous pushing margins. Although a role for BRCA1 and BRCA2 in non-inherited sporadic breast cancer is unclear, protein expression of BRCA1 is reduced in most sporadic advanced (grade III) ► ductal carcinomas.

**BRCA1 and BRCA2 as Caretakers of the Genome** To date, several biological roles for BRCA1 and BRCA2 have been demonstrated, and a number of observations indicate that they function in a similar pathway. Both maintain genomic stability



**BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk.** **Fig. 1** Overall penetrances of BRCA1 and BRCA2 for breast and ovarian cancer. Estimates were obtained by maximizing the LOD score with respect to all the different penetrance functions in those families with strong evidence of the breast and ovarian cancers being caused by the gene (done by linkage analysis). This is equivalent to maximizing the likelihood of

the marker data, which is determined only by disease phenotype data. This will give an unbiased estimation of the penetrance irrespective of ascertainment of families on the basis of multiple affected individuals. Data were compiled from Ford et al [2, 6]. The graphs can be read in such a way that, for example, an unaffected carrier of a BRCA1 mutation has a 50% risk to develop breast cancer before age 50



**BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk.** **Fig. 2** BRCA1- and BRCA2-related breast cancers are generally of higher grade than age-matched controls. Histological sections from 118 breast tumors attributable to BRCA1, and 78 attributable to BRCA2, were evaluated by five histopathologists, all experts in breast disease. Every slide was seen by two pathologists. An age-matched group of 547 apparently sporadic female breast cancer cases served as control. The overall grade of both BRCA1 and BRCA2 breast cancers was significantly higher than that of controls ( $p < 0.0001$  and  $p < 0.04$ , respectively). For BRCA1 breast cancers this was due to higher scores for all three grade indices, whereas for BRCA2 breast cancers the grade was only significantly higher for tubule formation. Data taken from The Breast Cancer Linkage Consortium [7]

through their involvement in ► homologous recombination repair, ► transcription-coupled repair of ► oxidative DNA damage, and ► DNA double-strand break repair. These roles are suggested by interactions of the

Brca1 and/or Brca2 proteins with proteins known to be involved in ► repair of DNA damage, most notably ► RAD50 and ► RAD51. Murine embryonic stem cells and mice in which both copies of BRCA1 or BRCA2 have been mutated show a repair deficiency and defects in cell-cycle checkpoints. BRCA1 and BRCA2 play a role as ► transcription factor, through interactions or complex formation with RNA polymerase II and various transcriptional regulators, although this is presently more firmly established for BRCA1 than for BRCA2. A transcriptional response to DNA damage is well-documented, and identification of downstream targets of BRCA1/2-mediated transcription regulation might help to further understand how BRCA1 and BRCA2 suppress tumor formation. ► Microarray-based screening of genes regulated by BRCA1 fall into two categories, cell-cycle control genes and DNA ► damage response genes.

### Clinical Relevance

#### When to Take the DNA Test?

Diagnosis of gene defects became possible after the identification of BRCA1 and BRCA2 in 1994 and 1995, respectively. In many countries, testing for mutations is being offered to women with a high priori ► familial risk in clinical genetic centers or multidisciplinary cancer family clinics. A few studies have presented models to determine the prior probability that the counselee is a BRCA mutation carrier, by

combining breast cancer and ovarian cancer family history data with results from comprehensive mutation testing. These models enable the genetic counselor to decide when a DNA test is indicated.

### Why Take the DNA Test?

A clear positive result of the DNA test, i.e., the presence of a deleterious mutation, is being used to enter these women into early-detection cancer screening programs or in the decision for or against prophylactic surgery. A woman in which breast cancer has just been diagnosed can benefit from knowledge about gene carrier status, since the risks to the ► **contralateral** breast and ovaria must be considered. The treatment of such cancer by lumpectomy will not reduce recurrence risks dramatically, as opposed to complete mastectomy. Healthy women who test positive can take action to prevent cancer developing, although the efficacy of the preventive options currently offered to a woman remains without formal supporting evidence.

► **Chemoprevention** is still controversial, and good prospective data on BRCA carriers will probably never become available, given the ethical and clinical difficulties surrounding ► **randomization**. Prophylactic surgery, intuitively the most secure way to reduce breast cancer risk to below population levels, is socially ill-accepted in many parts of the world, and formal proof of its preventive effect in BRCA carriers is also lacking. Clearly, this area is fraught with clinical dilemmas.

### Interpreting a Negative Test Result

Paradoxically, a negative test result (the absence of a deleterious mutation) presently still has limited power in excluding the presence of a strong ► **risk** ► **allele**. A negative test result is presently being found in 70–80% of all probands tested in most non-Ashkenazi Jewish populations. Among probands with a family history for ovarian cancer, a negative test result is found less frequently (although still in 40–60% of the cases). There are several levels of uncertainty.

- The first is technical: No single mutation-detection method is 100% sensitive, and therefore only exhaustive testing, using a range of different methodologies sensitive to various types of mutation mechanisms, and investigating the entire coding regions and regulatory domains, can detect any changes. This is obviously very cost- and labor-intensive.
- The second level of uncertainty relates to the interpretation of sequence changes that do not predict

### **BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk.** **Table 1** Mutation types in BRCA1 and BRCA2 and their predicted effects

<b>Mutation type</b>	<b>BRCA1</b>		<b>BRCA2</b>	
	% of total	% of distinct	% of total	% of distinct
Frame shifting	47.1	38.7	33.7	36.5
Nonsense	11.3	11.1	11.5	10.2
Splice site	4.4	7.9	2.2	3.6
In-frame del/ins	0.6	1.8	0.4	1.0
Missense	28.4	28.4	44.3	35.4
Neutral	3.5	3.9	3.1	5.5
Intronic change	4.7	8.3	4.9	7.8
<b>Mutation effect</b>				
Protein truncating	62.6	56.9	41.4	47.9
Missense	2.2	1.5	0.7	1.9
Neutral polymorphism	11.0	7.2	14.4	13.7
Unclassified variant	24.2	34.4	43.4	36.4

The entire Breast Cancer Information Core (BIC) database was downloaded on March 1, 2000, from [http://www.ncbi.nlm.nih.gov/Intramural\\_research/Lab\\_transfer/Bic](http://www.ncbi.nlm.nih.gov/Intramural_research/Lab_transfer/Bic). There were 3,086 *BRCA1* mutations and 1,892 *BRCA2* mutations. The total numbers of distinct changes were 724 and 670, respectively

a truncated protein. Of the almost 5,000 *BRCA1* and *BRCA2* mutations submitted to the Breast Cancer Information Core (BIC) database, about one-third are either missense, in-frame deletions or insertions, base-substitutions not leading to an amino acid change (neutral changes) or intronic changes with unknown effect on mRNA-processing (Table 1). Only a small proportion of these have been unmasked as ► **polymorphisms** unrelated to disease outcome. They include ► **missense mutation** and ► **intron** variants, but, intriguingly, also a ► **nonsense mutation** in *BRCA2*. The K3326X mutation was found in 2.2% of over 400 controls tested. Only a few missense mutations (e.g., *BRCA1*C61G) have been called a deleterious disease-related mutation, mainly because they reside in a validated functional domain of the protein or affect an evolutionary conserved residue. As a result, about 35% of all the distinct gene changes detected to date are lumped into the “unclassified variant” category, meaning that their relevance to disease outcome is uncertain. Almost certainly, a substantial proportion of these represent rare polymorphisms but equally certainly, a number of them will turn out to be true deleterious mutations.

- A third reason for a negative test result is that the familial clustering of breast cancer in a family is due to an unknown gene or in fact is a nongenetic chance event. The proportion of truly missed, deleterious mutations is therefore difficult to gauge. A study by the BCLC has suggested that a combination of incomplete testing and missed or misinterpreted gene changes causes false-negative test results in over 30% of all family types with some evidence of being linked to BRCA1. This proportion was independent of the mutation-screening methodology used.

► [Breast Cancer Genes BRCA1 and BRCA2](#)

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## BRCA1-associated Ring Domain (Gene/Protein) 1

► [BARD1](#)

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## BRCA2

### Definition

Breast cancer susceptibility gene 2.

► [Breast Cancer Genes BRCA1 and BRCA2](#)

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## BRCT Domain

### Definition

Named after BRCA1 c-terminal repeat. The domain consists of a 90–100 amino acid unit that occurs as a single element or as multiple repeats in several proteins involved in the DNA-damage response. Heterodimerization between BRCT repeats promotes protein–protein interactions. A subset of tandem BRCT repeats adopts a conserved head-to-tail structure. Such tandem repeats can function as a phospho-peptide binding module that binds proteins with specific phosphorylation motifs.

- [BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk](#)
- [BRIT1 Gene](#)
- [Fanconi Anemia](#)

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## BrdU

► [Bromodeoxyuridine](#)

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## Breakpoint

### Definition

Point of separation on a chromosome involved in translocation or other structural rearrangement.

- [Chromosomal Translocations](#)
- [E2A-PBX1](#)

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## Breakpoint Cluster Region

### Definition

A localized site of recurrent DNA breakage.

- [ALU Elements](#)
- [BCR-ABL1](#)

## Breast Cancer

Bill Gullick

Department of Biosciences, University of Kent at Canterbury, Canterbury, Kent, UK

### Definition

Breast cancer may originate from more than one cell type in the breast as a result of different subsets of molecular changes. It is therefore a collection of diseases with different characteristics, different risks, and different treatments. It occurs predominantly in women but it may also occur in men (0.5% of cases). In addition to invasive disease, several benign premalignant and noninvasive forms exist. Although the broad pathological categories are generally accepted there are several alternative systems of subcategorization.

- Benign conditions: Include sclerosing conditions and obliterative mastitis; mild, moderate, and severe hyperplasias and atypical hyperplasias; fibrocystic conditions, fibroadenomas, and related conditions (note that the *Oxford Textbook of Pathology* presents a “simplified nomenclature” of 50 subtypes of benign breast disease).
- Noninvasive carcinoma: Generally divided into lobular carcinoma in situ (LCIS) and ► **ductal carcinoma in situ** (DCIS). DCIS was originally subdivided into comedo, cribriform, papillary, solid, and clinging but has more recently been categorized as well, moderately, and poorly differentiated.
- Invasive carcinoma: Broadly defined as of “special type” (30% of cases) and “no special type” (70% of cases).

### Characteristics

#### Epidemiology

Breast cancer is the most common fatal malignancy in women in the Western world representing about 10% of all cancer deaths. It is however much less common in other countries probably as a result of environmental rather than genetic factors. Behavioral risk factors have been identified. Early pregnancy to term and multiple pregnancy are protective for breast cancer

incidence probably due to a reduced exposure to estrogens. There are several reports of weak associations with diet and use of oral contraceptives.

#### Screening

In some countries mammographic screening (► **Mammographic Breast Density and Cancer Risk**) is available to women to detect early disease, as it is likely that earlier treatment is beneficial for survival. Educational programs are also active, for instance, in promoting self-examination as a method of early detection. The value of these approaches for improving patient survival is not yet fully established.

#### Genetics

About 5–10% of breast cancer cases are associated with a genetic predisposition to the disease. Three genes have been identified where the inheritance of variants is associated with a very high incidence (► **Penetrance**) of the disease. The BRCA1 (► **Breast Cancer Genes BRCA1 and BRCA2**) gene was the first to be identified, and gene carriers are thought to have as much as a 70–80% chance of developing the disease, generally at an earlier age than women with “sporadic” (not genetically predisposed) disease. Subsequently a second gene called ► **BRCA2** was also found in other families that predisposes to breast cancer and ► **ovarian cancer**. The particular risk in an individual gene carrier may be determined by the nature of the particular gene defect and by environmental and hormonal factors and by their background genetic makeup (► **Breast Cancer Genes BRCA1 and BRCA2**). A further inherited condition, ► **Li–Fraumeni syndrome**, is associated with increased risk to breast cancer and many other types of cancers. This is due to the inheritance of a rare mutant copy of the ► **p53** gene.

#### Molecular Biology

Breast cancer is the most studied form of human cancer, due to its common occurrence and to the availability of many immortal breast cancer-derived cell lines that can be grown in tissue culture. About 60% of breast cancers at diagnosis express the ► **estrogen receptor**. Several genes have been found to be altered by mutation or ► **amplification** in invasive cancer and in ► **DCIS**. The ► **HER2** or ERBB2 gene (also known as c-erbB-2 and neu) is amplified in about 25% of invasive breast cancers leading to overexpression of

the growth factor receptor which it encodes. The ► **MYC oncogene** is similarly amplified in about 20% of breast cancer also resulting in overexpression of the c-myc protein. The ► **cyclin D** gene, which specifies a protein important in regulating the ► **cell cycle**, is also amplified in a proportion of breast cancers. The p53 gene is point mutated in approximately 20% of invasive breast cancers and overexpressed in about 50% of cases. Point mutations have been found also in the ► **E-cadherin** gene, which encodes a cell ► **adhesion** molecule. In this case the mutations are most common in lobular cancers. More subtle changes occur in the expression of apparently normal proteins including growth factors such as those in the epidermal growth factor (► **EGF**) family and the fibroblast growth factor (FGF) family of proteins (► **Fibroblast Growth Factors**), the receptor tyrosine kinase c-erbB-3 (► **Receptor Tyrosine Kinases**), and the ► **Src** tyrosine kinase. Some of these altered proteins represent targets for new forms of treatments.

### Treatment

Three methods of treatment are available: surgery, ► **radiotherapy**, and chemotherapy/► **hormonal therapy**. Benign disease and lobular carcinoma in situ are rarely treated but are observed, as they are associated with an increased risk of developing into breast cancer. ► **DCIS** of the breast has often been treated by ► **mastectomy** as it is frequently quite widespread within the breast but may also be treated by local surgery. It is possible that the different pathologically defined forms of DCIS may be associated with different relative risks of recurrence and recurrence as invasive disease. Invasive disease is generally first treated by surgery, and lymph nodes are sampled to determine, by pathological diagnosis, if there is evidence of tumor spread. This procedure may be limited to a single node (called the ► **Sentinel Node**) or may involve a greater degree of surgery. Patients with invasive breast cancer are often treated with radiotherapy to the breast to reduce the chances of local recurrence. Even if the cancer has not apparently spread, patients are sometimes offered preventative or ► **adjuvant therapy** using drugs, as this helps to prevent recurrence of the disease. Chemotherapy or ► **hormonal therapy** is generally offered to patients where metastatic spread of the disease has occurred. Hormonal therapy is frequently offered to women whose tumors express the ► **estrogen receptor**.

Specific methods of treatment still vary depending on the patient and the institution where it is given, although more generally agreed protocols are becoming accepted.

### New Treatments

Breast cancer is frequently a hormonally dependent disease. Thus treatments with drugs such as ► **Tamoxifen**, which binds to the estrogen receptor and reduces its activity, or other drugs that suppress the production of estrogen, such as aromatase inhibitors (► **Aromatase and Its Inhibitors**), are frequently employed. However, new drugs directed to known molecular changes in the cancer cells are under development. These include signal transduction inhibitors directed to molecules such as the epidermal growth factor receptor, monoclonal antibodies to the c-erbB-2 receptor, and drugs which inhibit proteolytic enzymes thought to be involved in the process of ► **metastasis**. Several of these are now in clinical trials that will determine their usefulness for the treatment of the disease.

- **Basal-like Breast Cancer**
- **BRCA1/BRCA2 Germ line Mutations and Breast Cancer Risk**
- **Breast Cancer Antiestrogen Resistance**
- **Breast Cancer Carcinogenesis**
- **Breast Cancer Drug Resistance**
- **Breast Cancer Epidemiology**
- **Breast Cancer Familial Risk**
- **Breast Cancer Genes BRCA1 and BRCA2**
- **Breast Cancer Immunotherapy**
- **Breast Cancer Multistep Development**
- **Breast Cancer New Therapies: HER2, VEGF, and PARP as Targets**
- **Breast Cancer Prognostic and Predictive Biomarkers**
- **Breast Cancer Prognostic Biomarkers**
- **Breast Cancer Rationally Designed Therapies**
- **Breast Cancer Stem Cells**
- **Breast Cancer Susceptibility Genes**
- **Breast Cancer Targeted Therapies**
- **Ductal Carcinoma In Situ**
- **Inflammatory Breast Cancer**
- **Mammographic Breast Density and Cancer Risk**
- **Metastatic Breast Cancer Experimental Therapeutics**
- **Oncoplastic Surgery**

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which is the primary mitogenic factor for ER alpha-positive breast cancer. ► **Tamoxifen** (commercial name Nolvadex), which prevents estrogen from binding to ER alpha, and aromatase inhibitors (► **Aromatase and Its Inhibitors**) such as ► **Ietrozole** and anastrozole, which prevent estrogen biosynthesis, are commonly used SERMs in the clinic. Aberrant ER alpha signaling and growth factor receptor mediated estrogen-independent growth are suggested mechanisms of anti-estrogen resistance. Various strategies to resensitize resistant cancers to anti-estrogens using growth factor receptor antagonists are being developed to treat anti-estrogen resistant breast cancer.

## Breast Cancer Anti-Estrogen Resistance

Harikrishna Nakshatri

IU Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN, USA

### Definition

Breast cells are programmed to respond to certain ► **hormones** as signals for growth and multiplication. The most prominent examples for these hormones are ► **estrogenic hormones** and ► **progesterone**. Many ► **breast cancer** cells retain hormones receptors, to which hormones can bind and execute their activities. The hormone receptors therefore make the cancerous cells responsive to these particular hormones. Most of the estrogen in women's bodies is made by the ovaries. Estrogen makes hormone-receptor-positive breast cancers grow. Reducing the amount of estrogen or blocking its action can reduce the risk of early-stage hormone-receptor-positive breast cancers coming back (recurring) after surgery. Hormonal therapy medicines can also be used to help shrink or slow the growth of advanced-stage or metastatic hormone-receptor-positive breast cancers. Hormonal therapy medicines are not effective against hormone-receptor-negative breast cancers. The term "anti-estrogen resistance" indicates the recurrence of breast cancer in patients with ► **estrogen receptor** alpha-positive breast cancer, and these patients should have received treatments called "► **selective estrogen receptor modulators**" (SERMs). SERMs inhibit estrogen signaling,

### Characteristics

The American Cancer Society estimates that 15% of 713,220 cancers in the USA in 2009 are breast cancers. Approximately 70% of these breast cancers, particularly if the cancer is in post-menopausal women, express ER alpha. ER alpha is a ► **transcription factor** that activates or represses genes in response to estrogen. Estrogen is the most important ► **mitogen** for normal breast as well as breast cancers. ER alpha-positive breast cancers are addicted to estrogen for survival and proliferation (► **Oncogene Addiction**); therefore, these cancers are susceptible to treatment with anti-estrogens. However, resistance to therapy is evident from the recurrence of tumor as a metastatic growth preferentially in bones, and resistance is observed in 30% of cases. Essentially, there are two forms of resistance:

1. De novo resistance
2. Acquired resistance

De novo resistance may be accompanied with loss of ER alpha expression while this is uncommon during acquired resistance. There are several mechanisms that may contribute to de novo or acquired resistance. Most of these resistance mechanisms are centered on the biology of ER alpha and/or ► **growth factor** receptors. ER alpha function is primarily influenced by ► **post-translational modification**, mostly ► **phosphorylation**, and by association with additional proteins of the transcription process. These factors, otherwise called coregulators, are sometimes overexpressed in cancers that fail anti-estrogen therapy. Thus, the magnitude of estrogen signaling in breast cancer is determined by the levels of ER alpha, kinases that phosphorylate ER

alpha, and coregulators that associate with ER alpha. Changes in the expression levels of these factors during the course of disease progression can play a role in acquired resistance.

### Breast Cancer Subtypes

Breast cancer is not a single disease. There are multiple subtypes. Previously, breast cancer was mainly classified into ER alpha-positive and ER alpha-negative types. ER alpha expression status along with nodal status and tumor grade influenced treatment decisions. Since 2000, this classification has been further refined into six subtypes:

1. Luminal type A
2. Luminal type B, both express estrogen ER alpha
3. HER2/Neu/ERBB2+
4. Basal-type
5. Claudin-low
6. Normal-like

Only luminal A and luminal B subtypes are relevant to anti-estrogen resistance.

#### Luminal Type A Subtype

Luminal subtype A cancers are generally considered to have the best good prognosis with a 90% 5-year survival rate followed by luminal B with 50%. Luminal type A tumors can be characterized by a hormonal signature comprised of the expression of three transcription factors: ER alpha, ► **FOXA1**, and GATA-3. Patients with tumors that express all three of these transcription factors display most favorable prognosis.

FOXA1 is a multifunctional transcription factor involved in activation as well as repression of transcription. It binds to target DNA sequences as a monomer, using a helix-turn-helix motif of ~110 amino acids (► **Helix-Loop-Helix Domain**). Unlike most transcription factors, FOXA1 binds to thousands of ► **enhancers** across the genome irrespective of ► **chromatin** organization. However, a substantial fraction of binding sites within a given cell type harbor relatively closed chromatin structure and lack apparent activity in positive gene expression. In actively transcribed regions, FOXA1 binding is associated with Histone H3 lysine 4 demethylation (H3K4me2; ► **Hypomethylation of DNA**) and H3K9 ► **acetylation**. In general, this cell type selective chromatin remodeling defines the active subset of FOXA1-bound enhancers. FOXA1 binding to these specifically marked chromatin regions enhances recruitment of ER

alpha to regions of chromatin that are enriched for both FOXA1 and ER alpha binding sites.

GATA-3 binding sites are enriched in regions that also bind to ER alpha. In ER alpha-positive breast cancer patients treated with tamoxifen, elevated expression of estrogen-regulated and GATA-3-regulated genes in primary tumor is associated with good prognosis. Loss of GATA-3 expression is associated with metastatic progression of breast cancer.

In the normal breast, ER alpha and FOXA1 are expressed in a small percentage of luminal epithelial cells. In contrast, ~30% of luminal epithelial cells express GATA-3. Considering similarity in FOXA1 and ER alpha expression pattern in normal breast, it is likely that there are at least four distinct ER alpha-positive breast epithelial cells:

1. ER alpha+/FOXA1+/GATA3+
2. ER alpha+/FOXA1+/GATA3-
3. ER alpha+/FOXA1-/GATA3+
4. ER alpha+/FOXA1-/GATA3-

These cell types are likely to exhibit distinct ER alpha binding pattern to genome and consequently estrogen-regulated gene expression. ER alpha-positive breast cancers expressing different levels of FOXA1 and GATA-3 are likely to express different set of estrogen-regulated genes, display variable degree of dependence on estrogen signaling for proliferation and survival, and hence to response to anti-estrogen.

#### Luminal Type B Subtype

Clinically, luminal B phenotype is associated with the expression of proliferation markers such as ► **Ki-67**. In fact, all ER alpha-positive breast cancers characterized by the expression of “proliferation signature” and associated poor prognosis may fall into this category. A small subfraction of these breast cancers also overexpress ► **HER2**. Growth factor signaling pathways are significantly active in these cells, suggesting that ER alpha:estrogen regulated signaling pathways and growth factor regulated signaling pathways are functionally redundant in these cancers. Cell line-based studies have provided some support for this possibility. The ER alpha-positive/HER2-positive cell line BT-474 treated with ► **lapatinib**, a HER2/EGFR growth factor pathway inhibitor, develops resistance. These resistant cells display functional ER alpha:estrogen signaling and are sensitive to combined lapatinib and anti-estrogen treatment. Therefore, the existence of redundant survival pathways may be

**Breast Cancer Anti-Estrogen Resistance.**

**Table 1** Differences between luminal A and luminal B breast cancers

Breast Cancer	Anti-Estrogen	Resistance.
Luminal type A	Luminal type B	
ER $\alpha$ and PR-positive	ER $\alpha$ or PR positive	
FOXA1-high	FOXA1-low or negative	
GATA-3-positive	GATA-3 $\pm$	
HER2-negative	Few HER2+ cases	
Low Ki67	High Ki67	
Hormone-dependent	Functional alternative growth factor pathway	
Low p53 mutation rate	40% p53 mutation	
95% 5-year survival rate	50% 5-year survival rate	
	Specific plasma proteome profile compared to luminal A or healthy	

responsible for the lack of response of luminal type B breast cancers to anti-estrogen therapy alone. **Table 1** provides a list of major differences between luminal type A and luminal type B breast cancers and **Fig. 1** provides schematic view of these differences, which can be utilized for therapeutic purposes.

### Gene Expression Signatures Predicting Response to Anti-estrogens

While luminal A and luminal B classification gives a simplistic view of ER alpha-positive breast cancers, tumors within these subtypes can show remarkable heterogeneity in the patterns at which individual genes are expressed. This heterogeneity of gene expression is likely to influence response to therapy and outcome. Predictive types of ► gene expression signature are ideally suited for further refining these subtypes. The field of breast cancer research has led the way in developing predictive gene expression signatures for solid tumors. Several of these predictive signatures have already entered clinical use. However, not surprisingly, there is no consensus on the signature that is most accurate.

At least three types of gene expression signatures have been described for ER alpha-positive breast cancers:

1. Predicts survival independent of endocrine therapy
2. Predicts response to endocrine therapy
3. Enables treatment decisions with respect to endocrine and chemotherapy

Luminal type A classification has been suggested to predict long-term survival independent of tamoxifen

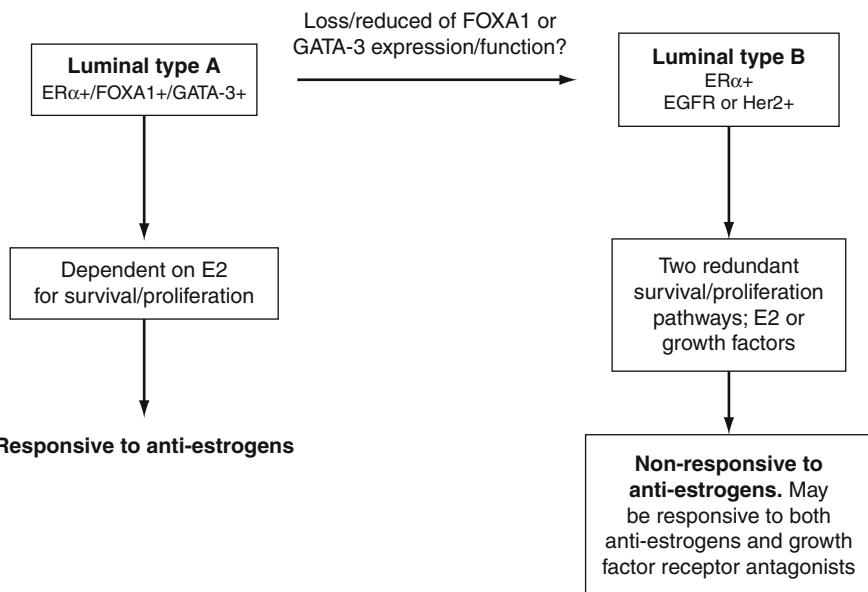
treatment. The 76-gene signature predicts outcome independent of tamoxifen treatment. Similarly, genomic grade signature helps to distinguish grade I tumors from grade III tumors at the molecular level, which may help in treatment decisions. This genomic grade index signature also cosegregates with poor response to tamoxifen treatment. The Oncotype Dx, which measures the expression of 21 genes (16 cancer-associated genes and five reference genes) enables clinicians to decide whether chemotherapy provides additional benefits to ER alpha-positive/node negative breast cancer patients treated with tamoxifen.

The number of gene expression signatures that predict response to tamoxifen treatment is growing day by day. Below are some of the examples:

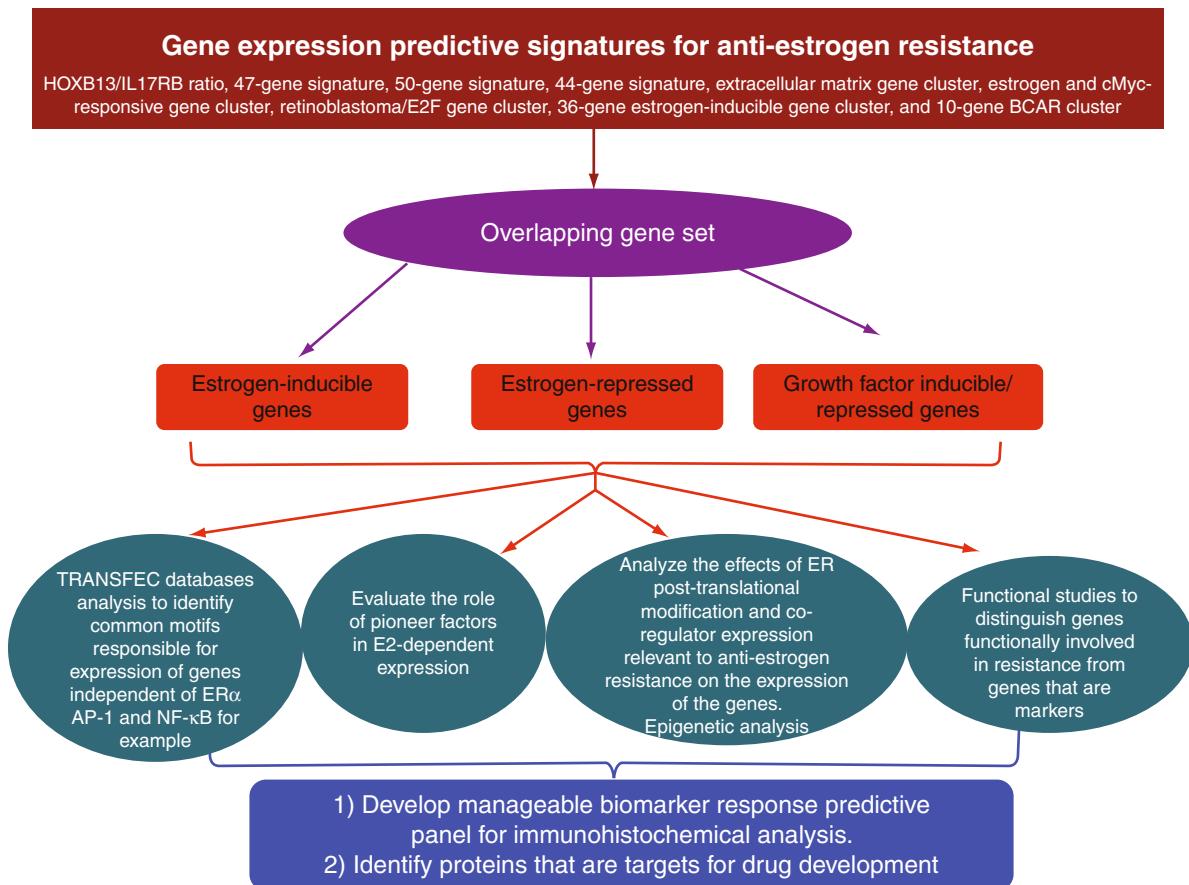
1. ► HOXB13/IL17RB expression ratio. A ratio of  $\leq 1.849$  is associated with better disease free survival among patients receiving tamoxifen treatment. IL17RB is an estrogen-inducible gene but lacks ER alpha binding sites. HOXB13 does not appear to be regulated by estrogen. Therefore, tumors with lower HOXB13/IL17RB ratio may have functional ER alpha network and expressing higher levels of estrogen-responsive genes.
2. A 47-gene signature identifies tumors that do not respond to tamoxifen treatment. In this signature, reduced expression of ER alpha, IGFBP4, ► Synuclein, ► BCL2 and ► FOS is associated with tamoxifen failure. Among these genes, estrogen positively regulates the expression of IGFBP4, BCL2, and FOS. Tamoxifen failure in these cases could be due to reduced activity of ER alpha, which forces cancer cells to adapt to alternative pathway of proliferation and survival.
3. A 50-gene signature that predicts response or de novo resistance to endocrine therapy. Patients who respond to treatment express high levels of a unique set of genes in their tumors prior to treatment and the expression of these genes in the tumors decline after treatment. S100P is expressed at 17.7-fold higher in untreated tumor and the expression decreases in responders after treatment. Cellular ► retinoic acid binding protein 2 (CRABP2), an estrogen-upregulated gene, is expressed at higher levels in responders and the expression decreases upon treatment. In contrast, perilipin is overexpressed in non-responders. Overall, similar to above two signatures, responders appear to have tumors with functional estrogen:ER alpha network.

**Breast Cancer Anti-Estrogen Resistance.**

**Fig. 1** Relationship between luminal type A and luminal type B breast cancers. There is no clinical evidence favoring or disproving progression of luminal A breast cancer to luminal B phenotype. E2 estrogen



4. A 44-gene signature that discriminates breast cancer patients with progressive disease and objective response to tamoxifen. Seventeen of these genes are involved in estrogen action; nine of them are up-regulated and eight of them are down-regulated in tamoxifen resistant tumors. Osteonectin (also called SPARC; ► **Secreted Protein Acidic and Rich in Cysteine**) is one of the genes up-regulated in resistant tumors. Estrogen:ER alpha suppresses the expression of this gene. TSC22D1 (► **Tuberous Sclerosis Complex**), a transcription factor overexpressed in tamoxifen resistant tumors, is generally suppressed by estrogen in cells that respond to tamoxifen treatment. Thus, loss of estrogen:ER alpha mediated suppression of these genes could potentially lead to tamoxifen resistance.
5. The ► **extracellular matrix** gene cluster comprising collagen 1A1 (COL1A1), ► **fibronectin** 1 (FN1), lysyl oxidase (LOX), secreted protein acidic cysteine-rich (SPARC, also called osteonectin), tissue inhibitors of metalloproteinases 3 (TIMP3), and ► **tenascin-C** (TNC). The expression levels of FN1, LOX, SPARC, and TIMP3 are associated with metastasis free survival in lymph node negative patients who received no adjuvant systemic therapy suggesting that the predictive value of these markers is independent of treatment. However, a high level of TNC is associated with a shorter metastasis free survival after ► **adjuvant** tamoxifen treatment.
6. Estrogen and MYC (► **MYC Oncogene**) responsive gene cluster. Estrogen-regulated genes can be subclassified into two categories: those that are also regulated by MYC ("Estrogen and MYC") and those that are not ("Estrogen but not MYC"). Elevated expression of a subset of estrogen-regulated genes that are also regulated by MYC and play a role in cell growth through ribosomal RNA and protein synthesis is associated with tamoxifen resistance. It is likely that deregulated expression/activity of MYC in the resistant tumors overcomes the need for ER alpha:estrogen for the expression of growth associated genes. In this respect, MYC controls the expression of several proliferation and growth associated genes and elevated expression of proliferation-associated genes is hallmark of anti-estrogen resistance and/or luminal B phenotype.
7. ► **Retinoblastoma**/► **E2F** target genes. Up-regulation of 59 genes regulated by retinoblastoma protein is associated with poor tamoxifen response. Loss of retinoblastoma protein results in elevated activity of E2F transcription factors. Estrogen:ER alpha up-regulates several members of E2F family transcription factors and these E2Fs mediate secondary estrogen response. Therefore, elevated estrogen secondary response genes may contribute to tamoxifen resistance. E2F family members control the expression of cell cycle, cell proliferation,



**Breast Cancer Anti-Estrogen Resistance.** **Fig. 2** Depiction of future studies essential for integrating clinical data with laboratory studies for elucidating the mechanisms of anti-estrogen resistance, biomarker development, and to identify therapeutic targets

and cell death (► Apoptosis) genes, and their deregulated activity could result in the expression of cell proliferation markers as evident in luminal B cancers.

8. A 36-gene signature derived from genes in the ► progesterone receptor (PR) pathway. All genes in this signature are induced by estrogen/ER alpha-positive cell lines and their expression in primary tumors correlates with PR expression. 30 and 28 of 36 genes of this cluster contain binding sites for ER alpha and FOXA1, respectively. Therefore, this 36-gene signature may be the most significant among all signatures in evaluating the function of ER alpha and FOXA1 in primary breast cancer and predicting response to tamoxifen in cancers that express variable levels of ER alpha and FOXA1.

9. A 10-gene BCAR (breast cancer anti-estrogen resistance) gene set. Genes in this set are ► AKT1, AKT2, BCAR1, BCAR3, ► EGFR, ► ERBB2,

GRB7, ► SRC, TLE3, and TRERF1. Among these genes, elevated levels of BCAR3 and TLE3 are associated with favorable prognosis, whereas elevated levels of ERBB2 and GRB7 are associated with poor outcome in patients treated with tamoxifen. Four among these genes have ER alpha and FOXA1 binding sites. Four among them are repressed by estrogen, while one of them is induced. Therefore, this signature mostly identifies genes repressed by estrogen and highlights how loss of estrogen-mediated repression contributes to tamoxifen resistance.

#### Challenges in Developing New In Vitro Models to Study Anti-estrogen Resistance

ER alpha-dependent and ER alpha-independent signaling events responsible for gene expression signatures in primary tumors can only be evaluated using in vitro model system. In this respect, it is important first to

identify a list of genes that overlap between these signatures and to determine whether expression of these genes are altered in ER alpha breast cancer cell lines that have acquired or intrinsically resistance to anti-estrogens. Several anti-estrogen resistant variants of breast cancer cell lines have been developed, and gene expression profiling data are available. Similar comparative gene expression profiling data on breast cancer cell lines that are sensitive to tamoxifen and de novo resistant to tamoxifen are also available. Importantly, genes regulated by estrogen in cell lines show similar expression pattern in primary breast cancers demonstrating relevance of these model systems. As in clinical breast cancers, the majority of cell lines with acquired or de novo resistance to tamoxifen express significant levels of ER alpha. The challenge is to combine gene expression signature available from different studies and identify common set of overlapping genes in these signatures. As several of the genes identified in the signatures appear to be also regulated by ► Myc, NF-kappaB, and ► AP-1, selective enrichment of binding sites for these transcription factors in genes associated with tamoxifen resistance is a likely possibility. At least three distinct pathways may emerge from this type of studies:

1. Genes that are enriched for ER alpha binding sites and are induced by estrogen and repressed by tamoxifen in sensitive tumors
2. Genes with or without ER alpha binding sites and are repressed by estrogen in vitro, and their expression is lower in responsive tumors
3. Genes that are up-regulated in non-responsive tumors and cell lines but lacking any relation to ER alpha and estrogen signaling

### Future Directions

There is an immediate need for comprehensive analysis of gene expression signatures from various studies to separate genes in the anti-estrogen response signature that are functionally linked to anti-estrogen resistance from those genes that only serve as biomarkers. Elucidating the role of FOXA1 and GATA-3 in establishing these signatures is also equally critical. As more data on specific types of ► histone modification associated with ER alpha:estrogen signaling become available, ► epigenetic events responsible for anti-estrogen resistance may be discovered. Such an effort is essential for mechanistic studies to better understand ER alpha biology, biomarker development

to distinguish acquired versus de novo resistance and to develop second-line targeted therapies for anti-estrogen resistant breast cancers (Fig. 2).

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### Breast Cancer Carcinogenesis

Beatriz Pogo and James F. Holland  
Tisch Cancer Institute Mount Sinai School of Medicine, New York, NY, USA

### Definition

Although ► breast cancer is still a major cause of morbidity and mortality in developed countries, the mortality rate has recently diminished due to earlier diagnosis and improved treatments. Incidence has risen, however, and one in eight American women are expected to develop breast cancer before age 90. Advances in biotechnology have allowed a better understanding of the molecular mechanism(s) involved in cancer initiation and progression, and have led to new therapeutic interventions.

### Characteristics

#### Breast Cancer Subtypes

In clinical practice, ► estrogen receptor (ER), ► progesterone receptor (PR) and epidermal growth factor

receptor (**► HER2**) status are determined by **► immunohistochemistry**. They identify the three major subgroups: a luminal group (E+/Pr+), an HER2+ group and a basal-like group which lacks ER and PR expression and HER2 over-expression, so-called **► triple negative breast cancer**. The terminal duct-tubular unit of the breast is the structure from which most cancers arise and they are mostly composed of two types of **► epithelial cells**: the inner or luminal cells which are surrounded by a basal layer of myoepithelial cells and the basal-like cells which express cytokeratins 5, 6, and 17 as do normal myoepithelial cells. By contrast, luminal tumors express more genes common to epithelial cells: **► E-cadherin** and cytokeratins 8 and 18. The advent of molecular profiling assays, especially for mRNA expression (**► Multigene Arrays**), has added more information about gene expression in subgroups. HER2+ cancers have the greatest frequency of high-level gene **► amplification** (independent of HER2 amplicon), while triple negative cancers show the highest overall frequencies of copy gain. These cancers also show more frequent loss of phosphatase and tensin homologue (**► PTEN**) and mutation of retinoblastoma protein 1 (**► RB1**). Amplification of **► cyclin D** (CCND1) and activating mutation of phosphatidylinositol 3-kinase (**► PI3K Signaling**) catalytic subunit alpha (PIK3CA) are mostly associated with ER and PR positivity. It has been concluded that all these changes may contribute to **► genomic instability** and that breast cancer subtypes are associated with distinct **► oncogene** pathways.

### Pathological Grade

The pathological grade is currently used as a prognostic **► biomarker** (**► Grading of Tumors**). Low-grade well-differentiated tumors (Fig. 1a) have a favorable prognosis, whereas poorly differentiated tumors (Fig. 1c) have a less favorable one. The group of intermediate-grade tumors makes determination of prognosis difficult. This pathological classification of breast cancer subtypes is based on tumor size, lymph node involvement, hormone receptor status, and **► HER2** expression. However, there is high heterogeneity at the molecular level, and the clinical outcomes and responses to treatment can be variable. It has been proposed that intermediate-grade tumors do not represent an independent subtype, but they are “clinical” and molecular hybrids between low- and

high-grade tumors. A linear model of progression from low to high was implied. Identification of the molecular changes involved in this progression may provide new molecular targets for therapeutic developments. Clinical subtypes and gene expression signatures have been fundamental for determining prognostic risk and treatment.

The natural history of breast cancer progressing from abnormal epithelial proliferation from *in situ* and invasive carcinoma to metastatic disease has been well documented. Transition from **► ductal carcinoma *in situ*** (DCIS) to invasive tumor is poorly understood, however. Using an experimental model, the progression to invasion has been shown to be promoted by fibroblasts and inhibited by normal **► myoepithelial cells**. Elimination of markers of myoepithelial differentiation leads to progression and invasion. Myoepithelial cells are considered to be natural tumor suppressors. The importance of the stroma in influencing progression of epithelial tumors has been shown. The **► gene expression profile** of tumor stroma (and derived gene expression signature) was found to be associated with clinical outcome. A new stroma-derived prognostic predictor (SDPP) has been introduced that helps to identify poor-outcome individuals from multiple clinical subtypes.

### Genetic Alterations

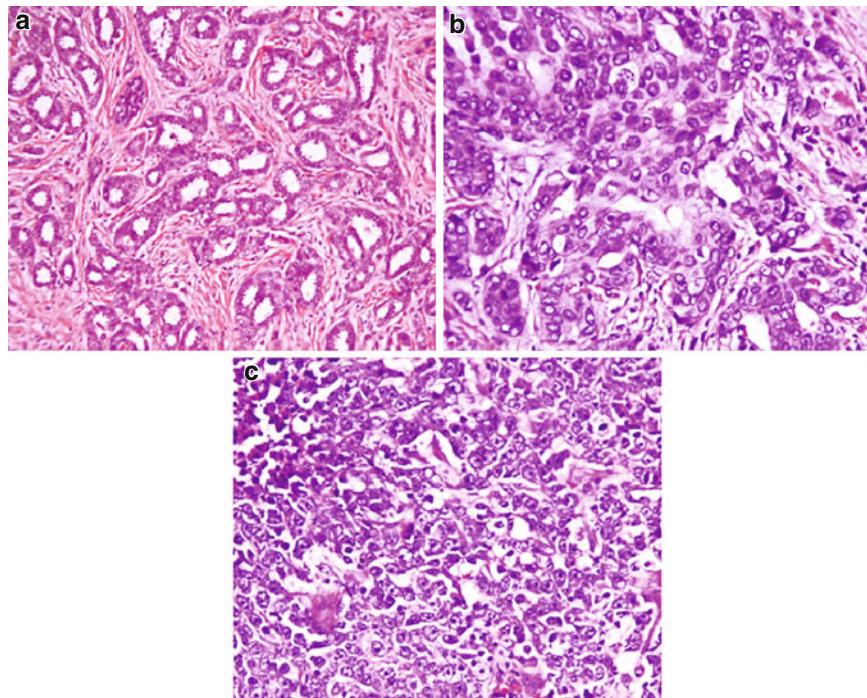
Known susceptibility genes account for less than 25% of family risk. Two genes are associated with familial breast cancer, the BRCA1 which is located in chromosome 17q21 and the BRCA2 located in chromosome 13q12 (**► BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk**). Studies on BRCA1 mutations have proved that BRCA1 is a **► tumor suppressor gene** and its function is related to **► DNA repair** of **► DNA damage**. The BRCA2 mutations are associated with early-onset cancer of the breast and ovary in females and breast cancer in males. It is also related to **► prostate cancer** and pancreas cancer (Pancreas Cancer Clinical Oncology). Breast cancer is also one cancer developing with the spectrum of cancers of the **► Li-Fraumeni Syndrome**, which results from germline mutations of the **► TP53** gene. High expression of BRCA1 is usually associated with a basal cell type breast cancer, luminal cell types have the **► KIT** receptor tyrosine kinase (**► Receptor Tyrosine Kinases**) overexpressed. This observation implicates luminal progenitor cell as a probable target population

### Breast Cancer Carcinogenesis.

**Fig. 1** Grading of Tumors.

Invasive breast carcinoma.

(a) Well-differentiated carcinoma (grade 1) composed of well-formed glands lined with cells that have uniform small nuclei. (b) Moderately differentiated carcinoma (grade 2) composed of glands and some solid sheets. Tumor cell nuclei are slightly enlarged and show mild pleomorphism. (c) Poorly differentiated carcinoma (grade 3) composed predominantly of solid cords and sheets. Tumor cell nuclei are enlarged and show pleomorphism



in BRCA1-associated and other types of ► [basal-like breast cancer](#).

In order to identify breast cancer risk loci, an extensive two-stage genome-wide association study of 4,398 breast cancers and 4,316 controls has been done which 30 single nucleotide polymorphisms (SNPs). It was estimated that there were SNPs in five novel independent loci that exhibited strong association with breast cancer. Four of the five loci contain putative causative genes, ► [fibroblast growth factor receptor 2](#) (FGFR2), trinucleotide repeat motive containing nine (TNRC9) probably a ► [transcription factor](#), ► [mitogen-activated protein kinase](#) (MAP3K), and lymphocyte-specific protein 1 (LSP1). Other loci previously identified were G2 ► [checkpoint kinase](#) (CHEK2; ► [Checkpoint Kinases](#)) and ► [ataxia telangiectasia mutation](#) (► [ATM](#)).

### Endocrine and Reproductive Risk Factors

Early onset of menarche and late onset of menopause, denoting many years of ovarian activity, increase the risk of breast cancer. Numerous studies indicate that there is strong correlation between estrogen level (► [Estrogenic Hormones](#)) and breast cancer development. ► [Breast cancer epidemiology](#) studies have shown that women who have their first pregnancy

before 18 years have 1/3 lower incidence of breast cancer than women who had their first child after 35. These findings are interpreted as due to stimulatory effect on an involuting epithelium. Other factors to be considered are number of pregnancies and lactation. Each birth increases the risk of breast cancer, but lactation for long periods seems to be a protective factor.

### Exogenous Hormones

The use of ► [hormone replacement therapy](#) (HRT) and breast cancer incidence has been studied by several groups. The Collaborative Group on hormonal factors in breast cancer found that a 1.35% modest increase of breast cancer was associated with 5 or more years of HRT. Other groups, Women's Health Initiative and the Million Women Study have found a higher increase, 26% after 5 years of HRT. Abrupt decrease in HRT in 2005 because of publicity led to a sharp drop in breast cancer incidence in the next 2 years, suggesting that HRT had been a promoting agent rather than an etiologic one. Other hormonal-related events like abortion and physical activity have been studied. No sufficient evidence exists that abortion plays a role. Strenuous physical activity in adolescence is related to a reduction of breast

cancer that may be correlated with retarding the onset of ovulation.

### Environmental Factors

Incidence of breast cancer varies in different parts of the world. It is high in North America and Western Europe, and lower in Asia and Latin America ([► Breast Cancer Epidemiology](#)). Changes in risk have been recorded when people migrate from one low-incidence country to a high-incidence one suggesting environmental factors. The most obvious change in risk factors appears to be a difference in diet. A high calorie intake rich in saturated fats may be linked to increased cancer risk. [► Alcohol consumption](#) is also a risk factor perhaps due to increased endogenous estrogen levels. The risk associated with alcohol can be reduced by intake of folate [► Cancer Causes and Control](#).

### Radiation Exposure

Exposure to [► ionizing radiation](#) is a factor; repeated fluoroscopic chest radiography increases risk. Mediastinal radiotherapy treatment for lymphoma increases breast cancer risk. Cigarette smoking and caffeine consumption have shown no definitive correlation with breast cancer risk.

### Viruses

Several viruses have been reported to be associated with breast cancer, including: [► Epstein–Barr Virus](#) (EBV), [► Human Papillomaviruses](#) (HPV), bovine leukemia virus (BLV), and the [► Mouse Mammary Tumor Virus](#) (MMTV). Also, the expression of human [► endogenous retrovirus](#) K-10 (MERK-10) has been correlated with breast cancer. Association does not mean causation, and until now, none of these agents has fulfilled the requirements for causation.

### Clinical Studies

Mammography, together with other imaging techniques, has been associated with decreased mortality from breast cancer, although many discovered tumors may be slow growing and of low virulence. Virulent rapidly growing tumors, particularly [► triple negative breast cancer](#), may become clinically evident between annual screenings and thus be discovered relatively late by the current imaging schedules. Adjuvant hormonal treatment ([► Adjuvant Therapy](#))

for ER + tumors has evolved from [► tamoxifen](#), a synthetic estrogen receptor modifier to aromatase inhibitors ([► Aromatase and its Inhibitors](#)) which diminish postmenopausal estrogen conversion from other steroid precursors. For both metastatic and postsurgical adjuvant treatments, aromatase inhibitors have shown better disease-free survival. Combination chemotherapy regimens for adjuvant treatment are better than single drugs. Such regimens for use after surgical excision have become more effective when they contain a [► taxane](#) and an [► anthracycline](#) or [► platinum complexes](#) than earlier formulas containing [► alkylating agents](#) and an [► anti-metabolite](#). Adjuvant chemotherapy regimens have improved survival by about 20%.

After [► fine needle aspiration biopsy](#) to establish a diagnosis, primary induction chemotherapy ([► Neoadjuvant Therapy](#)) has substantially decreased primary tumor size, thereby increasing the feasibility and frequency of lymphectomy rather than mastectomy. Compared to post-operative adjuvant chemotherapy, however, improved survival has occurred only in those patients whose primary tumor is completely eradicated by the treatment. This finding offers a valuable path forward: study of the primary tumor biopsy to select the optimal endocrine or chemotherapeutic treatment regimen based on genomic or proteomic data. Eventually, those who sustain complete pathologic eradication of the primary tumor may not require surgery.

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### Breast Cancer Contralateral

- [► Contralateral Breast Cancer](#)

## Breast Cancer Drug Resistance

Christina L. Addison and Miguel A. Cabrita  
Cancer Therapeutics Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada

### Definition

► **Breast cancer** drug resistance refers to the failure of chemotherapy at the outset of treatment or at some point after treatment has commenced. Resistance can thus be defined as intrinsic or acquired, depending upon presentation.

### Intrinsic Drug Resistance

Drug resistance that is present from the onset of therapy whereby a subpopulation of tumor cells are inherently resistant to the treatment regimen.

### Acquired Drug Resistance

An initial response to treatment followed by subsequent progression of disease. This occurs when a subpopulation of tumor cells develops resistance following exposure to selective pressures placed upon them in the presence of cytotoxic drugs.

### Additional Factors Contributing to Resistance

Resistance may also result from ► **microenvironment** influences that modulate the efficacy of a drug or the manner in which tumor cells respond to any given drug. Other factors that may influence responses to drugs are factors that may vary between individual patients such as differences in pharmacological clearance of the drug by the liver, poor tolerance of drug side effects that limit the tolerable dose administered and differences in immune and ► **inflammation** responses that may alter drug activities.

### Characteristics

Traditionally, breast cancer ► **drug resistance** has been described in terms of the available treatments. One mechanism can account for resistance to various therapeutic agents (i.e., cross-resistance), especially if they are derived from the same general class of compounds or have a similar target. Understanding breast cancer

drug resistance requires knowledge of current breast cancer drug regimens and their targets. Surgery is routinely the first step in treatment followed by systemic therapy in the form of cytotoxic ► **chemotherapy**, ► **hormonal therapy**, and/or ► **immunotherapy** (Table 1). In addition to these different regimens, breast cancer may also be treated by ► **radiotherapy** alone or in combination with systemic therapy.

### Mediators of Drug Resistance

► **Multidrug resistance** (MDR) is a limiting factor to the efficacy of systemic therapy and is a very serious concern in the treatment of breast cancer. Cancer cells may be intrinsically resistant to various drug agents. Alternatively, as they are challenged by different drugs, tumor cells may mutate and become refractory to various therapies as a result of selective pressures, thus rendering the therapies ineffective. The end result is that patients may have to change to a different drug regimen or stop therapy altogether (i.e., treatment failure).

### Drug Efflux Transporters

MDR was first reported to be mediated by overexpression of ► **P-glycoprotein** (MDR1), a 170-kDa multiple transmembrane domain protein initially characterized in cultured cells. MDR1 is a member of the ATP-binding cassette (ABC) family of transporters, a large superfamily now known to encompass at least 49 ► **ABC transporter proteins**. MDR1 acts as an efflux pump by removing a variety of structurally diverse compounds from the cell (► **Membrane Transporters**) including many therapeutic agents used to treat breast cancer such as ► **taxanes**, ► **vincristine**, ► **doxorubicin**, and ► **etoposide**. Many ABC proteins, including MDR1 (ABCB1), multidrug resistance-associated protein (MRP1; ABCC1) and ► **breast cancer resistance protein** (BCRP; ABCG2), have been extensively studied in tumor cell lines and in breast cancer patients. However, while there have been numerous studies examining the relationship between the expression levels of these efflux pump proteins and drug resistance in breast cancer patients, the results have not been consistent. The fact that positive correlation between resistance to therapy and ABC transporter expression does not always occur clinically could be due to many factors including the heterogeneity of breast cancers, the methods used to examine expression levels, differences in the patient

**Breast Cancer Drug Resistance. Table 1** Common forms of systemic therapy used in the treatment of breast cancer

Type of systemic therapy	Mode of action	Mechanism of resistance
► Anthracyclines (e.g., ► doxorubicin, ► epirubicin)	Intercalates in DNA and hence inhibits DNA Topoisomerase II activity thus inducing apoptosis, activation of signaling pathways, and production of reactive oxygen intermediates.	Increased expression or gene amplification of the P-170 multidrug resistance protein (MDR) or the Multidrug resistance-associated protein (MRP); altered topoisomerase II
► Taxanes (e.g., ► paclitaxel)	Blocks appropriate microtubule function thus inhibits cell division (mitosis).	Changes in expression of beta-tubulin isoforms; increases in MDR expression
Antimetabolites (e.g., 5-► fluorouracil, ► methotrexate)	Blocks enzymes involved in nucleic acid metabolism (e.g., dihydrofolate reductase, DHFR), thereby inhibiting DNA replication and cell proliferation.	Changes in expression or activity of enzymes involved in drug metabolism, for example, ► amplification of DHFR gene; defective folate transporter
► Nucleoside analogs (e.g., ► capecitabine, ► gemcitabine)	Inhibits activity of DNA polymerase alpha thus prevents DNA chain elongation, hence replication and cell proliferation	Defects in nucleoside transport; altered activity of enzymes involved in nucleoside metabolism (e.g., kinases and ribonucleotide reductase)
► Alkylating agents and ► platinum drugs therapies (e.g., ► cisplatin, ► carboplatin)	Alters structure of DNA thereby inhibiting replication and cell proliferation	Altered drug accumulation; drug inactivation in the cytosol; increased repair of ► DNA damage; reduction in apoptosis
► Vinca alkaloids (e.g., ► vincristine, ► vinblastine, vinorelbine)	Prevents polymerization of ► tubulin, hence cell division.	Changes in expression of alpha and beta tubulin; increased expression or gene ► amplification of MDR
► Hormonal therapy (e.g., ► Tamoxifen)	Estrogen receptor (ER)	Altered or lack of ER expression; increased expression levels of ErbB2; altered pharmacology of drug due to genetic factors.
► Trastuzumab	Blocks signaling activity of the receptor tyrosine kinase ErbB2 (► HER-2/neu), leading to down regulation of ErbB2 expression or activity, thus decreased cellular proliferation and increased cellular ► apoptosis	Increased expression of ► epidermal growth factor receptor; increases in ► insulin-like growth factor-1 expression, increased expression of beta 1-► integrin

populations studied or the variability in the chemotherapeutic drugs administered.

### Cyclin E

► Cell division involves a series of precisely controlled steps that can be subdivided into four stages: cell growth (G1-phase), monitoring (G2-phase), DNA synthesis (S-phase), and mitosis (M-phase). These phases are controlled by a number of different proteins including a group of cell cycle regulatory proteins called ► cyclins, which in turn are regulated by a family of serine/threonine protein kinases (► Serine/Threonine Kinase), the ► cyclin-dependent kinases (Cdk). Cyclin E is an important G1-associated cyclin that associates with Cdk2 in late G1-phase to regulate the transition to S-phase. Cyclin E is synthesized during the early stages of G1-phase and is degraded during the S-phase. When this synthesis/degradation pattern becomes aberrant, the cell cycle becomes altered and deregulated.

Deregulation of Cyclin E is an important ► prognostic biomarker in early stage breast cancer and may contribute to resistance of some forms of therapy. Specifically, breast tumors can generate hyperactive low molecular weight (LMW) forms of Cyclin E for which Cdk2 has a higher affinity. This complex of Cdk2 with LMW isoforms of Cyclin E leads to increased Cdk2 activity, resulting in profound effects on cells including reduced cell size, lower cell growth rates, and growth factor-independent proliferation. The subsequent deregulation of the cell cycle causes the cells to be refractory to many types of chemotherapeutic treatments, since the majority of these are targeted to rapidly proliferating cells.

### Transcription Factors

**p53** ► p53 is a ► transcription factor that controls the expression of a large number of genes associated with cell growth. It is mutated in 50% of all cancers, and p53 mutations contribute to drug resistance (► TP53).

Furthermore, certain types of p53 mutation and/or ► polymorphism correlate with positive and negative responsiveness to various breast cancer therapies. For example, mutations in the DNA binding domain of p53 alter its transcriptional activity and correlate with poor response to ► tamoxifen. However, patients carrying a common polymorphism at ► codon 72 are more likely have to a good pathological response to standard chemotherapy compared to those that lack the polymorphism. Thus, knowing the type of p53 mutation/polymorphism of a particular tumor can be important in predicting response to therapy.

**BRCA1** BRCA1 is another nuclear protein that controls transcription (positively and negatively) and is involved in chromatin remodeling and DNA damage repair. Mutations in BRCA1 (► *Breast Cancer Genes BRCA1 and BRCA2*) cause familial breast cancer risk (about 10% of all breast cancers), but BRCA1 can also be lost or downregulated in cases of sporadic breast cancer. Individuals with these mutations have an enhanced sensitivity to chemotherapeutic agents that target DNA, such as ► cisplatin and ► anthracyclines. Since BRCA1 plays a crucial role in the repair of ► DNA damage, these findings are not surprising. However, patients that lack or have decreased amounts of BRCA1 are also more resistant to therapies that target ► microtubules (e.g., ► taxanes and ► vincristine). Moreover, BRCA1 binds to gamma-► tubulin and thus regulate ► centrosomes and the mitotic checkpoint. Thus, patients with BRCA1 mutations are predicted to be more responsive to DNA damaging agents. However studies have shown that the responses to these agents are mixed (i.e., some tumors are more sensitive while others do not respond).

**Epidermal Growth Factor (EGF) Receptor Family** The ► epidermal growth factor receptor family of proteins includes EGFR (ErbB1), ErbB2 (► HER-2/neu), ErbB3, and ErbB4. These transmembrane proteins are overexpressed in numerous breast cancers including many estrogen receptor negative tumors, which tend to be the most clinically aggressive. Members of this receptor family can homo- or heterodimerize. EGFR binds to EGF, while ErbB2 is an ► orphan receptor (i.e., has no known ligands) and exists in an open conformation. ErbB3, although catalytically inactive, binds to many ligands and can heterodimerize with any of the other family members

to influence their signaling. ErbB4 binds to neuregulins and has a functional tyrosine kinase domain. In breast cancer triage, ErbB2 (HER-2/neu) status is another widely used classifier. Tumors overexpressing ErbB2 tend to be resistant to agents that damage DNA, perturb microtubule networks or induce apoptosis. It has been proposed that ErbB2 can inhibit paclitaxel-mediated apoptosis by preventing activation of p34/Cdc2 kinase (directly or indirectly). Despite clinical and laboratory evidence, the mechanism by which ErbB2 overexpression mediates drug resistance is controversial as there are studies which suggest the opposite. However, tumors overexpressing ErbB2 are more sensitive to trastuzumab (► Herceptin), an antibody that binds to the extracellular domain of ErbB2, and in vitro ► trastuzumab has been shown to sensitize breast cancer cells to other therapeutic agents. In clinical studies, some patients have shown therapeutic benefit from inclusion of trastuzumab in an ► adjuvant setting.

**Estrogen Receptors** Tamoxifen is the most common anti-estrogen and one of the most effective treatments given to estrogen receptor (ER)-positive breast cancer patients. However, hormone resistance and relapse is routinely observed in patients treated with tamoxifen for extended periods of time (i.e., 5 years or greater). A variety of mechanisms have been postulated to explain this refractory to hormone therapy. After prolonged periods of exposure to anti-estrogens, the balance of proliferative and apoptotic signals in tumors can be altered so as to promote tumor growth. Furthermore, ER activity is modulated by many coactivators and corepressors whose expression levels are modified after extended treatment with tamoxifen. In addition, hormone resistance has been linked to the increased activity and expression of EGFR and ErbB2 upon exposure to tamoxifen. Activation of other signal transduction pathways, such as MAPK and PI3K/AKT, have also been implicated in hormone resistance, however the exact molecular mechanisms have not yet been elucidated.

#### Tumor-Stroma Interaction-Induced Resistance

Extracellular Matrix Composition and Integrin Signaling  
The composition of the ► extracellular matrix (ECM) within tumors is very different from that of normal counterpart tissues. In breast cancer, increases in

collagen I and fibronectin deposition and decreases in normal basement membrane collagen IV levels have been documented. Furthermore, dramatic changes in the ECM, particularly the collagenous stroma, occur in the normal mammary gland as it transitions to ductal infiltrating carcinomas of the breast. Expression of various ECM components has been correlated with patient survival and recurrence risk in breast cancer. The major cellular receptors that interact with ECM are the heterodimeric integrins. ECM-integrin signals are bidirectional. Following binding of ECM ligands, conformational changes in integrins result in activation of various downstream kinases, a process termed “outside-in signaling,” which leads to signal transduction events that control various aspects of tumor cell biology including cell differentiation, migration, adhesion, proliferation, survival, and response to chemotherapeutic drugs. As an example, it has been shown that integrin-laminin binding can induce polarization of breast tumor cells, which was sufficient to mediate resistance to chemotherapy-induced apoptosis. Integrins can also be induced to undergo conformational changes following activation stimuli in cells, such as EGFR activation, that result in increased affinity of integrins for binding ECM ligands, a process termed “inside-out signaling.” Inside-out signaling may thus alter the adhesive or invasive properties of tumor cells and contribute to their aggressiveness and response to therapy. ECM-integrin binding has also been shown to “cross talk” with other signaling pathways such as those induced by growth factor receptors (GFR). For example, Src can be activated downstream of both integrins and various GFR such as EGFR, and activation of Src downstream of certain integrin-ECM ligands can result in enhanced duration and intensity of EGFR signaling, thereby contributing to EGFR-mediated resistance pathways.

In experimental models of acquired drug resistance, many changes in tumor cell produced ECM proteins and expression of their integrin receptors are known to occur, suggesting that tumor cells may actively reorganize their ECM microenvironment and their response to it. As an example, MCF7 tumor cells that were made multidrug resistant following exposure to increasing concentrations of multiple drugs (including paclitaxel, docetaxel, vincristine, and doxorubicin) had upregulated the expression of ~25 different ECM or integrin genes compared to the drug-sensitive parental control cells. Given that many of these interactions

are favorable for tumor cell survival, it is likely that a process of selection for these ECM-producing and ECM-responsive tumor cells occurs in the presence of cytotoxic drugs. This would result in a process of clonal selection and expansion of tumor cells with decreased apoptosis and hence increased drug resistance following treatment. Different ECM proteins also have different affinities for binding and possibly sequestering various drugs and proteins. Therefore, the composition of the tumor ECM may affect the efficacy of drug treatment by altering its ability to be delivered to tumor cells.

#### Vessel Integrity, Angiogenesis, and Hypoxia

Tumor ► angiogenesis results from a stimulation of resident endothelial cells to form new blood vessels and also from the recruitment of circulating endothelial progenitor cells to the tumor site where they become incorporated into the newly forming vessels. As the angiogenic process is exacerbated in tumors, the tumor vessels themselves are quite abnormal and tortuous, leading to overall reduced blood flow and hence impeding drug delivery to tumors and hence contributing to lack of clinical response. The reduced blood flow also leads to increased tumor ► hypoxia. Hypoxia inhibits the response of tumor cells to both chemo- and radiotherapeutic regimens and occurs primarily in response to increased activity of hypoxia inducible factor (HIF). HIF is a transcription factor that is responsible for production of proteins designed to scavenge and remove oxygen free radicals. As many chemotherapy agents rely on oxygen radicals to induce significant cellular damage, their efficacy is negatively impacted by HIF activity. HIF also increases the expression of many of the drug efflux transporters, hence resulting in reduced drug levels within tumor cells. HIF also modulates DNA repair pathways thereby leading to increased ► genomic instability and a mutator state in cells, all of which may contribute to selection of mutated tumor cells with drug resistant phenotypes. Furthermore, when a tumor region is hypoxic, usually due to its increased distance from tumor vessels, the amount of drug delivered to these regions is inherently decreased as many drugs are delivered intravenously.

#### Tumor-Stromal Cell Interactions

Tumors are comprised of not only tumor cells and extracellular matrix, but many other cell types and

proteins commonly referred to as the tumor ► **stroma**. Stromal cell types include cancer associated fibroblasts (CAF), immune cells such as ► **macrophages**, and ► **endothelial cells** that contribute to tumor-associated angiogenesis. Moreover, the tumor stroma can comprise ~50–90% of the tumor itself. Factors produced by the tumor cells, such as ► **transforming growth factor beta** (TGF $\beta$ ) and ► **platelet-derived growth factor** (PDGF) lead to recruitment to and activation of stromal cells within the tumor. Moreover, these factors can induce stromal cells to secrete other growth factors, remodel the ECM microenvironment, and induce immune tolerance to tumor antigens within infiltrating immune cells. All of these processes can alter the response of tumor cells to various therapeutic strategies. Increased stromal macrophage density has been associated with poor prognoses in breast cancer. Macrophages increase the invasiveness of tumor cells and can contribute to enhanced tumor-associated angiogenesis via production of various growth factors. These in turn may activate survival pathways in tumor cells, making them more refractive to apoptotic signals. In addition to this, CAF represent a large proportion of the tumor cellular stroma. These cells resemble myofibroblasts and are usually  $\alpha$  smooth muscle actin-positive, and histologically they resemble fibroblasts observed in normal tissue healing wounds. CAF also contribute to aggressive tumor growth in part by their ability to secrete stromal-derived factor 1 alpha (SDF1 $\alpha$  which, in addition to recruiting circulating endothelial progenitor cells to tumor sites and promoting tumor-associated angiogenesis, has profound effects on tumor cell proliferation itself, for example, as shown for breast tumor cells harboring the SDF1 $\alpha$  receptor, CXCR4. The importance of CAF has been demonstrated by studies in which they have been co-implanted with tumor cells in in vivo models. The presence of CAF induced much more aggressive tumor growth than did co-implantation of normal fibroblasts, and CAF also stimulated tumorigenic growth from non-tumorigenic epithelial cells upon co-implantation. Although currently there is no evidence suggesting a direct influence of CAF in mediating drug resistance, their contribution to tumor aggression and angiogenesis suggests that they play an important role in modulating the tumor microenvironment and hence drug delivery and response to therapy in general.

## Cancer Stem Cells and Resistance

Over 150 years ago, it was first proposed that cancers arise from germ or stem cells, however this hypothesis has now become a major focus of investigation in the biology of tumorigenesis and also in the response of cancers to various therapies. In part, the renewed focus of investigation is a result of advances in technology and in understanding the biology of putative stem cells. Stem cells are defined by their ability to undergo self-renewal and their pluripotency (ability to differentiate into multiple cell lineages). The discovery of proteins preferentially expressed in stem cells has allowed the identification and purification of these cells from various tissue sources (► **Stem Cell Markers**). Human breast cancers possess a subpopulation of cells, characterized by expression of certain cell markers that display stem cell properties (► **Breast Cancer Stem Cells**). These cancer stem cells (CSC) have the ability to form tumors following transplantation into ► **xenograft** animal models, which then display the phenotypic heterogeneity of the cell types within the original tumor from which they were isolated. Transplantation of as few as 20–200 CSC results in subsequent generation of these tumors. The presence of CSC in human tumors has significant implications for the diagnosis and clinical management of cancer patients. As stem cells generally have reduced proliferative capacities, they may be refractory to most conventional cancer therapies that are designed to target rapidly proliferating cells. Given the possibility that they can produce a tumor with as few as 20 remaining cells, the presence of CSC may be a critical mediator of cancer recurrence following treatment.

### Resistance of Cancer Stem Cells to Conventional Cancer Therapies

CSC isolated from human tumors are fairly resistant to conventional chemotherapy and radiation therapy as compared to the non-CSC populations (► **Breast Cancer Stem Cells**). This may be explained in part by the fact that CSC commonly express proteins known to modulate response to drugs, including drug efflux pumps and thus have enhanced intrinsic capabilities to remove drugs or overcome their inhibitory activities. CSC are also more adept at stimulating ► **angiogenesis** within tumors and therefore can contribute to more aggressive tumor growth both before and after therapy.

**Aldehyde Dehydrogenase** CSC express higher levels of metabolic mediators such as aldehyde dehydrogenase (ALDH1; ► **Detoxification**). ALDH1 has been used as a marker for selection and isolation of CSC from tumor homogenates. As ALDH1 confers resistance to ► **cyclophosphamide** in normal stem cells, it is likely that the same is true for CSC. Reduction of BRCA1 expression (► **Breast Cancer Genes BRCA1 and BRCA2**) in normal breast epithelial cells resulted in increased expression of ALDH1 in conjunction with decreased expression of ER, suggesting that loss of BRCA1 may contribute to tumorigenesis and resistant cancers in part from generation of more stem-like cancer cells. In a similar manner, overexpression of ErbB2 (► **HER2/neu**) increases the proportion of ALDH1-positive CSC, further suggesting that the resistance mechanisms imparted by many previously identified factors may be due to increases in the CSC component of tumors. ALDH1 is also associated with poor prognosis in cancer patients, suggesting that drug resistance mediated by CSC may directly influence overall patient response to therapy.

**Enhanced DNA Repair Mechanisms** The majority of the experimental evidence for enhanced ► **DNA repair** capacity in CSC is in the context of response to radiation damage. However, many of the DNA repair enzymes implicated are also involved in response to DNA damage induced by some chemotherapeutic agents and thus may also play a role in mediating CSC drug resistance. In general, CSC repair DNA damage more rapidly than non-CSC tumor cells due in part to an ability to more readily activate DNA damage ► **checkpoint** mechanisms. CSC have a basal activation of this checkpoint and thus are primed to rapidly induce repair mechanisms in response to ► **genotoxic** stress. Two of the ► **checkpoint kinases**, ► **CHK1** and ► **CHK2**, are critical mediators of resistance to radiation in CSC, and inhibition of these two kinases by specific small molecule inhibitors resulted in sensitization of cells to the genotoxic stress. As many chemotherapy agents commonly used to treat breast cancer (see **Table 1**) also primarily damage DNA, therapeutic agents that target these DNA damage checkpoint regulatory proteins may become extremely useful in ► **adjuvant** therapies to help overcome intrinsic chemoresistance of CSC.

**Altered Transcription Factor Activity** Many ► **transcription factor** pathways known to control stem cell self-renewal and differentiation are also found to be overexpressed and active in CSC. Some of these, such as the canonical Wnt (wingless-type mouse mammary tumor virus integration site) pathway (► **Wnt Signaling**), result in increases in the proportion of tumor cells expressing stem cell markers in ► **transgenic** animal models. Wnt binds to cell surface receptors of the Frizzled family, which results in the downstream translocation of ► **Beta-Catenin** to the nucleus where it activates the LEF1/TCF family of transcription factors, many of which are required for embryonic mammary development from normal stem cells. In addition to its role in modulating increases in the proportion of CSC within tumors, ► **DNA damage** may induce activity of the Wnt/β-catenin pathway thus enhancing its CSC-promoting activities. As the Wnt/β-catenin pathway also promotes ► **genomic instability**, its activation in CSC populations treated with DNA damaging agents may additionally promote mutation within these populations in response to selective pressures which may thus contribute to induction of acquired resistance. Other proteins that play an important role in stem cell self-renewal and differentiation are the Notch family of proteins (► **Notch/Jagged Signaling**). Upon activation by ligand binding, proteolytic cleavage of the Notch receptors results in translocation of the cytoplasmic domain of Notch to the nucleus and subsequent induction of downstream transcriptional targets. During development, Notch plays a role in regulating asymmetric cell division and hence promotes self-renewal of stem cell precursors. Approximately 40% of human breast cancers have reduced expression of the Notch inhibitor NUMB, suggesting a role for activated Notch pathways in human breast cancer. Notch activation occurs also in CSC in response to DNA damaging agents such as radiation. Therefore, in addition to promoting CSC self-renewal, Notch may also contribute to resistance to genotoxic stress. A link between Notch activation and ErbB2 (HER2/neu) overexpression has been demonstrated whereby inhibition of Notch signaling resulted in decreased ErbB2 (► **HER2/neu**) expression. Given the demonstrated role of ErbB2 (HER2/neu) in breast cancer progression and response to various therapies, which may in part be a result of its increased expression in the CSC population of breast

tumors, targeting Notch may be a viable therapeutic option to target CSC and tumor recurrence post-treatment.

### The Future of Breast Cancer Therapy: Pharmacogenetics and the Promise of Personalized Medicine

Through the use of new high-throughput DNA technologies, a thorough understanding of how breast cancers evolve and which treatments work best for particular types of breast cancer is emerging. For example, scientists have been able to track changes in the ► **gene expression signature** of a cancer from a single patient as it evolved from a primary to a metastatic lesion. Such studies lead one to think that in the future, a personalized treatment regimen may be achievable. In some cases, this is already occurring, although to a limited degree. ► **Pharmacogenetics** studies use information from a patient's genetic traits to predict responses to various therapies. For example, some women are unable to metabolize tamoxifen properly due to inactive forms of a gene encoding a liver enzyme required for its proper activation. As a result, many women are now being tested for this liver insufficiency prior to administration of tamoxifen. Moreover, from many breast cancer patients now an mRNA ► **gene expression signature** is established (e.g., <http://www.oncotypedx.com/>) to determine the likelihood that they will respond to certain breast cancer therapies (► **Breast Cancer Anti-Estrogen Resistance**).

Thus, while breast cancer drug resistance continues to be a challenge in the clinic, technological and biological advances and the advent of personalized medicine will make a difference in mitigating the effects of resistance in future therapeutic strategies.

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## Breast Cancer Epidemiology

Randall E. Harris

Director Center of Molecular Epidemiology,  
The Ohio State University, Columbus, OH, USA

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### Definition

► **Breast cancer** is a multifactorial disease promoted by sustained heightened exposure to endogenous or exogenous ► **estrogens**. Rates of breast cancer vary widely and are higher in developed countries such as the United States and the United Kingdom and lower in developing countries such as India and China. Breast cancer risk appears to increase with high intake of essential polyunsaturated fats that promote ► **inflammation** and estrogen biosynthesis. Reproductive risk factors include early menses, nulliparity, late first pregnancy and late menopause, all of which increase exposure to endogenous estrogens. ► **Estrogen replacement therapy** (ERT) and high body mass increase breast cancer risk in postmenopausal women. Identifiable genetic factors account for only a small fraction of breast cancer cases. Studies in cancer control show that annual screening with mammography after age 50 is highly effective in detecting early breast lesions when they can be surgically excised with a high probability of long term survival. Breast cancer prevention may be aided by taking synthetic or natural compounds with anti-inflammatory or antiestrogenic activity. Additional studies in molecular epidemiology are needed to more clearly delineate the way in which breast cancer risk factors interact to impact the natural history of this disease.

### Characteristics

#### Global Impact of Breast Cancer

Among developed countries, the United States has the highest annual incidence rates of breast cancer exceeding 100 cases per 100,000. The lifetime risk of breast cancer for American women is approximately 1 in 8 compared to a lifetime risk of only 1 in 66 for Chinese women. Breast cancer mortality rates show a narrower range than incidence rates ranging from

**Breast Cancer Epidemiology. Table 1** Annual incidence and mortality rates of breast cancer per 100,000 women

Nation	Incidence	Mortality
China	18.7	5.5
Africa (Zimbabwe)	19.0	14.1
India	19.1	10.4
Japan	32.7	8.3
Brazil	46.0	14.1
Singapore	48.7	15.8
Italy	74.4	18.9
Switzerland	81.7	19.8
Australia	83.2	18.4
Canada	84.3	21.1
Netherlands	86.7	27.5
United Kingdom	87.2	24.3
Sweden	87.8	17.3
Denmark	88.7	27.8
France	91.9	21.5
United States	101.1	19.0

Data Resource: [1]

5.5 deaths per 100,000 Chinese women to 27.8 deaths per 100,000 Danish women. The incidence and mortality rates of breast cancer tend to be higher for women in developed countries compared to those in underdeveloped countries.

Breast cancer strikes 1.3 million women and results in 465,000 deaths annually throughout the world. It is the most commonly diagnosed cancer and the second leading cause of cancer death among women (only ► [lung cancer](#) causes more deaths). Breast cancer incidence is highly variable among populations ranging from low rates of 19 per 100,000 women in China, Africa, and India to high rates exceeding 80 cases per 100,000 in Scandinavian and European countries, the United States, and Great Britain ([Table 1](#)).

### Breast Cancer Detection, Staging, and Survival

Mammography is a radiographic imaging process using low-dose X-rays to assist in the detection and diagnosis of breast cancer. The goal of mammography as a screening tool is to detect breast tumors early in their growth and development so they can be completely excised by qualified breast surgeons. Screening mammography together with effective biopsy (► [Fine Needle Aspiration Biopsy](#)), accurate pathologic evaluation, and surgical excision of breast tumors has been shown to reduce the mortality from

breast cancer by approximately 30% in women over the age of 50 years. Because of the difficulty in discriminating normal active mammary glands from abnormal neoplastic growths in women during their reproductive years, there is controversy about the value of screening for breast cancer by mammography in premenopausal women (before age 50). Currently, the American National Cancer Institute recommends that women initiate biannual screening for breast cancer by mammography at age 40–49, whereas after age 50, screening is recommended on an annual basis (► [Mammographic Breast Density and Cancer Risk](#)). Other imaging techniques such as ultrasound, ► [Magnetic Resonance Imaging \(MRI\)](#) and ► [Positron Emission Tomography \(PET\)](#) are now being widely used by physicians to assist in the evaluation and diagnosis of breast tumors. Breast-self-examination (BSE) and physician examination are also considered essential components of regular breast care.

### Pathology

Tumor staging (► [Staging of Tumors](#)) refers to the microscopic evaluation of tissue by a pathologist to assess size, exact anatomic location, growth, and spread of a cancerous lesion. While imaging procedures are important for the identification of suspicious lesions, the ultimate diagnosis of breast cancer (or any other malignant neoplasm) must be confirmed by microscopic examination of cancerous tissue (obtained by ► [biopsy](#)) by a qualified pathologist.

Breast cancer survival is highest when tumors are detected prior to invading contiguous tissues or lymph nodes (Carcinoma in situ, Stage I; ► [Ductal Carcinoma In Situ](#)), whereas survival is lowest with late detection after tumors have spread (metastasized; ► [Metastasis](#)) to other sites ([Table 2](#)). Early-stage breast cancer is effectively “cured” by complete surgical excision with clear margins (no evidence of spread beyond the surgical margins), whereas later stage disease usually requires additional treatment by ► [chemotherapy](#), radiation therapy (► [Ionizing Radiation Therapy](#)), and hormonal therapy (► [Endocrine Therapy](#)).

### Mechanisms of Breast Carcinogenesis

Breast carcinogenesis is most probably due to stimulation of ► [epithelial cells](#) that line the breast ducts by estrogens (► [Estrogenic Hormones](#)). The major evidence for this is that breast cancer primarily occurs

### Breast Cancer Epidemiology.

**Table 2** Breast cancer survival by stages at detection

Stage at diagnosis	Description of stage at diagnosis	Five year survival (%)
0	Carcinoma in situ (no invasion)	100%
I	Tumor <2 cm with no lymphatic spread	100%
IIA	Tumor $\leq$ 2 cm with no lymphatic spread	92%
IIB	Spread to axillary lymph nodes	81%
IIIA	Spread to axillary and other lymph nodes	67%
IIIB	Spread to lymph nodes and opposite breast	54%
IV	Widespread metastatic cancer	20%

Source: American Cancer Society: 2005

in women, although occasionally breast tumors do develop in men, particularly in association with Klinefelter syndrome, where there is an extra X chromosome in the karyotype (XXY) or by ingestion of synthetic estrogens such as diethylstibesterol in the treatment of prostate cancer (► [Prostate Cancer Clinical Oncology](#)).

Several theories have been proposed to explain breast carcinogenesis. Perhaps the best known of these relates breast cancer risk to the sustained stimulus of estrogen over many years. The “estrogen-stimulus” theory of breast cancer postulates that the risk is enhanced with a sustained continuum of estrogen cycles unbroken by pregnancy or other mechanisms of estrogen ablation such as ovariectomy. Both endogenous and exogenous factors may potentially increase estrogen stimulus of the mammary gland in association with breast cancer development.

### Risk Factors

Several “risk factors” have been identified that increase a woman’s chance of developing breast cancer. Nevertheless, cause and effect cannot be established in most individual cases. The classical risk factors of breast cancer include familial and genetic predisposition, early menses, delayed reproductive history, nulliparity, late menopause, and the natural process of aging.

### Hormones

During the reproductive years, estrogens are produced by the ovaries; whereas after menopause, the source of circulating estrogens is biosynthesis in fat and muscle cells by the enzyme ► [aromatase \(Aromatase and its Inhibitors\)](#). The risk of premenopausal breast cancer increases two- to threefold with either nulliparity or “late” first pregnancy (after age 30). Parous women who do not breast feed are also at increased risk.

### Family History

A strong family history (breast cancer in a first-degree or second-degree relative) increases the risk of breast cancer by three- to fivefold. Genetic or familial predisposition is identifiable for approximately 5–10% of women diagnosed with breast cancer. Two heritable genetic mutations have been identified that predispose to familial breast cancer, BRCA1 and BRCA2 (► [Breast Cancer Genes BRCA1 and BRCA2](#)). The BRCA1 gene predisposes ► [heterozygous](#) female carriers to both breast cancer and ► [ovarian cancer](#), while the BRCA2 gene predisposes heterozygous female carriers to breast cancer only. Hallmarks of familial predisposition to breast cancer include early age of onset, an excess of bilateral disease, and breast cancer in familial association with other malignancies such as ovarian cancer and ► [endometrial cancer](#). ► [BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk](#)

### Estrogen Replacement Therapy

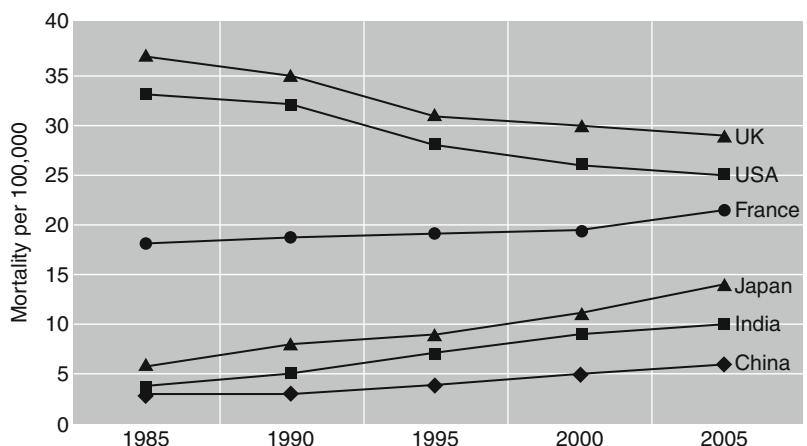
Approximately 75% of breast cancers are diagnosed in women after they undergo menopause. A number of investigations have examined the association between ► [estrogen replacement therapy](#) (ERT) and postmenopausal breast cancer risk. There is a general consensus that ERT (with or without ► [progesterone](#)) elevates the risk of postmenopausal breast cancer by two to three-fold. Several studies show consistency in observing an interaction between body mass and ERT in elevating the risk of breast cancer in postmenopausal women. Specifically, lean women who receive ERT after menopause have been found to be at significantly higher risk for the development of breast cancer. One possible explanation for this is the relatively higher concentration of ERT in women of smaller body mass.

### Body Mass Index

Body Mass Index (BMI) shows differential effects on premenopausal versus postmenopausal breast cancer

## Breast Cancer Epidemiology.

**Fig. 1** Trends in breast cancer mortality



risk. Before menopause, BMI shows little association with risk, whereas after menopause, the risk of breast cancer increases two to threefold among women with high BMI, presumably due to heightened estrogen biosynthesis.

### Diet

From an etiologic standpoint, rates of breast cancer are changing in populations that historically have been at low risk, whereas the rates have remained relatively constant in populations at higher risk. For example, breast cancer mortality rates among Japanese, Indian, and Chinese women have increased approximately threefold in the past two decades, whereas the United States, the United Kingdom, and European (French) rates have remained constant or slightly declined (Fig. 1).

Concurrently, the Japanese, Indian, and Chinese diets have also changed dramatically with higher intakes of fat and calories. However, other risk factors may also be involved since birth rates are declining, age at first pregnancy is being delayed, and nulliparity is increasing in these populations.

Various hypotheses postulate that dietary factors are related to the initiation and promotion of breast cancer. One such hypothesis states that breast cancer development is due to intake of certain types of essential fatty acids that increase inflammation and estrogen biosynthesis and thus promote breast cancer development. However, there is controversy among epidemiologists regarding the role of dietary fat or other dietary factors in the development of breast cancer (► **Cancer Causes and Control**).

The highest risk target organ for development of breast cancer is the contralateral (opposite) breast of a woman who has already manifested unilateral disease (► **Contralateral Breast Cancer**). In addition, the familial breast cancer patient has a markedly enhanced risk for development of malignancy in the contralateral breast (about 50% over 20 years, post-► **mastectomy**).

Many studies in biochemical epidemiology have been performed with the objective of identifying a biochemical marker of breast cancer risk. The various subtypes of estrogens (estradiol, estrone, and estriol) and their ratios, ► **androgens** and other steroids, polypeptide hormones such as prolactin, and various indices of these parameters have been tried; however, no single parameter or index of parameters has been developed which accurately predicts an individual's risk of developing cancer of the breast.

### Prevention

Breast cancer specimens ascertained by biopsy or surgical procedures (mastectomy) are routinely subjected to laboratory analysis of estrogen receptors (ER) and progesterone receptors (PR). Breast tumors that are positive for ER/PR may respond to hormone therapy by administration of antiestrogenic compounds such as ► **Tamoxifen**. Tamoxifen is now being offered to women treated for early stage breast cancer for ► **chemoprevention** against the development of second primary cancer in the contralateral breast.

Large independent clinical trials have been performed to examine the preventive activity of Tamoxifen. While a US trial showed beneficial effects, the results of two European trials were negative. Despite

this apparent discrepancy of results, the US FDA has approved Tamoxifen for use as a preventive agent in high risk women. This action tends to disregard adverse side effects of the drug, including increased risks of endometrial cancer, ER negative breast cancer, ► colon cancer, and pulmonary embolus.

Many epidemiologic studies have noted a significant preventive effect of ► nonsteroidal anti-inflammatory drugs (NSAIDs) against breast cancer. These investigations suggest that the risk of breast cancer is reduced by 20–30% with regular use of common over the counter NSAIDs such as ► aspirin and ibuprofen. Studies in ► molecular epidemiology and in animals suggest that this effect is manifest due to blockade of cyclooxygenase isozymes of the inflammatory cascade, particularly the inducible isoform, ► Cyclooxygenase-2.

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## Breast Cancer Familial Risk

Barbara Burwinkel<sup>1</sup> and Yang Rongxi<sup>2</sup>

<sup>1</sup>Divison Molecular Biology of Breast Cancer,  
University of Heidelberg, Heidelberg, Germany

<sup>2</sup>Molecular Epidemiology Unit German Cancer  
Research Center, Heidelberg, Germany

## Definition

Familial breast cancer is characterized by multiple affected individuals in one family. The patients with breast cancer are normally diagnosed in a relatively younger age compared to the sporadic ones. Familial breast cancers account for approximately 5–10% of all breast cancers. The closer and the younger the relatives affected by breast cancer are, the higher the breast cancer risk to the individuals in the same family will be. Moreover, multiple affected individuals in one family will increase the risk of breast cancer.

## Characteristics

A twin study based on a large population indicated that breast cancer in general owns to the combination of one third of genetic background and two thirds of environmental factors. Familial aggregation can be attributed to shared genes and shared physical environment and lifestyles. Considering the familial aggregation, hereditary breast cancers are mainly due to ► germline mutations in ► tumor suppressor genes or ► oncogenes transmitted from one generation to another. The breast cancer susceptibility genes can be stratified by risk profile into three tiers: high-► penetrance genes (contribute to approximately 25% of all familial breast cancer cases), intermediate-penetrance genes (accounts for about 5% of familial breast cancer risk), and low-penetrance genes (the known genes account for about 5% of familial breast cancer risk).

Most of the risk factors are still unknown, and the vast majority of breast cancers are presumed to be due to an undefined number of additional inherited susceptibility factors with various degrees of ► penetrance, exposure to hormonal and environmental factors and stochastic genetic events (Fig. 1).

## High-Penetrance Breast Cancer Susceptibility Genes

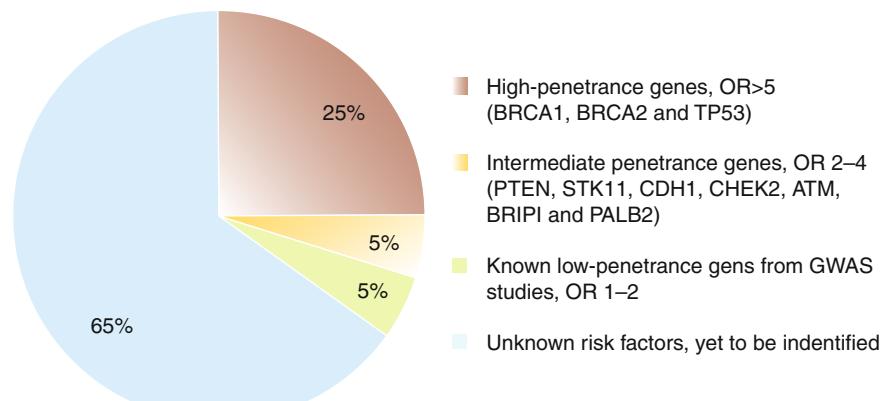
High-penetrance breast cancer susceptibility genes are genes that confer a greater than fivefold relative risk of breast cancer. This group includes three genes, BRCA1, BRCA2, and TP53.

## BRCA1 and BRCA2

Breast cancer 1 gene (BRCA1) and breast cancer 2 gene (BRCA2) are identified as two major breast-cancer-susceptibility genes (► Breast Cancer Genes BRCA1 and BRCA2). The two genes are known as ► DNA repair genes, and play roles as tumor suppressor genes. They both are involved in a cluster of processes in the cells, including ► cell-cycle checkpoint control, protein ► ubiquitination, chromatin remodeling, and the maintenance of genomic stability. Mutations in the BRCA1 and BRCA2 tumor-suppressor genes are the strongest indicators of risk for ► breast cancer and/or ► ovarian cancer. Women with mutations in either of the two genes have a lifetime risk of breast

### Breast Cancer Familial Risk.

**Fig. 1** Genetics of familial breast cancer. The percentages present the contribution of each group of genes to familial breast cancer



cancer of 60–85% and a lifetime risk of ovarian cancer of 15–40%.

Mutations in BRCA1 and BRCA2 have a considerable contribution to familial breast cancer aggregation. It has been estimated that 0.7–29% of familial breast cancers are accounted for by mutations in BRCA1, and 1.5–25% are accounted for by mutations in BRCA2. Although the contribution of BRCA1 and BRCA2 mutations to cancer risk is varied in different populations, the true degree of these differences is difficult to be estimated given the wide variety of patient inclusion criteria, mutation ascertainment methods and limited sample size used in different studies. Thus, the data from different studies might show very different frequencies of BRCA1/2 mutations even in the same cohorts of patients. In general, inherited germline mutations in BRCA1/2 occur in about 20–30% of all familial breast cancer cases and in 2–3% of all breast cancers. Some founder mutations are relatively frequent in particular ethnic groups. For example, BRCA1 185delAG, BRCA1 5382insC, and BRCA2 6174delT are observed in about 59% of familial breast-ovarian cancer patients in Ashkenazim Jewish population, comparing to 2% in normal population. The BRCA2 999del5 mutation, the sole high-frequency founder mutation in Iceland, is found in about 10.4% of breast cancer cases unselected for family history and 38% of male breast cancers, whereas is only detected in 0.6% of unaffected Icelanders. Furthermore, BRCA1 mutations are more penetrant at a younger age, while BRCA2 variants are more likely to account for cases diagnosed above the age of 50 years old. BRCA2 is also associated with a 6% lifetime-risk for male breast cancer and increased ovarian cancer risk.

### TP53

► **TP53** was recognized as a tumor suppressor gene and central to multiple cellular pathways. Loss of p53 function by somatic mutations occurs frequently in tumors. Germline mutations of TP53 are correlated with the ► **Li-Fraumeni syndrome** (LFS). LFS is characterized by ► **autosomal dominant** inheritance and early onset of tumors, such as breast cancer, ► **soft tissue sarcomas**, ► **osteosarcomas**, ► **brain tumors**, ► **leukemias**, and ► **adrenocortical carcinomas**. The pattern of breast cancer in LFS families is remarkable. In a study on 28 LFS families including 148 cancer affected individuals, 38 women were diagnosed with breast cancer. The breast cancer patients in LFS are more likely to be diagnosed at a younger age (younger than 30 years old) and with bilateral disease. As Li-Fraumeni syndrome is rare and leads to multiple tumors, not only breast cancer, the contribution of mutations in TP53 to the familial breast cancer is very low.

### Intermediate-Penetrance Breast Cancer Susceptibility Genes

In addition to BRCA1, BRCA2, and TP53, some other genes are also considered as well established breast cancer susceptibility genes. Germline mutations in ► **PTEN**, ► **STK11**, and ► **CDH1** have been identified as causes of some syndromes that are associated with an increased risk of familial breast cancer. Mutations in CHEK2, ► **ATM**, ► **BRIPI**, and ► **PALB2** are rare and confer a relative risk of breast cancer of two- to fourfold. However, all these intermediate-penetrance genes only account for less than 5% of all familial breast cancer cases.

## PTEN

Phosphatase and tensin homolog (► PTEN) gene encodes a lipid phosphatase and functions as a tumor suppressor by leading to cell-cycle arrest and ► apoptosis. Germline mutations in PTEN cause ► Cowden syndrome, a rare autosomal dominant syndrome that is characterized by an increased familial cancer risk especially in breast and thyroid, and together with multiple clinical features. The lifetime risk of breast cancer in women with Cowden syndrome is estimated to be as high as 50%, comparing to 11% in the general population. The breast cancer carriers in Cowden syndrome are normally diagnosed at a younger age and with the average age of 36–46 years old.

## STK11

Serine/Threonine Protein kinase 11 (► STK11) encodes a serine/threonine kinase that can inhibit cellular proliferation, control cell polarity, and interact with the ► mTOR pathway. Mutations in STK11 lead to ► Peutz-Jeghers syndrome (PJS), which is a rare autosomal dominant hereditary disease leading to a predisposition to benign and malignant tumors of many organs, including breast and ovary.

## CDH1

Cadherin 1 (CDH1) gene encodes ► E-cadherin, which is important for the cell ► adhesion. The mutation in CDH1 have shown dominantly inherited predisposition for ► gastric cancer. Multiple lobular breast cancer carriers are also observed in families with germline mutations in CDH1.

## CHEK2

► Checkpoint kinase 2 (CHEK2) encodes protein CHK2, a ► protein kinase that is activated in response to ► DNA damage and is also involved in cell cycle arrest. The inherited mutations in CHEK2 are found in a portion of the autosomal dominantly inherited Li-Fraumeni syndrome. The CHEK2 Breast Cancer Case-Control Consortium has found that CHEK2\_1100delC is associated with a more than twofold increased breast cancer risk in the population. Some other variants in CHEK2, such as I157T, S428F, and P85L, have also been reported to be associated with increased breast cancer risk.

## ATM

Ataxia-telangiectasia mutated gene (ATM) encoded protein is involved in DNA repair and/or cell cycle

control. The mutations in ATM contribute to ► Ataxia Telangiectasia (TA) which is an autosomal recessive syndrome with progressive cerebellar ataxia, immune deficiency, and cancer predisposition. Some mutations in ATM have shown a larger than twofold of relative risk with familial breast cancer.

## BRIP1

BRCA1-interacting protein 1(BRIP1) is a member of the DEAH helicase family. It interacts directly with BRCA1 to form BRIP1-BRCA1 complex and contributes to the key BRCA1 activity. The truncation mutations in BRIP1 were found to be more frequent in familial breast cancer cases than in the controls. It has been estimated that BRIP1 mutations confer a twofold higher risk of breast cancer. Some other mutations in BRIP1, such as P47A and M299I, are identified in early-onset breast cancer individuals with family history of breast and ovarian cancer.

## PALB2

Partner and localizer of BRCA2 (PALB2) gene encodes protein PALB2 that promotes localization and stability of BRCA2 in the nucleus and enables the ► DNA repair and checkpoint functions of BRCA2. Monoallelic ► truncating mutation of PALB2 confers a 2.3-fold higher risk for familial breast cancer. The 1583delT variation is associated with significant increased breast cancer risk in Finnish population (fourfold in unselected breast cancer individuals and about tenfold in familial cases).

## Low-Penetrance Breast Cancer Susceptibility Alleles

Low-penetrance alleles are variants or ► polymorphisms that may be associated with a small increased relative risk to cancer, with the odd ration of less than 2, and mostly less than 1.5. The frequency of the variants in low-penetrance genes in all is higher in general population than that of high-penetrance genes. According to the polygenic model of inherited breast cancer, unfavorable combinations of polymorphic genetic variants in low-penetrance susceptibility genes contribute to the excess familial breast cancer risk. Most of the low-penetrance susceptibility genes have not been discovered yet. A large number of genetic polymorphisms contribute to low-penetrance breast cancer genes. The low-penetrance genes are mainly detected by association studies, where the

frequencies of candidate alleles are compared between cases and controls.

Genome-wide association studies (GWAS) have emerged as a powerful approach to identify susceptibility loci. By utilizing genotyping platforms that can type hundreds of thousands of single nucleotide polymorphisms (SNPs) simultaneously, it is possible to conduct association studies using sets of SNPs that tag most of the known common variants in the genome. The GWAS studies have already identified several breast cancer susceptibility alleles.

Most of these alleles are located in a linkage disequilibrium block with known cancer related genes, such as FGFR2, CASP8, and MAP3K1; some are located in regions that are far away from any known genes, such as rs13387042 in 2q35 and rs13281615 in 8q24. So far, the biological characteristics of these SNPs are still unknown. Although, these low-penetrance genes or variants have explained a number of sporadic breast cancer cases, their contribution to familial breast cancer is low. Five loci are estimated to account for a modest 3.6% of the excess familial risk of breast cancer in European populations. All the identified and well verified low-penetrance alleles might account for about 5% of all familial breast cancers in European population.

### Interaction Between Breast Cancer Susceptibility Genes

There is etiological evidence that cancer is the result of accumulated mutations in genes under the pressure of combination of genetic and environmental risk factors. The interaction and collaboration between breast cancer susceptibility genes are important aspects for familial breast cancer risk. Recent studies have suggested that breast cancer risk in BRCA1 and BRCA2 mutation carriers are modified by other genetic or environmental factors that cluster in families. For example, the minor alleles of SNP rs2981582 and rs889312 are associated with increased breast cancer risk in BRCA2 mutation carriers (rs2981582, Ptrend = 1.7 × 10 – 8 and rs889312 Ptrend = 0.02, respectively) but not in BRCA1 carriers. Genotype and haplotype analyses of TP53 in the Spanish population revealed that the haplotype with the variant allele for the Arg72Pro but without 97–147ins16bp in TP53 is associated with an earlier age of onset in BRCA2 mutation carriers. Further investigations are necessary to reveal whether similar interactions exist between other susceptibility genes.

### Conclusion and Outlook

Although GWAS studies have revealed many genetic factors contributing to sporadic breast cancer risk, most of the familial breast cancer related inherited factors are still unknown. Far more rare genetic variants/mutations conferring an intermediate to high breast cancer risk might contribute to a larger portion of familial breast cancer than assumed before. The association of these rare variants with breast cancer can hardly be detected by GWAS studies. Next generation sequencing approaches will be helpful to reveal these variants. Furthermore, DNA copy number variations (CNV), which are genomic structural aberrations occurring in the population, might contribute to cancer risk.

## Breast Cancer Genes BRCA1 and BRCA2

Ashok R. Venkitaraman

Hutchison/MRC Research Centre, Cambridge, UK

### Definition

► *BRCA1* and ► *BRCA2* are cancer-predisposition genes, germ line mutations which are associated with a high risk of developing ► *breast cancer*, ► *ovarian cancer*, and other cancers. Much information has been accumulated on the function of their large, nuclear-localized protein products, which implicates them in the cellular response to ► *DNA damage*, the control of mitotic ► *cell division*, and the regulation of gene ► *transcription*. *BRCA1* and *BRCA2* are very distinct genes, despite the similarity in their acronyms.

### Characteristics

Roughly one-tenth of all ► *breast cancer* cases exhibit a familial pattern of inheritance. Of these familial cases, ► *germ line mutation* in either one of two genes, *BRCA1* or *BRCA2*, occur in 20–60% (that is, in 2–6% of all cases). Mutations in *BRCA1* or *BRCA2* in ► *somatic tissue* do not appear to be a feature of nonfamilial (that is, sporadic) breast cancer, but there is evidence that ► *epigenetic* suppression of *BRCA*

gene expression, or genetic alterations affecting the biological pathways in which they participate, can occur in sporadic breast cancer.

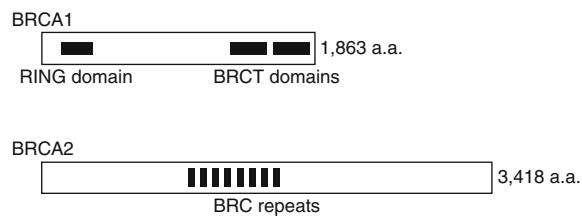
*BRCA1* and *BRCA2* were first identified in 1994–1995 through the analysis of families exhibiting a predisposition to early-onset breast cancer. ► **Founder** mutations affecting these genes occur in Iceland and among the Ashkenazim, where they confer a highly penetrant risk of breast cancer, ovarian cancer, and other cancers (including cancers of the male breast, ► **pancreas cancer**, and ► **prostate cancer**). In other populations, germ line *BRCA1* or *BRCA2* mutations are found in the great majority (up to 80%) of families that suffer from multiple occurrences of breast cancer plus ovarian cancer. Germ line *BRCA2* mutations affecting both alleles also occur in the rare D1 complementation group of ► **Fanconi anemia**.

The *BRCA1* and *BRCA2* genes have been assigned to human chromosomes 17q and 13q, respectively. In both genes, ► **exon** 11 (3.4 kb in *BRCA1*, and 5 kb in *BRCA2*) encodes a large portion of the protein. Overall, the murine and human genes are no more than 60% identical at the amino acid level, although small regions exhibit a much higher degree of conservation.

## Protein

*BRCA1* and *BRCA2* encode large proteins (human *BRCA1* is 1,863 amino acids long; and human *BRCA2* is 3,418 amino acids) that localize to the nucleus in mitotic and meiotic cells (Fig. 1). They bear little resemblance to proteins of known function. At its N-terminus *BRCA1* protein contains a ► **RING** domain known to mediate heterodimerization with the RING domain of ► **BARD1**, forming an active E3 ► **ubiquitin** ligase. At its C-terminus, *BRCA1* includes two copies of an approximately 95 amino acid motif (the ► **BRCT domain**, for *BRCA1* C-terminal) later detected in a number of different proteins implicated in ► **DNA repair** and ► **cell-cycle** ► **checkpoint** control. This domain, whose atomic structure has been elucidated, mediates a number of protein–protein interactions with phosphorylated targets by serving as a phosphopeptide-binding module.

Eight repeated sequences (the BRC repeats), each of about 30 amino acids, are encoded in *BRCA2* exon 11. The BRC repeats, but not their intervening sequences, are conserved between several mammalian species suggestive of a conserved function. The interaction of *BRCA2* protein with ► **RAD51**, a



**Breast Cancer Genes BRCA1 and BRCA2.** Fig. 1 Structural features of the BRCA1 and BRCA2 proteins (not drawn to scale)

mammalian homologue of bacterial ► **RecA** essential for genetic ► **recombination**, occurs through the BRC repeats. There is good evidence from genetic, structural, and biochemical studies that the BRC repeats regulate the activity of RAD51 in reactions that lead to DNA repair by recombination. Two other regions of *BRCA2* have been implicated in the control of recombination. A domain carboxyl-terminal to the BRC repeats interacts with the small protein Dss1 to form a structure capable of binding junctions between single-stranded and double-stranded DNA, which can displace the ssDNA-binding protein RPA from recombination substrates, whereas an additional RAD51-binding region of uncertain function is located near the extreme C-terminus of *BRCA2*.

## Cellular and Molecular Regulation

The transcripts and protein products encoded by *BRCA1* and *BRCA2* are expressed in dividing cells of many types. Expression is also high in meiotic cells. These expression patterns speak to the possible functions of *BRCA1* and *BRCA2* proteins.

### Role in the Cellular Response to DNA Damage

Both *BRCA1* and *BRCA2* proteins localize to the nucleus. In meiotic cells, colocalization has been reported to the synaptonemal complexes of developing axial elements. This is suggestive of a role in meiotic recombination, a process that is initiated by DNA double-strand DNA breakage. Similarly, there is good evidence that *BRCA1* and *BRCA2* are essential in mitotic cells for the repair of DNA double-strand breaks by homologous recombination.

Several lines of evidence are indicative of such a role:

- Cells in which *BRCA1* or *BRCA2* or their homologues in other species have been inactivated exhibit ► **genotoxin** hypersensitivity and ► **chromosomal**

instability suggestive of defects in ► DNA double-strand break repair.

- Second, ► homologous recombination repair of double-strand DNA breaks introduced into chromosomal substrates is impaired by the disruption of BRCA1 or BRCA2, although pathways for ► nonhomologous end joining remain largely unaffected.
- Finally, BRCA1 and BRCA2 localize after DNA damage to nuclear foci where they interact with molecules implicated in DNA recombination, including RAD51, and the ► Fanconi anemia proteins.

The precise mechanisms that may underlie such a function remain to be determined. BRCA2 interacts directly, and at a relatively high stoichiometry, with RAD51, a protein essential for DNA repair by recombination, to modulate RAD51 activity or availability. The interaction of BRCA1 with RAD51 is less well defined, although both proteins colocalize – along with BRCA2 – to discrete nuclear foci following DNA damage. BRCA1 may participate in the cellular mechanisms that sense and signal DNA damage, culminating in the activation of cell-cycle checkpoints and the machinery for DNA repair. The protein kinases ► ATM (encoded by the gene mutated in ► Ataxia telangiectasia), ► ATR, ► CHK1, and CHK2 (mutated in ► Li–Fraumeni syndrome) are proximal components of these sensing/signaling mechanisms. ATM, CHK1, and probably the other checkpoint kinases, phosphorylate BRCA1 following DNA damage, a modification essential for its proper function. These observations are important because they place BRCA1 – and by extension, possibly BRCA2 – in the same pathway as genes such as ATM (► ATM Protein), germ line mutations in which are also associated with an increased risk of breast cancer and other cancers. Thus, a common DNA ► damage response pathway may be defective in a significant fraction of breast cancers.

BRCA1 and BRCA2 have also been implicated in the enforcement of cell-cycle checkpoints during the G2 and M phases, and in the regulation of ► centrosome number. Additional functions have recently been described in the control of ► mitosis. BRCA1 regulates proteins such as MAD2 that act in the ► mitotic spindle assembly checkpoint, and has an essential function in directing the correct formation and function of the mitotic spindle, whereas BRCA2-deficient cells exhibit defects in the completion of cell division

by ► cytokinesis. Thus, BRCA1 and BRCA2 appear to work in multiple processes responsible for maintaining the integrity of chromosome number as well as structure in dividing cells, which may help to explain why they are potent ► tumor suppressors.

#### Other Functions

It is difficult to reconcile the disparate nature and severity of the cellular and developmental defects induced by the disruption of murine homologues of BRCA1 and BRCA2, with functions exclusively in the response to DNA damage. Evidence is accumulating that BRCA1, in particular, can control gene ► transcription. Several proteins that interact with BRCA1 are known to regulate transcription or mRNA processing. Moreover, at least a fraction of the total intracellular pool of BRCA1 is linked to the general transcription machinery – the RNA polymerase II holoenzyme – through its RNA helicase subunit.

BRCA1 has been implicated in the control of X-inactivation in female cells, a process whose dysregulation is associated with breast cancer predisposition. How this function may be exerted is not clear, but it may work through the control of localization of the Xist product.

In addition, roles for BRCA1 and possibly BRCA2 have been reported in the control of ► estrogen receptor expression and signaling.

#### Tumor Suppression by BRCA1 and BRCA2

Inheritance of a single defective copy of *BRCA1* or *BRCA2* confers cancer predisposition in humans. However, the second allele is almost invariably lost in the cancers that arise in predisposed individuals, indicating that *BRCA1* and *BRCA2* behave in some respects as ► tumor suppressor genes.

Abnormalities in growth or in DNA repair have not yet been reliably detected in murine or human cells heterozygous for *BRCA1* or *BRCA2* mutations. Thus, there is currently little to suggest that cancer predisposition arises solely from ► haploinsufficiency, or a trans-dominant deleterious effect induced by a single mutant *BRCA1* or *BRCA2* ► allele. Rather, as has been proposed for other tumor suppressor genes, ► germ line mutation in one allele may simply increase the likelihood that the gene is wholly inactivated by loss of the second allele through somatic mutation. However, ► aneuploidy as a consequence of abnormal

► **cytokinesis** has been reported in cells and tissues ► **heterozygous** for *BRCA2* mutations, and the possibility that this effect of ► **haploinsufficiency** contributes to carcinogenesis remains to be determined.

Whatever the events that lead to ► **loss of heterozygosity**, inactivation of *BRCA1* or *BRCA2* would initiate ► **genetic instability** by destabilizing ► **chromosome** structure and number, allowing the rapid evolution of tumors due to increased somatic alterations in genes that control ► **cell division**, ► **cell death**, or life span. Thus, *BRCA* genes are proposed to work as ► “**caretakers**” of genetic stability. This “caretaker” role is most likely to arise through the function of the *BRCA* proteins in ► **DNA repair** and ► **mitosis**. Cells that harbor disruptions in *BRCA1* or *BRCA2* accumulate aberrations in chromosome structure, reminiscent of diseases like ► **Bloom syndrome** or ► **Fanconi anemia**, where ► **chromosomal instability** is associated with cancer predisposition. They also exhibit ► **aneuploidy** and defects in cell division. These defects could together elevate the rate of genomic instability, leading to somatic mutations or alterations in gene copy number that promote carcinogenesis.

It is unclear why carcinogenesis accompanied by loss of the second *BRCA* gene allele in individuals who inherit one mutant allele should occur preferentially in tissues such as the breasts or ovaries. Both *BRCA1* and *BRCA2* are widely expressed and appear to perform functions essential to all tissues. Currently there is little evidence to help distinguish between the several possible explanations that can be advanced.

The chronology of the molecular events during carcinogenesis in *BRCA* gene mutation carriers is not known. Loss of the second allele is clearly very frequent, but it is unclear at what stage in tumor evolution this may occur. However, the catastrophic cellular consequences of ► **homozygous** inactivation of *BRCA1* or *BRCA2*, which quickly lead to ► **cell death**, does emphasize that other genetic alterations will be necessary. Current evidence favors the notion that the inactivation of cell-cycle ► **checkpoint** genes, particularly those that enforce mitotic checkpoints, is an important additional step during carcinogenesis in *BRCA* gene mutation carriers.

Viewed in this way, it is conceivable that the tissue specificity of carcinogenesis represents differences in the ability of cells which have lost both alleles of *BRCA1* or *BRCA2* to survive for long enough to acquire

these additional genetic alterations. For instance, *BRCA*-deficient cells in epithelial tissues such as the breast and ovary may take advantage of hormonal or local intercellular interactions to support survival until the accumulation of additional genetic alterations allows outgrowth. By contrast, *BRCA*-deficient cells in nontarget tissues may quickly be eliminated.

### Clinical Relevance

Germ line mutations in *BRCA1* or *BRCA2* are frequently associated with ► **familial**, early-onset breast cancer and ovarian cancer, particularly in those families that suffer from multiple cases of cancer in both sites. This has obvious important implications for genetic testing and counseling in the clinic. The mutations have been estimated to carry a cumulative lifetime cancer risk of between 40–70%.

There is some evidence that the pathological features of breast cancer and ovarian cancer associated with *BRCA1* or *BRCA2* mutations differ from those of sporadic tumors. So far, these differences seem to be insufficiently well-marked to be of diagnostic significance. One notable association is that *BRCA1*-deficient cancers often exhibit a “basal-like” phenotype usually characterized by the expression of specific markers, and negativity for ► **estrogen receptor** expression.

It is also unclear if the prognosis of breast cancer and ovarian cancer associated with *BRCA1* or *BRCA2* mutations will differ significantly from that of sporadic cases. Conflicting results have been reported in the literature, their interpretation made difficult by the varied study designs and by the relatively small numbers of cases that have been compared. Similarly, the value of prophylactic interventions, whether surgical or drug-based, in *BRCA* gene mutation carriers awaits evaluation.

Emerging evidence suggests that the DNA repair defect inherent in *BRCA*-mutant tumors can be exploited in cancer therapy. Thus, both *BRCA1* and *BRCA2*-deficient cancers appear to be hypersensitive to the effect of DNA cross-linking agents such as ► **carboplatin**, and also to novel chemical inhibitors of ► **poly(ADP-Ribose) polymerase** (► **PARP**); ► **Breast Cancer Targeted Therapies**.

► **BRCA1/BRCA2 Germ line Mutations and Breast Cancer Risk**

## Breast Cancer Immunotherapy

Silvia von Mensdorff-Pouilly

Department of Obstetrics and Gynaecology, Vrije Universiteit Medisch Centrum (VUmc), Amsterdam, The Netherlands

### Definition

Active specific ► immunotherapy of cancer endeavors to direct the host's own immune system against an ► antigen expressed by tumor cells to create an ► immune response that will destroy the established tumor. The same immune response induced in an ► adjuvant setting targets and intends to eliminate isolated disseminated tumor cells (► Micrometastasis) to prevent disease recurrence and to create a state of immune surveillance (► Immune Surveillance of Tumors) that will eliminate tumor cells as they arise. Passive immunotherapy uses monoclonal antibodies (MAb; ► Monoclonal Antibody Therapy) that bind to receptors or antigens on the tumor cell surface blocking receptor-ligand interactions and recruiting immune effector cells against the tumor. ► Trastuzumab (► Herceptin®), a monoclonal antibody to human epidermal growth factor receptor 2 (HER2; ► Epidermal Growth Factor Inhibitors), is already part of the standard treatment of patients with breast carcinomas expressing the antigen.

### Characteristics

One major difficulty faced by ► immunotherapy of cancer in general, and that of ► breast cancer in particular, is that there are very few antigens that are specific to tumor cells. Tumor-specific antigens (TSA) are for the most part oncogenic viral antigens, among others ► Epstein-Barr Virus (EBV in Burkitt lymphoma) and ► Human T-Cell Leukemia Virus (HTLV in T-cell leukemia/lymphoma), which are not relevant to breast cancer and adenocarcinomas. On the other hand, numerous ► tumor-associated antigens (TAA) that are expressed by carcinomas have been described, and their number continues to increase with the use of new methods to define them, such as recombinant expression cloning (► SEREX),

serological proteome analysis (SERPA), genomics, and proteomics. Immunotherapy exploits the fact that TAAs are present in greater amounts and in a different cell distribution in cancer than in normal cells. This is the case with members of the epithelial growth receptor family, such as ► HER2 and ► EGFR. Additionally, some TAAs, such as the mucin ► MUC1 and ► gangliosides, are aberrantly glycosylated and express tumor-associated ► glycans that provide a good target for immunotherapy. Other TAAs represent mutated versions of self (► P53), constitute oncofetal antigens (► carcinoembryonic antigen, CEA; alpha fetoprotein, αFP; ► Alpha Fetoprotein Diagnostics) with restricted expression in mature tissues, or, like the cancer-testis antigens (► Cancer Germline Antigens), their expression in normal tissues is limited to the testes (melanoma antigen 1, ► MAGE-1). An advantage of many TAAs, such as MUC1, CEA, and HER2, is that they are shared antigens expressed by many types of tumors, thus broadening the applicability of a particular vaccine. However, TAAs are in essence self-antigens, and therefore have been subjected to thymic selection to eliminate high-avidity T-cells that otherwise might induce ► autoimmunity. This makes them weak antigens, and partly explains the tolerance exhibited to growing tumors. Because many of the targeted TAAs are aberrant self-antigens, one important safety issue of cancer vaccines is that they induce immune responses to tumor cells but not to normal cells. ► Transgenic mice expressing either human ► MUC1, ► CEA, or ► HER2 that were vaccinated with the corresponding antigen showed tumor rejection and no autoimmunity. Cancer vaccines based on TAAs that have been tested in clinical trials in human subjects differ in vectors, carrier proteins, and adjuvants but are similar in showing low toxicity, the adjuvant in question being mainly to blame for the local and constitutional symptoms associated with the vaccine. No autoimmune reactions have been reported to the moment.

Another hurdle that cancer immunotherapy has to overcome is that carcinomas use a variety of immunosuppressive mechanisms to defeat potentially effective immune responses, such as induction of immunosuppressive immune cells (T regulatory cells, Treg; ► Regulatory T Cells), elaboration of immunosuppressive ► cytokines (TGF-β, ► Interleukin-4 (IL-4), ► Interleukin-6 (IL-6), and IL-10), and loss of major-histocompatibility-complex (MHC) class I

(► **HLA Class I**) expression. Conversely, immune recognition of tumors frequently occurs in cancer-bearing hosts: high titre IgG antibodies to tumor-associated gene products regularly accompany cancer development, and their presence has been associated with favorable outcome of disease in several tumor types, including breast; ► **cytotoxic T-cells** derived from patients with breast cancer react with tumor antigens present on malignant cells; ► **tumor-infiltrating lymphocytes** (TILs) are present in various types of tumors, and the composition and localization of the tumor infiltrate seems to be crucial for good or bad prognosis. The ultimate goal of tumor vaccines is to induce antigen-specific immune effector cells (► **CD8<sup>+</sup>** ► **cytotoxic T-cells**) that will kill tumor cells. Cytotoxic T-cells are capable of recognizing defined tumor antigens in the context of MHC class I, and are indispensable for clinically significant antitumor responses in advanced metastatic disease. Additionally, there is an increasing awareness of the importance of inducing CD4<sup>+</sup> helper T cells (► **Helper CD4 T-Cells**), given the key role that these cells play in the control of immune responses and in the induction of cytotoxic responses, as well as stimulating B-cells to produce antibodies specific to the tumor. Dissemination of disease occurs early in breast cancer: isolated disseminated tumor cells can be found at the time of primary surgery in the bone marrow of 30% of lymph node negative breast cancer patients, and their presence is associated with poor outcome. Antibodies, particularly of the IgG1 isotype, may be effective in eradicating these tumor cells by ► **antibody-dependent cellular cytotoxicity** (ADCC), antibody-dependent cellular ► **phagocytosis** (ADCP), and by ► **complement-dependent cytotoxicity** (CDC), and prevent recurrence of disease.

Immunotherapeutic strategies in breast cancer include the use of MAbs to target antigens on breast cancer tumor cells (HER2, EGFR) and ► **vascular endothelial growth factor** (VEGF) and numerous cancer vaccine constructs, illustrated in **Figs. 1** and **2**.

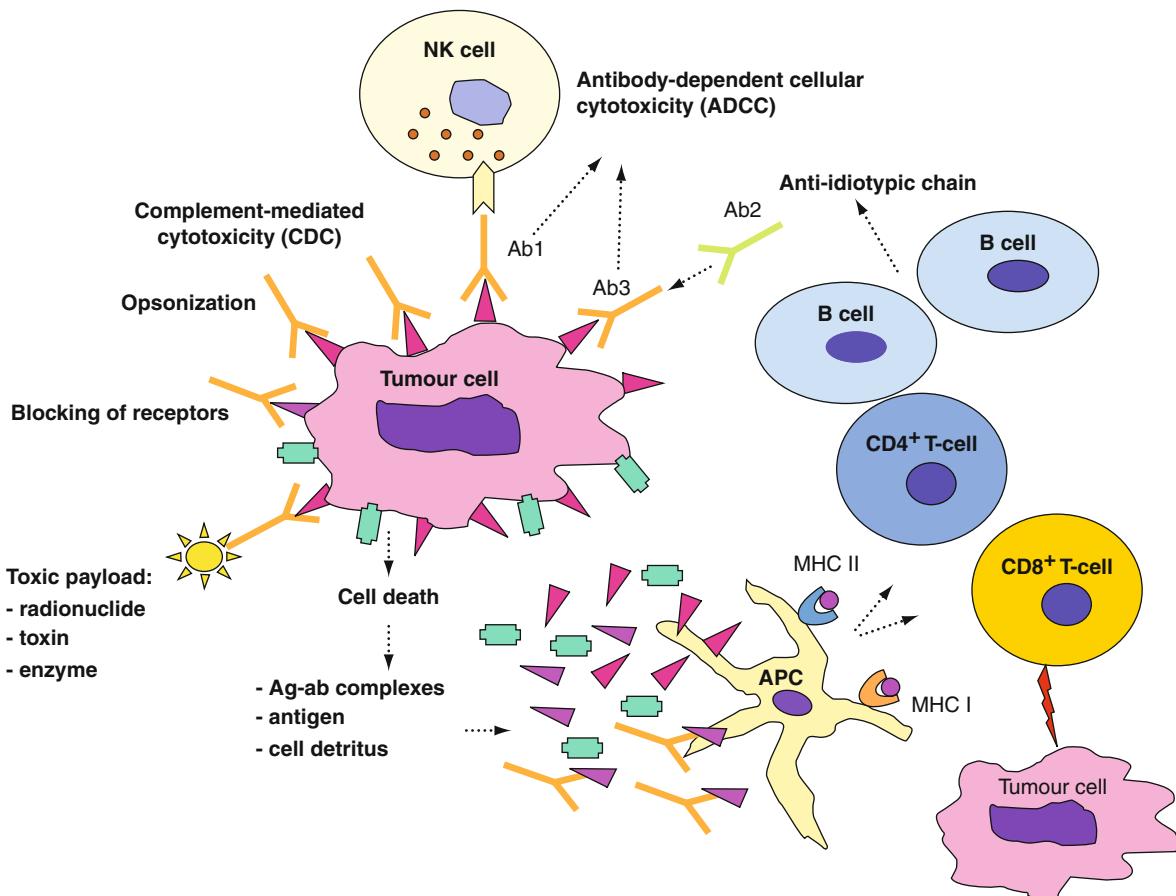
### Monoclonal Antibodies for the Immunotherapy of Breast Cancer

The best results so far in the immunotherapy of ► **breast cancer** have been obtained with MAbs, in general used in combination with conventional chemotherapy rather than as monotherapy. MAbs with clinical activity in breast cancer not only block receptor-ligand

interactions but also mediate immune responses, such as Antibody-Dependent Cellular Cytotoxicity/antibody-dependent cellular cytotoxicity (ADCC). Antigen released from cells dying after exposure to the MAb or to chemotherapeutic agents forms immune complexes with the MAb that can stimulate an immune response through the intermediary of Antigen-Presenting Cells/antigen-presenting cells (APC). The beneficial effect on breast cancer of Trastuzumab/trastuzumab, a monoclonal antibody against ► **HER2**, is associated with the induction of T-cell responses against HER2. Furthermore, antigenic determinants (► **Idiotype**) in the variable region of MAb are recognized by the host immune system as foreign and can trigger an idiotypic cascade. After administration of MAb for diagnosis or therapy, some patients with cancer develop anti-idiotypic antibodies that can be associated with a good clinical response. Anti-idiotype antibodies, mostly murine, but also human, that mimic tumor antigens have been used to elicit tumor-antigen-specific immune responses (**Fig. 1**). The effect of MAb monotherapy may be greatly enhanced with adjuvants that stimulate the innate immune system by way of the ► **toll-like receptors** (TLR). Similar to bacterial DNA, synthetic oligodeoxynucleotides (ODN) that contain a high frequency of CpG motifs (► **CpG Islands**) act as TLR agonists and enhance both NK cell cytotoxicity (► **Natural Killer Cell Activation**) against MAb-coated cells (ADCC) and NK cell cytokine production (IFN- $\gamma$ ; ► **Interferon Gamma**). IFN- $\gamma$  is an important mediator of antitumor responses by enhancing ► **phagocytosis** by Macrophages/macrophages, inhibiting tumor growth and promoting recognition of tumor cells by immunologic effectors, and plays a role in immunosurveillance. Additionally, MAb conjugated to radionuclides, toxins, and ► **prodrug**-converting enzymes can be used to deliver a toxic load to the tumor.

#### HER2 and EGFR

HER2 (erbB2) and EGFR (HER1 or erbB1) are members of the family of transmembrane protein kinase receptors, known as the erbB or HER receptor family, that also includes HER3 and HER4. HER2 and EGFR are expressed in a variety of tumors, and their activation promotes processes responsible for tumor growth and progression. HER2 and EGFR are transmembrane glycoproteins that consist of an extracellular ligand-binding domain, a hydrophobic transmembrane



**Breast Cancer Immunotherapy.** Fig. 1 Monoclonal antibodies (MAbs) as immunotherapeutic agents. MAbs bind to cell surface antigen blocking receptors responsible for constitutive growth signals (e.g., EGFR, HER2). MAbs are able to mediate antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and opsonization,

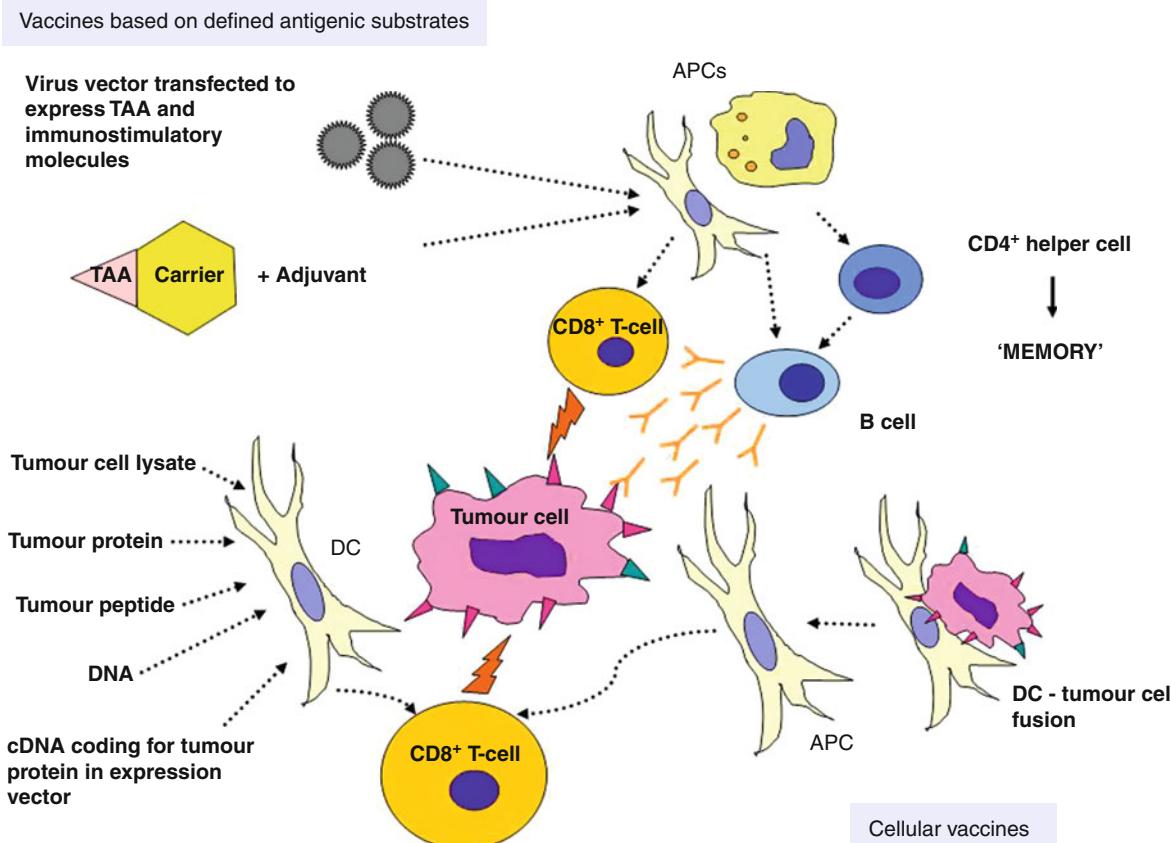
which lead to cell lysis. Antigen processing and presentation via MHC class I or class II molecules on antigen-presenting cells (APC) induces antitumor immunity in the host. Immune responses include cytotoxic T-cells and antibodies to the tumor (idiotypic cascade)

region, and an intracellular domain with tyrosine kinase activity for ► signal transduction. Ligands to EGFR are epidermal growth factor (EGF; ► **Epidermal Growth Factor Inhibitors**), transforming growth factor- $\alpha$  (TGF- $\alpha$ ; ► **Transforming Growth Factor Alpha**), ► **amphiregulin**, ► **betacellulin**, and ► **epiregulin**; the ligands to HER2 are not known. Binding of ligands to the ► **extracellular domain** induces homodimerization of the receptors, or heterodimerization with the other ErbB family members, inducing activation of the tyrosine kinase domain, and setting in motion signaling cascades involved in cell growth, proliferation, and survival. MAbs to these receptors (► **Trastuzumab**, ► **Cetuximab**) block ligand binding and induce receptor internalization and degradation without

stimulating receptor phosphorylation, resulting in downregulation of surface expression of the receptors. Antitumor efficacy of trastuzumab and cetuximab results from multiple mechanisms that include inhibition of cell cycle progression, promotion of ► **apoptosis**, ► **angiogenesis**, and ► **ADCC**.

#### Trastuzumab

Trastuzumab (Herceptin®), a humanized monoclonal antibody that targets HER2, is standard treatment in patients with HER2 positive breast cancer, preferably in combination with nonanthracycline based chemotherapy. HER2 is expressed in low levels in several normal tissues and is overexpressed in 20–30% of primary breast carcinomas. Overexpression is



**Breast Cancer Immunotherapy. Fig. 2** Vaccines based on defined substrates (often synthetic peptides) and cellular vaccines for the immunotherapy of breast cancer. Antigen presentation in an adequate cytokine environment provoked by

immunostimulatory adjuvants in the vaccine formulation lead to antigen processing and presentation by dendritic cells (DC) and induction of a tumor-specific immune response

associated with poor prognosis. Pivotal phase III trials showed that trastuzumab in combination with cytotoxic chemotherapy prolongs overall survival in patients with HER2 positive breast cancer in both the metastatic and the adjuvant setting. Treatment with trastuzumab may be complicated by an increased risk of congestive heart failure that is higher with advanced age and concurrent treatment with trastuzumab and ► **anthracyclines**. Trastuzumab is most active in patients with tumors that have 3+ HER2 staining on immunohistochemistry (IHC) or have HER2 gene ► **amplification**.

The trials led to the approval of trastuzumab as a first-line treatment in combination with ► **paclitaxel** for HER2-positive metastatic breast cancer by the U.S. Food and Drug Administration (FDA) in 1998. In 2004, it was approved for the ► **adjuvant therapy** of HER2-positive breast cancer, either in combination

with chemotherapy, or as a single agent, for patients with node negative disease with high risk features or with node positive breast cancer. Trastuzumab is being extensively investigated in combination with different chemotherapy or hormone therapy agents to refine treatment combinations and schedules, as well as in combination with other approved Mabs (► **Cetuximab**, ► **Bevacizumab**, ► **Avastin**), with ► **tyrosine kinase inhibitors** of EGFR and HER2 (► **Lapatinib**), or with immunostimulatory agents (► **Interleukin-12**; IL-12; CpG). Continuing trastuzumab in combination with other treatments may have clinical benefits despite tumor progression on prior trastuzumab treatment. An antibody-drug conjugate (ADC) of trastuzumab and the maytansinoid DMI, a cytotoxic drug that acts by binding to (Tubulin/tubulin) and inhibiting tubulin polymerization, exhibited antitumor activity in patients with

metastatic breast cancer progressing under prior HER2 targeted therapy.

#### Cetuximab

Cetuximab (Erbitux<sup>®</sup>) is a recombinant chimeric IgG1 MAb that binds specifically to the extracellular domain of EGFR. Cetuximab is approved for the treatment of ► irinotecan-refractory metastatic ► colon cancer in combination with irinotecan, and for the treatment of locoregional advanced head and neck cancer as monotherapy or in combination with radiation. Dermatological toxicity is a limiting factor to the use of Cetuximab. Hypersensitivity to cetuximab (rash, urticaria, fever, dyspnea, and hypotension) is frequent in certain regions and has been related to the presence of IgE antibodies specific for an oligosaccharide, galactose- $\alpha$ -1,3-galactose, which is present on the Fab portion of the cetuximab heavy chain. Cetuximab is under investigation in combination with chemotherapy (► Carboplatin or ► Irinotecan) in pretreated triple negative breast cancer (TNBC) with advanced disease. TNBC is estrogen and progesterone receptor negative, as well as HER2 negative, and therefore is not amenable to treatment with ► hormonal therapy or with trastuzumab. Furthermore, several phase I/II studies with cetuximab in combination with cytotoxic agents or with other targeted therapies, such as trastuzumab, are currently ongoing.

#### VEGF

Vascularization, i.e., the formation of blood vessels, is essential for the growth of clinically relevant invasive carcinomas and metastasis. Expression of high levels of ► hypoxia-inducible factor 1 in tumor cells in response to reduced oxygen availability, as well as oncogenetic alterations, leads to the production and secretion of many different angiogenic factors by tumor cells, including ► vascular endothelial growth factor (VEGF). Once produced, angiogenic factors stimulate ► angiogenesis by binding to their cognate receptors on peritumoral vascular endothelial cells promoting neo-capillary formation. Furthermore, the secreted angiogenic factors bind to cells located at distant sites, including the bone marrow, and stimulate their homing to the tumor, where they contribute further to promote vascularization. VEGF expression is increased in many tumor types, including breast cancer, and this increase is associated with poor clinical outcome. ► Bevacizumab (► Avastin<sup>®</sup>) is a

recombinant humanized monoclonal antibody against vascular endothelial growth factor (VEGF) that blocks binding of VEGF to its receptor on vascular endothelium limiting angiogenesis and, consequently, tumor growth. Additionally, restoration of vessel structure and permeability decreases intratumoral interstitial fluid pressure, increasing delivery of cytotoxic drugs into tumors. Bevacizumab has shown antitumor efficacy, which was stronger in combination with chemotherapy, in many tumor types. Two pivotal phase III trials investigated ► capecitabine alone or combined with bevacizumab in patients with metastatic breast cancer who had prior anthracycline and ► taxane-based chemotherapy, and bevacizumab in combination with ► paclitaxel in patients with previously untreated metastatic breast cancer. Paclitaxel plus bevacizumab significantly increased median progression-free survival and objective response rate; median overall survival did not differ between the two groups. Grade 3/4 toxicities were more frequent in the combined treatment arm, including hypertension, proteinuria, headache, and thrombotic events. ► Bevacizumab offers an alternative treatment for HER2 negative tumors, and it has been approved for first-line treatment of metastatic breast cancer in combination with paclitaxel. Multiple trials using bevacizumab as part of ► adjuvant therapy are ongoing, including a phase II trial evaluating ► neoadjuvant ► cisplatin and bevacizumab in ► triple negative breast cancer (TNBC). Toxicity associated to bevacizumab may be a limitation to its use in an adjuvant setting.

#### MUC1

HuHMFG1 (AS1402), a humanized IgG1 antibody to MUC1, is capable of effecting ADCC that is enhanced by cytokines that stimulate NK cells. HuHMFG1 has been investigated in phase I clinical trials in patients with advanced breast cancer: The antibody was well tolerated and five cases of prolonged stable disease were seen, supporting its advancement to phase II clinical trials. However, a randomized phase II clinical trial evaluating the addition of huHMFG1 to endocrine therapy with ► letrozole in postmenopausal women receiving first-line treatment for advanced breast cancer was discontinued after 10 months. No safety concerns were identified, but reviewing of the data led the sponsor to conclude that the trial would be very unlikely to give sufficiently positive efficacy findings. The failure of this trial illustrates the difficulties of

testing the efficacy of MAbs in advanced disease and the importance of trial design.

### Vaccines for the Immunotherapy of Breast Cancer

Strategies for ► vaccine therapy (► Colon Cancer Vaccine Therapy) are numerous, ranging from cellular vaccines to vaccine constructs based on specific antigens with a variety of vectors and carriers, immunologic adjuvants, and modes of administration (Fig. 2; ► Cancer Vaccines).

#### Cellular Vaccines

An advantage of whole tumor cell vaccines is that the target antigen need not be identified before vaccination, as they present a whole range of antigens to the immune system. A strong disadvantage is that preparation of the vaccines is labor intensive, time-consuming, and costly, especially for personalized vaccines based on autologous material. This approach remains restricted to particular centers. The majority of studies with cellular vaccines have been carried out in melanoma, colorectal cancer (Colon Cancer Vaccine Therapy) and renal cell carcinoma. Cellular vaccines based on autologous tumor cells (from the patient's tumor), or allogeneic tumor cells (using established tumor cell lines), combined with an immunological adjuvant or a cytokine, or genetically engineered to secrete a cytokine, are being tested in clinical trials in advanced breast cancer, with some results.

**Dendritic Cell Vaccines** ► Adoptive immunotherapy involves the ex vivo expansion and activation of autologous immune cells that are then administered back to the patient. Several phase I/II clinical trials have been carried out in breast cancer with ► dendritic cells (DCs) cultured ex vivo from the patients' peripheral blood mononuclear cells (PBMCs) and pulsed with ► MUC1 peptides, ► HER2 protein, or tumor lysates. The vaccines have low toxicity and do not induce autoimmunity. Immune responses to the tumor, as well as clinical responses were observed in a subset of patients. A limiting factor to the clinical applicability of these vaccines may be posed by the technical challenges faced in their development. Vaccines based on tumor cells fused to allogeneic DCs that provide ► cytokines for adequate antigen presentation by the hosts' DCs may be more amenable to production than autologous DC vaccines.

### Vaccines Based on Defined Antigenic Substrates

One approach to vaccine design is to conjugate synthetic antigenic molecules to carrier protein, e.g., keyhole limpet hemocyanin, KLH, and administer the conjugate together with an immunologic adjuvant such as ► Bacillus Calmette-Guérin (BCG), ► DETOX, or ► QS-21 to augment the immunogenicity of the antigen and elicit an immune response to it. Another current approach includes the insertion of antigens of interest into a wide array of vectors (DNA, bacteria, viruses, or yeast), capitalizing on the vectors' innate and adaptive immunostimulatory characteristics to potentiate an immune response to the antigen. Viral vectors infect professional ► antigen-presenting cells (APCs), which in turn present peptides derived from the transgene antigen molecule in the context of HLA class I and II to T cells. An inflammatory reaction to the virus vector further amplifies the immune response to the transgene antigen. Virus vectors that actively replicate in the host, such as vaccinia virus, can present high levels of transgene antigen to the host immune system over a period of approximately 1 week, substantially increasing the potential for immune stimulation. Generation of host immune responses against the virus vector limits the efficacy of multiple vaccinations and has led to the development of vaccines that use different vectors for priming and for boosting the immune response.

#### HER2

Patients who have tumors overexpressing ► HER2 exhibit increased frequencies of T-cells to HER2 peptides and/or serum antibodies to HER2. Vaccines based on HER2 peptides are being tested in phase I clinical trials. A vaccine based on a HER2 protein and two epitopes from tetanus toxin formulated with aluminum hydroxide and administered with ► QS-21, a purified saponin that has been shown to increase both B-cell and T-cell responses, was tested in a phase I trial in 14 patients with locally advanced or metastatic breast cancer expressing HER2. The vaccine was nontoxic and induced antibodies to HER2. A chimeric peptide vaccine consisting of two HER2 epitopic sequences linked to a T-helper epitope derived from the measles virus (MVF-HER2 vaccine) was tested in a phase I trial in 27 patients with HER2-overexpressing metastatic tumors. The vaccine induced strong and long-lasting anti-HER2 antibody

responses, and two patients experienced complete remission of their tumor.

#### CEA

► **Carcinoembryonic antigen (CEA)** is a 180 kD glycoprotein expressed in normal fetal colon. It is a member of the immunoglobulin superfamily involved in intercellular recognition and ► **adhesion**. In the adult, it is expressed in normal colonic mucosa and is overexpressed primarily in colorectal adenocarcinomas, but also in other adenocarcinomas, including breast. One promising approach to CEA vaccination being tested in phase I/II clinical trials in metastatic breast cancer uses two poxvirus-based vaccines (Vaccinia-CEA-TRICOM and Fowlpox-CEA-TRICOM vaccine). These recombinant virus vector vaccines contain genes for human CEA and three costimulatory molecules that are capable of providing activating signals to antigen-specific T cells: B7.1, intercellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3). Granulocyte-macrophage colony-stimulating factor (GM-CSF) is coadministered at the vaccination site to promote the activation, maturation, and migration of APCs, such as ► **dendritic cells**. One study is being carried out in combination with dose-intensive induction chemotherapy, another in combination with ► **docetaxel**.

#### MUC1

Human MUC1 mucin is a major component of the ductal cell surface of normal glandular cells that is overexpressed and aberrantly glycosylated in virtually all adenocarcinomas. It is a multifunctional protein involved in the protection of mucous membranes, signal transduction, and modulation of the immune system. MUC1 is a high-molecular-weight (over 400 kD) type I transmembrane glycoprotein with a large, highly glycosylated extracellular domain that consists mainly of numerous peptide repeats, varying in number among the different alleles. Tumor-associated MUC1 has shorter glycans attached to the extracellular peptide core, which leads to the exposure of core protein epitopes, as well as to the presence on the molecule of tumor-associated glycans, such as the blood-group-related antigens, and sialyl Tn, as well as the Thomsen-Friedenreich (TF or T) antigen. The restrictive distribution of these antigens in normal tissues and their extensive expression in a variety of epithelial

cancers make them good targets for immunotherapy. MUC1 favors tumor progression and metastasis: loss of polarity and overexpression of MUC1 on cancer cells interferes with cell–cell adhesion and shields the tumor from immune recognition by the cellular arm of the immune system. At the same time, carcinoma-associated MUC1 induces cellular and humoral immune responses to the tumor. IgG antibodies to MUC1 are associated with a benefit in survival in patients with early stage breast cancer; they bind to MUC1 expressed on breast cancer cells and mediate ADCC in vitro. Several vaccine formulations based on MUC1 have been tested in patients with breast cancer, and immune responses and some clinical responses have been reported. A recombinant vaccinia virus expressing human MUC1 and interleukin-2 genes (TG1031) tested in phase I/II clinical trials in patients with advanced breast cancer induced some proliferative T-cell responses to MUC1 and stabilization of disease in a small number of patients, which lasted for some months. A recombinant vaccinia and fowlpox vaccine expressing both CEA and MUC1 and TRICOMTM administered with GM-CSF (PANVAC-VF) was tested in a pilot study in 25 patients with metastatic carcinoma, including breast cancer. The vaccine induced T-cell responses to CEA and to MUC1 in half of the patients, and two prolonged clinical responses. A phase I trial in metastatic breast cancer with a similar vaccine (without CEA) is in progress.

Vaccination of breast cancer patients with MUC1 peptides conjugated to KLH and administered with QS-21-induced high titres of MUC1 antibodies to the naked peptide with only moderate binding to glycosylated forms of the peptide, suggesting that glycopeptides, rather than naked peptides, constitute a better vaccine substrate. Vaccination with MUC1 glycopeptides conjugated to KLH, alone or in combination with other tumor antigens, are been tested in phase II trials. Most clinical studies with (glyco)peptide vaccines (► **Peptide Vaccines for Cancer**) have been carried out in advanced disease, whereas this form of therapy may be effective only in an adjuvant setting, in patients with a low tumor burden (► **Minimal Residual Disease**), and is probably not useful in advanced metastatic disease. In this respect, the results of a small phase III randomized study (31 patients) performed in stage II breast cancer patients with no evidence of disease using a MUC1

fusion protein coupled to oxidized mannan (M-FP) or *placebo* are encouraging. Mannan is a polysaccharide made up of mannose subunits present in the cell wall of microorganisms and yeast that binds to the mannose receptor on dendritic cells (DCs). In preclinical studies, oxidized mannan was able to steer the immune response toward the Th1 type (high cytotoxic T-cells, low antibody, and IL-12 and IFN- $\gamma$ ). The vaccine induced antibodies to MUC1 in most patients, and MUC1-specific T-cell responses in a small number of them. After a median follow-up of more than 5 years, the recurrence rate in the *placebo* group was 27%; those receiving immunotherapy had no recurrences. A vaccine to sialyl-Tn (STn), a glycopeptide expressed on MUC1, was tested in phase I, II, and III clinical trials. In phase II studies in breast cancer, the SialylTn-KLH combined with DETOX vaccine (THERATOPE<sup>®</sup>), induced antibody responses to STn and to ovine submaxillary mucin (OSM) that were improved by pretreatment with low-dose intravenous cyclophosphamide, and showed median survival rates nearly three times that of patients in a retrospective, frequency-matched, control group who received conventional therapies. A phase II study in 33 patients with breast cancer who were treated with THERATOPE<sup>®</sup> following high-dose chemotherapy provided evidence for the induction of T-cell immunity. A phase III randomized clinical study in 1,028 patients with metastatic breast cancer who had either no evidence of disease or nonprogressive disease after first-line chemotherapy did not result in longer overall survival (OS) and time to progression (TPP). Antibody titres against OSM, but not against STn or KLH, were correlated with survival. As with MUC1 glycopeptide vaccines, minimal tumor burden settings might be more suitable for eliciting a clinical benefit with this type of vaccine immunotherapy. Patients with metastatic disease may not have time to mount an effective immune response before tumor burden either exceeds the therapeutic potential of immunotherapy or compromises the patient's immune responsiveness.

## Perspectives

In general, the current studies argue for vaccines to be used as early on in the disease as therapeutically possible, as opposed to end stage disease after other modalities have failed. Patients included in experimental vaccine therapy have late-stage disease with high tumor burden and are the least likely to benefit from

vaccination. Monotherapies tested in this setting run the risk of being discarded early on, when they may well have been effective in an adjuvant setting. Vaccines that are effective in an adjuvant setting may have applicability as prophylactic vaccines to reduce the risk of breast cancer in BRCA1/2 mutation carriers (► **BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk**). Furthermore, there is a substantial body of evidence to suggest that some combinations of chemo- or radiotherapy and vaccine treatment are synergistic, as well as increasing evidence that the standard chemotherapy approaches may target regulatory cells. Combining such approaches with immunotherapy may result in enhanced tumor-specific immune responses and clinical efficacy. Multimodal treatments that affect several aspects of the immune system may improve the efficacy of cancer immunotherapy.

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## Breast Cancer Multistep Development

Dihua Yu and Jing Lu

Departments of Molecular and Cellular Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

### Definition

A ► **multistep development** of breast cancer involves increasingly abnormal stages during ► **breast cancer progression** as illustrated in Fig. 1.

### Characteristics

Breast cancer is well-recognized as a heterogeneous disease. It can be categorized as five subtypes based on gene expression profiles as determined by ► **multigene arrays**: luminal A, luminal B, ► **HER2+/-/estrogen receptor (ER)-**, basal-like, and normal breast-like [1]. Based on ► **epidemiological** and histological observations of mostly the luminal A and luminal B subtypes, these steps can be defined as a series of morphological changes beginning with ► **hyperplasia**, followed by atypical hyperplasia, ► **carcinoma in situ**, invasive ► **carcinoma**, ending with ► **metastatic** breast cancer, the major cause of most breast cancer related deaths. This seemingly continuous but nonobligatory progression can occur over long periods of time, decades in many cases, and many patients can live with the early stage noninvasive lesions through a normal life span, without being diagnosed or treated.

### Multistep of Breast Cancer Progression

#### Hyperplasia

Hyperplasia refers to the increased ► **proliferation** of normal-looking mammary ► **epithelial cells** within the breast. As a benign, noncancerous disease, hyperplasia can be caused by delayed ► **differentiation** rather than the essential alterations that will obligatorily lead to breast cancer. However, statistical studies have indicated that women with hyperplasia have a twofold increase in the risk of developing breast cancer.

#### Atypical Hyperplasia

Atypical hyperplasia (AH) is characterized as a condition when breast cells appear abnormal in size, shape,

number, or growth pattern. It is found in approximately 15% of breast biopsies following the identification of suspicious microcalcification. According to the location of these abnormal cells within the breast tissue, the lobules or the ducts, AH can be further divided into atypical lobular hyperplasia (ALH) or atypical ductal hyperplasia (ADH).

#### Carcinoma In Situ

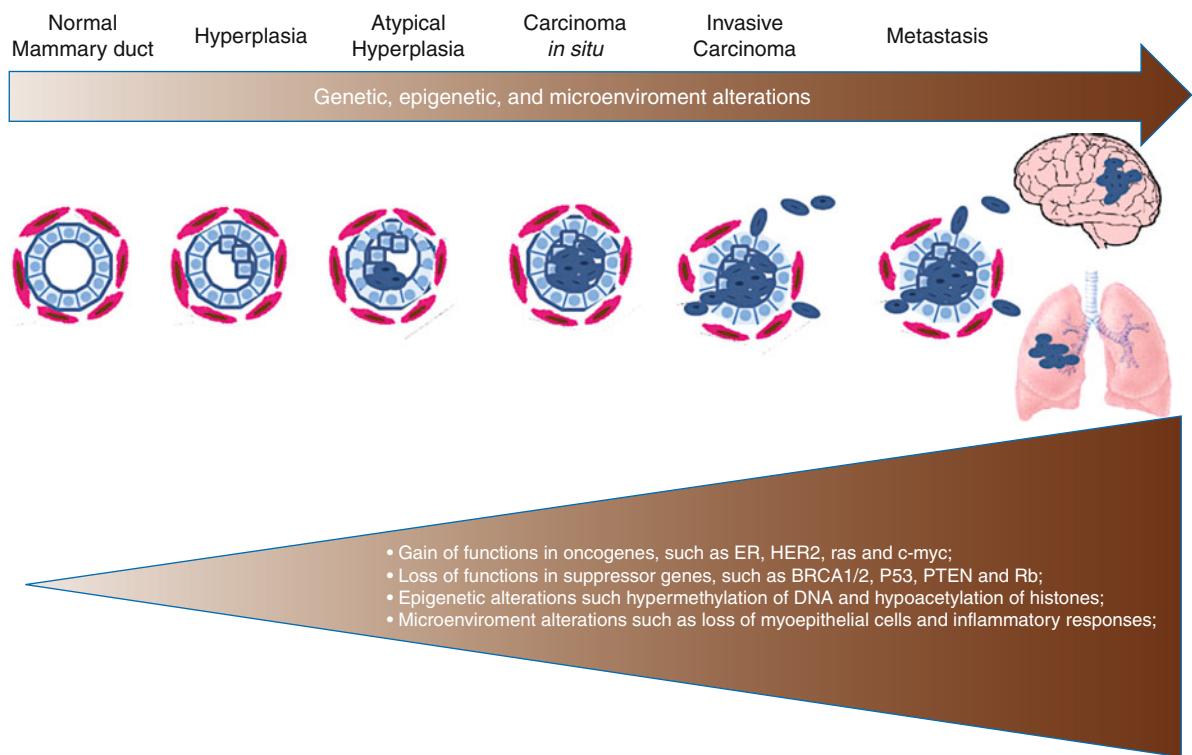
► **Carcinoma in situ** is the first malignant step in the progression of breast cancer. It is defined by the clonal proliferation of malignant cells that are restrained within the lumen of mammary ducts (termed ► **ductal carcinoma in situ**, or DCIS) or lobules (termed lobular carcinoma in situ, or LCIS). DCIS and LCIS have been indicated to evolve from ADH and ALH, respectively. In both cases, there is no invasion into surrounding stroma.

DCIS is the most common type of noninvasive breast cancer in women, accounting for 25% of all breast cancer diagnoses. As an intermediate stage in breast cancer progression between ADH and invasive cancer, DCIS represents a spectrum of heterogeneous breast diseases which vary both morphologically and biologically, and therefore remain a challenging task for its classification and clinical management. Traditionally, DCIS classification has been mainly based on architectural growth pattern and thus divided into comedo, solid, cribriform, papillary, micropapillary, clinging, hypersecretory, and apocrine variants. However, this classification does not allow prediction of the clinical behavior of DCIS, particularly its potential for progression into life-threatening invasive disease. To generate better correlation with the clinical outcome of DCIS, several new criteria have been proposed and most of them are based primarily on nuclear grade (high, intermediate, and low) and secondarily on cell polarization (architectural differentiation) and absence or presence of ► **necrosis**. These classifications are more predictive of disease recurrence after surgical resection.

LCIS is relatively rare compared to DCIS and usually shows a low proliferation rate. In many cases, LCIS is diagnosed in patients before menopause, and the lesions are usually multifocal and bilateral (► **Contralateral Breast Cancer**).

#### Invasive Carcinoma

Invasive carcinoma is defined as cancerous cells having spread beyond the mammary ducts or lobules and



**Breast Cancer Multistep Development.** **Fig. 1** Schematic representation of the multiple-step model of breast cancer development. These steps can be defined as a series of

increasingly abnormal stages, including hyperplasia, atypical hyperplasia, carcinoma *in situ*, invasive carcinoma, and metastatic breast cancer

invaded into the surrounding ► **stroma**. There are many subtypes of invasive carcinoma in the breast, with the invasive ductal carcinoma (i.e., malignant cells have penetrated through the ► **basement membrane** of mammary duct and invaded the fatty tissue of the breast) as the most common type, accounting for three-quarter of all cases. The second most common subtype is invasive lobular carcinoma, which is characterized as cancerous cell invading through the lobules of breast. Other rare forms of invasive breast cancer include inflammatory carcinoma, medullary breast cancer, and adenocystic breast cancer. Pathologic/clinical and molecular studies have strongly supported the *in situ* carcinoma as the precursor lesion of invasive carcinoma.

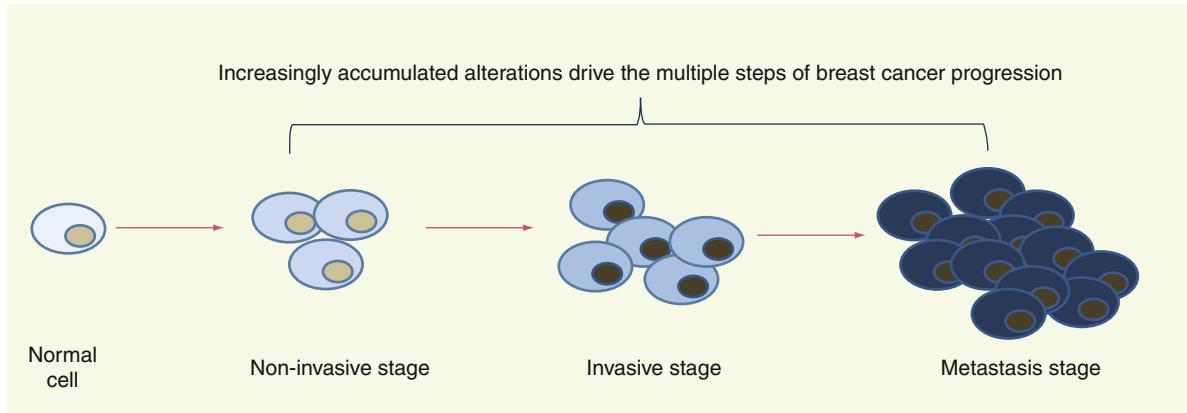
### Metastasis

The end stage of breast cancer as a progressive disease is ► **metastasis**, when breast cancer cells gain the capability to escape the restrain of primary site, metastasize, and colonize a secondary site. Metastasis is extremely devastating to patients because the vast

majority of breast cancer mortality is due to metastasis, not the primary tumor. Metastasis is a multistep cascade involving at least the following crucial events: dissemination from original tissue architecture, increased ► **matrix metalloproteinases** expression to degrade extracellular matrix barrier, elevated ► **motility** and ► **invasion**, ► **intravasation** into the blood or lymphatic vessels and survive in the circulation, extravasation, and adapt to a foreign microenvironment of distant organs for metastatic growth. The most common sites of breast cancer metastasis are the bones, brain, liver, and lungs.

### Mechanisms That Drive Multistep Development of Breast Cancer

It is commonly accepted that the multistep development of breast cancer is driven by progressively accumulated genetic, epigenetic, and microenvironmental alterations (Figs. 1 and 2). Numerous studies have confirmed the essential role of genetic abnormalities in breast cancer progression. Two categories of genetic abnormalities are the ► **gain-of-function mutation** in



**Breast Cancer Multistep Development.** **Fig. 2** Linear Multistep model of breast cancer development. The increasingly accumulated alterations at genetic, epigenetic, and

microenvironment levels gradually drive the progression from normal breast tissue to noninvasive stage, to invasive stage, and ultimately metastatic breast cancer

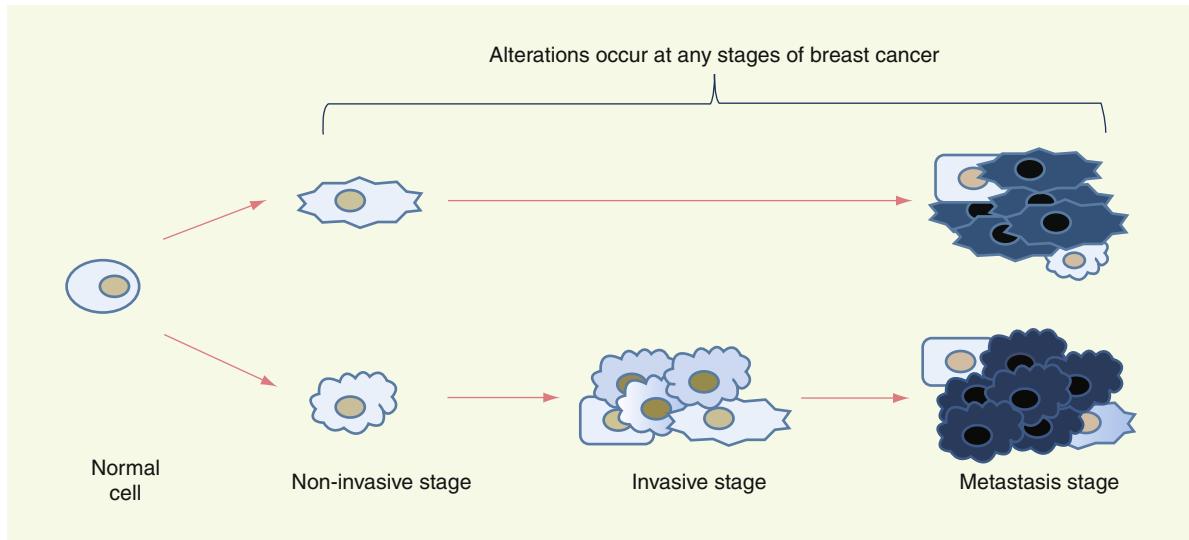
proto-oncogenes and the loss-of-function mutation in tumor suppressor genes. Some well-known oncogenes involved in breast cancer include HER2 (or ErbB2), RAS (RAS Genes), the MYC oncogene, and many others. The activation of proto-oncogenes can occur through gene amplification, rearrangement by chromosomal translocation, and mutation. Critical tumor suppressor genes in breast cancer include BRCA1 and BRCA2 (Breast Cancer Genes BRCA1 and BRCA2), P53, PTEN, the retinoblastoma gene RB1, and others. Inactivation is frequently caused by mutation, deletion, or allelic loss. These diverse genetic events contribute to the disruption of normal cellular physiology in various perspectives, such as uncontrolled proliferation, insensitivity to stimuli to undergo apoptosis, increased potential for migration, and eventually lead to the ultimate transition to a malignant mammary epithelial cell.

The contribution of epigenetic changes to breast cancer development and progression is increasingly recognized over the past decade. Different from genetic alterations, epigenetic events, such as hypermethylation of DNA and hypoacetylation of histones, can substantially alter gene expression by modifying chromatin structures (Histone Modification). Genes affected by epigenetic alterations in breast cancers include HOXA5, P21, gelsolin, E-Cadherin, and others.

The essential role of the microenvironment along breast cancer progression has also been gradually established. The fact that malignant breast cancer

cells could dwell in a dormancy state over long period of time clinically, and numerous elegant experimental models demonstrating the failure of many tumor cells to thrive in a new environment in spite of high rates of arriving at the secondary organs, effectively reveal the protective role of normal microenvironment in preventing breast cancer development and progression. The suppressive role of the microenvironment during breast cancer progression is perhaps best reflected at its last stage – metastasis. It has been demonstrated that the distinct organ pattern of breast cancer metastasis is highly dependent on the intricate interactions between breast tumor cells and the microenvironment of particular target organs, not a random process. Therefore, it is increasingly accepted that the progression of breast cancer through the multiple steps is accompanied by tumor cells gradually acquiring capability to convert an oppressive microenvironment to a permissive microenvironment. A distinct example of the microenvironment components in regulating breast tumor progression is the suppressive role of myoepithelial cells in preventing the transition of ductal carcinoma in situ (DCIS) to invasive breast cancer. Emerging data strongly suggested that the layer of myoepithelial cells surrounding mammary ducts functions as a barrier to inhibit the escape of malignant breast tumor cells to other tissues or organs.

Unlike the noninvasive breast lesions, which have favorable prognosis if diagnosed and intervened clinically, invasive carcinoma and metastasis significantly contribute to the morbidity and mortality of breast cancer patients. Therefore, extensive efforts in both



**Breast Cancer Multistep Development.** Fig. 3 Selection model of breast cancer development. Diverse genetic, epigenetic, and microenvironment alterations can occur at any stage of breast cancer development. The successfully established

clinical and basic research have been attributed to better understand the transition from noninvasive carcinoma *in situ* to invasive carcinoma. Various alterations at the genetic, ► **epigenetic**, and microenvironment levels collaborate to increase the intrinsic cell ► **migration** ability and decrease the rigid intracellular restraints exerted on by both cell–cell and cell–matrix ► **adhesion**, to ultimately convert the noninvasive breast tumor to life-threatening invasive/metastatic breast tumor.

#### Alternative Model of Breast Cancer Development

As the central paradigm of breast cancer development, the linear multiple-steps model reflects both the pathological observations and the genetic/epigenetic alterations found in patients and experimental models. However, due to the heterogeneous nature of breast cancer and the enormous number of factors involved in the breast cancer progression, this model mainly applies to the luminal A and luminal B subtypes of this disease, but cannot summarize all subtypes of breast cancer. The massive diversity in both phenotype and genotype of a certain stage of breast tumor formulates an alternative model of breast cancer development and progression: the diversity selection model (Fig. 3). This model proposes that the various subtypes of breast cancer are the results of selective expansion of altered stem or progenitor cells in the breast. And the

tumor is the result of selection pressures from the environment and/or clinical treatments, but does not necessarily go through all the steps

tumor does not necessarily go through all the linear stages. These two models are not intrinsically incompatible. Multiple genetic/epigenetic alterations can also gradually accumulate in stem cell or progenitor cells (► **Breast Cancer Stem Cells**), which may contribute to the intratumoral heterogeneity. Also, somatic breast epithelial cells can acquire genetic/epigenetic alterations to obtain stem cell or progenitor cell properties.

#### Conclusion

In summary, breast cancer ► **multistep progression** has been significantly elucidated over the past decade. However, more in depth investigations are imperative to identify key players in this process. The goal is to develop strategies to detect the early events of breast cancer multistep progression and to intervene effectively this dreadful process.

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## Breast Cancer New Therapies: HER2, VEGF, and PARP as Targets

Shaheenah Dawood<sup>1</sup> and Massimo Cristofanilli<sup>2</sup>

<sup>1</sup>Department of Medical Oncology, Dubai Hospital, Dubai, UAE

<sup>2</sup>Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, USA

### Definition

The last two decades have seen an explosion of information in the treatment of both early and advanced stage ► **breast cancer**. The Early Breast Trialists' Collaborative group 15-year update clearly demonstrates that 6 months of ► **adjuvant** ► **anthracycline**-based polychemotherapy reduces the annual breast cancer death rate by 38% and 20% for women younger than 50 years and those aged 50–69 years respectively. The recognition and understanding of the biological subtypes of breast tumors has helped move its management toward a more personalized approach further improving these figures. ► **Gene expression profiling** has identified at least six subtypes of breast tumors including luminal subtypes (hormone receptor positive), ► **HER2** subtype, and a ► **basal-like subtype**. In parallel has been the development and implementation of specific targeted therapies that has not only allowed for more treatment options to be available but has altered the natural history of the disease positively impacting survival outcomes.

### Characteristics

#### Anti-HER2 Therapy

► **HER2** protein overexpression or gene ► **amplification** occurs in approximately 20–25% of breast cancers

and is a ► **biomarker** of a more aggressive disease associated with an adverse prognostic outcome. Several agents have been developed that abrogate HER2 mediated signaling pathways with two agents currently approved for clinical use. ► **Trastuzumab**, a humanized ► **monoclonal antibody**, targeting the extracellular component of the HER2 receptor, is approved for use in both the adjuvant and metastatic setting. In the pivotal phase III ► **clinical trial** by Slamon and colleagues that randomized 469 patients with HER2 positive metastatic breast cancer to receive first line treatment with either ► **chemotherapy** alone or chemotherapy and trastuzumab, the investigators reported a significant improvement in median overall survival from 20.3 months to 25.1 months. Four large randomized clinical trials evaluated the role of trastuzumab in the adjuvant setting among women with node positive or high risk node negative breast cancer. A combined analysis of the NSABP-31 and the NCCTG N9831 studies, in which women with early stage HER2 positive breast cancer treated with adjuvant ► **doxorubicin** and followed by ► **paclitaxel** with or without 1 year of trastuzumab, demonstrated a 52% increase in disease free survival and 35% increase in overall survival with the addition of trastuzumab. The HERA study randomized a similar cohort of 5,102 women with HER2 positive early stage breast cancer who had completed standard chemotherapy to 1 or 2 years of trastuzumab versus observation. At a median follow-up of 3 years, the investigators reported a significant increase in disease free survival by 36% and overall survival by 34% among women who had received 1 year of trastuzumab compared to observation. In the BCIRG 006 study, 3,222 women with early stage HER2 positive breast cancer were randomized to receive either anthracycline-based regimen (adriamycin and ► **cyclophosphamide** followed by ► **docetaxel**), a non-anthracycline-based regimen with 1 year of trastuzumab (trastuzumab, docetaxel, and ► **carboplatin**), or an anthracycline-based regimen with 1 year of trastuzumab (► **adriamycin** and ► **cyclophosphamide** followed by docetaxel and trastuzumab). The investigators reported an improvement in disease free survival with the addition of trastuzumab by 39% and 33% in the anthracycline and non-anthracycline containing arms of the study respectively compared to the group of women who did not receive trastuzumab. In contrast to these large-scale trials that evaluated 1 year of trastuzumab, the FinHer study assessed the efficacy of 9 weeks of adjuvant

trastuzumab in a group of node positive or high risk node negative women with HER2 positive early stage breast cancer. At a median follow-up of 62 months, the authors reported that the addition of trastuzumab resulted in a reduced risk of distant recurrence or death compared to the group who did not receive trastuzumab (hazard ratio with adjustment for presence of axillary nodal metastases was 0.57, p = 0.047). There is currently an ongoing phase III trial evaluating 1 year of trastuzumab compared to 9 weeks of trastuzumab in the adjuvant setting.

The main side effect of the use of trastuzumab is cardiotoxicity. In a pooled analysis of the four large adjuvant studies grade III or IV cardiotoxicity was reported for 4.5% of patients receiving trastuzumab compared to 1.8% of patients. In a separate meta-analysis of over 11,000 patients, the relative risk of cardiotoxicity associated with the adjuvant use of trastuzumab versus no trastuzumab was 5.59 (95% CI 1.99–15.7, p = 0.011). Similar observations were noted in the metastatic setting as well with an important observation that the rate of cardiotoxicity substantially increased with the combination of trastuzumab and anthracyclines. Based on such observations guidelines are now available for cardiac monitoring of patients receiving trastuzumab in either the adjuvant or metastatic setting.

The second anti-HER2 agent approved for clinical use is the reversible ► **tyrosine kinase inhibitor** ► **lapatinib** that targets intracellular ► **tyrosine kinase** component of both the ► **HER2** receptor and the ► **epidermal growth factor receptor** (EGFR). In phase III randomized clinical trial over 300 women with metastatic HER2 positive breast cancer who had progressed after receiving ► **anthracycline**- , ► **taxane**- , and ► **trastuzumab**-based regimens were randomized to receive either ► **capecitabine** alone or capecitabine and ► **lapatinib**. The investigators reported a significantly improved median time to progression in the combination arm versus the monotherapy arm of the study (8.4 months versus 4.4 months). A randomized phase III trial has also evaluated the combination of lapatinib and trastuzumab compared to lapatinib alone in a cohort of heavily pretreated women with HER2 positive breast cancer demonstrating significantly improved progression free survival in the combination arm.

Pertuzumab, like trastuzumab, is a ► **monoclonal antibody** that binds HER2. However, in contrast to

trastuzumab it binds to a different ► **epitope** disrupting HER2 dimerization. Phase I and II trials have demonstrated good tolerance and clinical benefit in a heavily pretreated population. It is currently being evaluated in the phase III CLEOPATRA trial. ► **Trastuzumab** –DM 1 is trastuzumab that is bound to an inhibitor of tubular polymerization. In the phase II setting trastuzumab – DM1 when administered to a cohort of women with HER2 positive metastatic breast cancer who had progressed on prior anti-HER2 therapy resulted in an overall response rate of 38.2%. This agent is currently being tested in the phase III setting.

### Anti-VEGF Therapy

Tumor ► **angiogenesis** is an important step in the development of breast tumors and is regulated by a number of pro angiogenic factors including ► **vascular endothelial growth factor** (VEGF). ► **Antiangiogenesis** agents abrogate signaling pathways promoted by these receptors. ► **Bevacizumab** is a humanized anti-VEGF antibody that is approved for use in the treatment of women with ► **HER2** negative metastatic breast cancer. In the phase III ECOG 2100 trial, 722 women with HER2 negative metastatic breast cancer were randomized to receive first line treatment with either ► **paclitaxel** alone or paclitaxel and bevacizumab. The investigators reported an significant improvement in median time to progression (11.8 months versus 5.9 months, p < 0.001) and overall response rate (36.9% versus 21.2%, p < 0.001) in the combination arm compared to the group of patients who received paclitaxel alone. Overall survival however was similar between the two groups (26.7 months vs. 25.2 months, p = 0.16). In the phase III AVADO trial, a similar cohort of women were randomized to receive first line treatment with docetaxel alone or in combination with bevacizumab. A significant improvement in progression free and overall survival was observed. Recent results from the Ribbon 1 and 2 studies have also demonstrated the efficacy of bevacizumab in combination with a variety of chemotherapeutic agents in both first and second line setting respectively.

A number of phase II and III clinical trials are exploring novel combinations with bevacizumab. The CALGB is conducting a phase III clinical trials of the combination of an ► **aromatase inhibitor** with bevacizumab in an attempt to overcome or delay endocrine resistance. The combination of

bevacizumab with anti-HER2 agents is also being explored. Other anti-VEGF agents such as tyrosine kinase inhibitors ► **sorafenib** and ► **sunitinib** are also being evaluated in patients with HER2 negative metastatic breast cancer.

### PARP Inhibitors

► **Poly(ADP-ribose) polymerase** (PARP) is a nuclear enzyme that plays a critical role in cell ► **proliferation** and ► **DNA repair**, and therefore inhibition of PARP has been explored in a number of ► **phase I and II trials**. The PARP inhibitor BSI-201 has been evaluated among women with ► **triple negative breast cancer** in a randomized phase II setting in combination with ► **gemcitabine** and ► **carboplatin** where a significant improvement in clinical benefit rate, progression free survival, and overall survival was observed compared to chemotherapy alone. The oral PARP inhibitor ► **olaparib** has in the phase II setting demonstrated 38% response rate as a single agent in a cohort of women with chemotherapy refractory ► **BRAC1 or BRCA2 mutated** metastatic breast cancer.

### Future Directions

The use of targeted therapies in the treatment paradigm of patients' breast cancer has been revolutionary in the management of this disease. Current adjuvant and metastatic trials are focused on incorporated these novel agents. Other novel agents such as the ► **mammalian target of rapamycin** (mTOR) inhibitor ► **rapamycin** and the heat shock protein 90 (► **Hsp90**) inhibitor tanespimycin (that interacts with HER2 through its kinase domain and has a stabilizing effect on it) are also currently being investigated. Ultimately, the goal is to improve prognostic outcomes with minimal toxicity by individualizing treatment using ► **targeted therapies** based on the breast tumor subtype presentation.

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## Breast Cancer Prognostic and Predictive Biomarkers

Michael Z. Gilcrease

Department of Pathology, Breast Section, M. D. Anderson Cancer Center, Houston, TX, USA

### Definition

A biomarker is a body substance or component that can be objectively measured to indicate the status of a biological (usually pathological) process. Normal genes and gene products can serve as biomarkers, as well as alterations in or modifications of normal genes and gene products. Combinations of substances that together indicate a particular biological function can also serve as biomarkers, as well as entire cells. Biomarkers that indicate how a disease will progress in an individual patient are referred to as prognostic biomarkers, whereas those that predict how a disease will respond to a particular therapy are termed predictive biomarkers. A number of prognostic and predictive biomarkers are currently used clinically or are under investigation to guide therapy for ► **breast cancer** patients.

### Characteristics

#### Established Prognostic Biomarkers in Breast Cancer

Well-established prognostic factors for invasive breast carcinoma include the histologic type, tumor grade, presence or absence of lymphovascular invasion, tumor size, and lymph node status. These traditional prognostic markers, although based on the microscopic

assessment of the tumor or regional lymph nodes, are sometimes not regarded as biomarkers per se, as they do not entail the quantitative measurement of a single biological substance. Nevertheless, they are biomarkers in a broad sense, and they have well-established prognostic utility. Other prognostic biomarkers are useful only if they provide additional information about disease outcome that is independent of that provided by these well-established prognostic factors.

Favorable histologic types of invasive breast carcinoma include tubular carcinoma, mucinous carcinoma, medullary carcinoma, low-grade adenoid cystic carcinoma, low-grade adenosquamous carcinoma, and fibromatosis-like metaplastic tumor. Unfavorable histologic types of invasive breast carcinoma include invasive micropapillary carcinoma, some forms of metaplastic breast carcinoma, centrally necrotizing breast carcinoma, and invasive breast carcinoma with a “large central acellular zone.” Invasive micropapillary carcinoma tends to be high stage at presentation but does not clearly have a worse prognosis than stage-matched invasive ductal carcinomas. Some metaplastic breast carcinomas, particularly those with a predominant sarcomatoid morphology, are aggressive tumors that behave like true sarcomas. Carcinosarcomas are similarly clinically aggressive tumors but have a greater likelihood of axillary lymph node involvement than predominantly sarcomatoid carcinomas. Both centrally necrotizing carcinomas and those with large central acellular zones have a tendency to metastasize to lungs and brain and have a particularly poor prognosis.

The grade of breast cancer is a measure of potential aggressive behavior based on the histologic appearance of well-defined cytological parameters. The Nottingham combined histologic grading system is recommended by the College of American Pathologists for grading invasive breast carcinomas. This grading system takes into account the degree of nuclear pleomorphism of invasive tumor cells, the mitotic rate of the invasive tumor, and the degree of tubule formation by the invasive tumor cells. Tumor grade is reported as grade 1 (low grade), grade 2 (intermediate grade), or grade 3 (high grade). Tumor grade is an independent prognostic factor. High-grade tumors have a worse prognosis than low- and intermediate-grade tumors.

Lymphovascular invasion also portends a worse prognosis. The College of American Pathologists recommends using the terminology “vascular invasion” when tumor cells are identified within either lymphatic or blood vascular channels. (It is not necessary to distinguish between the two.) Lymphovascular invasion should be evaluated in the peritumoral breast tissue. It is present in approximately 20% of primary invasive breast carcinomas, and its presence is an adverse prognostic factor, independent of other prognostic factors. Lymphovascular invasion is independently associated with local tumor recurrence and patient survival.

The size of an invasive breast carcinoma should be reported at least for the greatest single dimension. The prognostic significance is based on the size of the invasive component only. Associated carcinoma *in situ* (carcinoma that has not invaded beyond the basement membrane of the normal breast duct system) is not included in the size of the invasive breast carcinoma. Only 10–20% of patients with invasive breast carcinomas measuring less than 1 cm have axillary lymph node metastases. The recurrence-free survival at 10 years for patients with negative axillary nodes is approximately 90% when the tumor size is less than 1 cm.

The lymph node status has long been regarded as the single most important prognostic factor in breast cancer. Only 20–30% of patients with negative lymph nodes develop tumor recurrence within 10 years, compared to almost 70% of patients with positive lymph nodes. Patients with four or more positive lymph nodes have a worse prognosis than those with three positive nodes or less. The prognostic significance of micrometastases is not clearly established but appears to be worse than complete absence of metastasis. The significance of isolated tumor cells in the axillary lymph nodes, now staged separately from micrometastases, is even less clear.

### Prognostic Markers Following Breast Conservation Surgery and Neoadjuvant Chemotherapy

With increasing use of breast conservative surgery and neoadjuvant chemotherapy (chemotherapy before surgical excision of the primary tumor), additional important prognostic markers include margin status and pathologic response to neoadjuvant chemotherapy (► [Neoadjuvant Therapy](#)). A positive margin (invasive

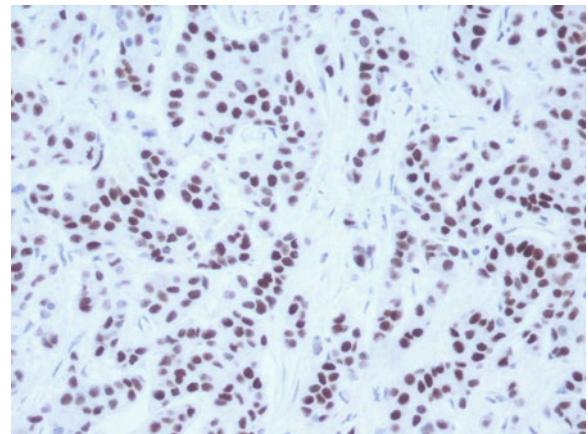
tumor at the surgical margin of the excised breast tissue) has been shown to be an independent predictor of decreased survival ( $RR = 3.9, P = 0.011$ ). Breast conservative surgery, therefore, requires negative margins. Subsequent radiation therapy is also required, even when negative margins are achieved, to reduce the risk of tumor recurrence following breast conservative surgery.

A pathologic complete response (pCR) to neoadjuvant chemotherapy is a favorable prognostic factor. It is defined as a complete eradication of invasive carcinoma cells following chemotherapy. In a recent study of 1,731 patients treated with neoadjuvant chemotherapy, a pCR was observed in 13%; 8% of hormone-receptor-positive patients had a pCR, while 24% of hormone-receptor-negative patients had a pCR. In hormone-receptor-positive patients, 5-year survival was 96.4% versus 65.3% with and without a pCR, respectively. In hormone-receptor-negative patients, 5-year survival was 83.4% versus 67.4% with and without a pCR, respectively. Because a pCR is an important prognostic factor for patients treated with neoadjuvant chemotherapy, it is important that the tumor site be sampled correctly by the pathologist. It is useful to place a metallic marker in the tumor if a response is observed after initiating chemotherapy to facilitate identification and correct sampling of the tumor site in the surgical excision specimen in the event of a pCR or near-complete response.

### Established Predictive Biomarkers in Breast Cancer

Clinically useful prognostic and predictive biomarkers should have biologic relevance and well-defined scoring criteria. They should be reproducible in different laboratories, confirmed independently by multiple investigators, and validated in large prospective studies. Most reported markers for breast cancer do not yet meet these criteria. As a result, only a few are currently recommended for routine practice.

Hormone receptor staining is routinely performed more for its utility in predicting response to hormonal therapy than for its prognostic significance. (There are mixed data on the prognostic significance of hormone receptor expression in invasive breast carcinoma.) A quantitative value for ► [estrogen receptor](#) (ER) and ► [progesterone receptor](#) (PR) expression is routinely reported for all invasive breast carcinomas, as response to ► [endocrine therapy](#) has been shown to be



### Breast Cancer Prognostic and Predictive Biomarkers.

**Fig. 1** Nuclear expression of estrogen receptor in invasive breast carcinoma

proportional to the degree of hormone receptor positivity. Completely negative staining or weak staining in less than 1% of invasive carcinoma cells is regarded as a negative test for estrogen or progesterone receptor. Any degree of staining greater than this is now regarded as a positive test, and the likelihood of response to ► [hormonal therapy](#) appears to be directly related to the amount of nuclear staining for ER and PR in the invasive tumor cells (Fig. 1).

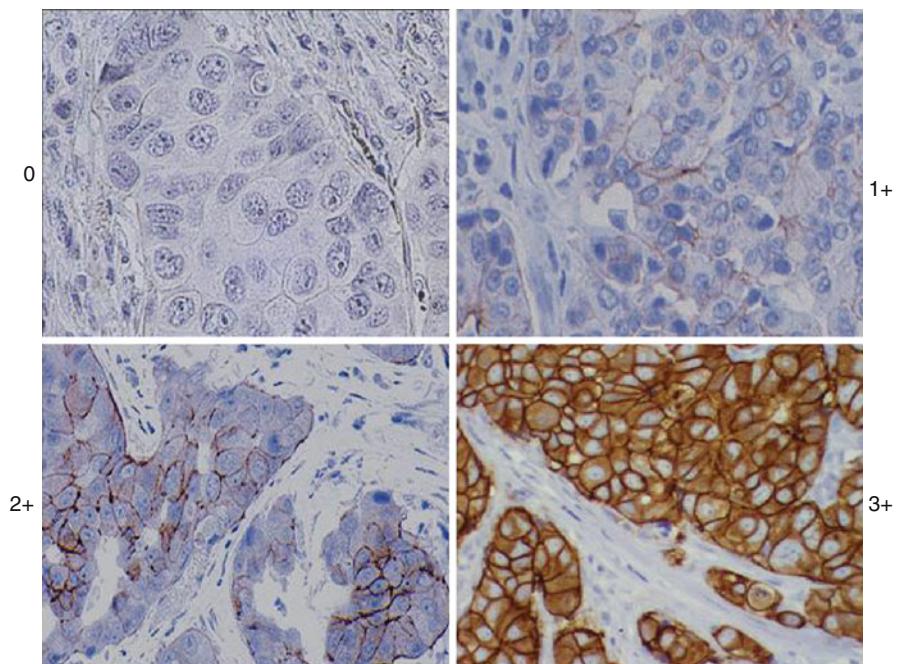
► [HER2](#) (c-erbB-2) is a member of the ► [Epidermal Growth Factor Receptor](#) (EGFR) family of growth factor receptors. Overexpression of the protein and/or ► [amplification](#) of the HER2 gene have been shown to be an adverse prognostic factor in node-positive breast cancer patients, but evaluation of HER2 status is routinely performed on all invasive breast carcinomas more for its utility in predicting response to anti-HER2 therapy, such as ► [trastuzumab](#) (► [Herceptin](#)) or ► [lapatinib](#). In experienced labs, 3+ HER2 staining by ► [immunohistochemistry](#) correlates well with HER2 gene ► [amplification](#) as determined by ► [fluorescence in situ hybridization](#) (FISH) (Fig. 2). Tumors with 3+ HER2 expression or HER2 gene amplification show the greatest response to trastuzumab therapy, and they are also more sensitive to ► [anthracycline-containing](#) ► [chemotherapy](#).

### Proposed Biomarkers in Breast Cancer

A variety of tumor markers have been proposed, most of which are analyzed by ► [immunohistochemistry](#) assays. A few of these show promise as potentially

**Breast Cancer Prognostic and Predictive Biomarkers.**

**Fig. 2** Membranous expression of HER2 in invasive breast carcinoma. Scores of 0 and 1+ are negative, 2+ is equivocal, and 3+ is positive for ► HER2 overexpression



useful prognostic markers but have not yet been adopted in routine practice. Several new molecular tests are also reported to have both prognostic and predictive utility.

The ► **Ki-67** antigen is expressed in late G1, S, and early G2/M phases of the ► **cell cycle**. Immunohistochemical staining for Ki-67 is more sensitive than S-phase analysis or mitotic figure counting for assessing proliferation. Ki-67 analyses, however, lack standardization. The College of American Pathologists recommends reporting mitotic figure counts for every invasive breast carcinoma and designates the use of MIB-1 immunohistochemistry (for detection of Ki-67) as optional.

► **Urokinase-Type Plasminogen Activator** (uPA), a serine protease, is a promising prognostic marker for breast cancer. uPA and its inhibitor, Plasminogen Activator Inhibitor 1 (PAI-1; ► **Plasminogen-Activating System**), stimulate the ► **adhesion**, migration, and proliferation of cells and the degradation of matrix proteins. Elevated levels of uPA and/or PAI-1 consistently correlate with tumor recurrence and decreased patient survival. Some studies also show that elevated levels of these markers predict response to chemotherapy. In a study of more than 3,400 patients with invasive breast carcinoma, uPA/PAI-1 levels correlated with response to chemotherapy.

In a subsequent pooled analysis of 8,377 patients with invasive breast carcinoma, except for lymph node status, a high level of uPA or PAI-1 was the strongest prognostic factor identified. High levels of uPA or PAI-1 correlated with reduced survival in both lymph-node-positive and lymph-node-negative subgroups. In particular, uPA or PAI-1 levels had prognostic significance in lymph-node-negative patients that received no adjuvant systemic therapy. Unfortunately, uPA and PAI-1 levels are currently evaluated by ELISA, and reliable immunohistochemical assays for uPA and PAI-1 for clinical use are still lacking. This has hindered acceptance of uPA and PAI-1 as routine prognostic markers in the USA.

**Bcl-2** belongs to a family of proteins that regulates cell survival. Bcl-2 inhibits apoptosis in vitro. Some reports show a correlation between bcl-2 and ER expression and response to tamoxifen. Some data also show that bcl-2 expression appears to be a favorable prognostic factor in lymph-node-negative patients. The College of American Pathologists currently does not recommend use of bcl-2 expression as a prognostic factor because of insufficient data. However, in a recent study published after the latest CAP recommendations, multiple tumor markers were evaluated on tissue microarrays from 930 invasive breast carcinomas, and the most powerful marker to

predict survival at 10 years was bcl-2. Moreover, its prognostic significance was independent of the Nottingham Prognostic Index. A large prospective study is needed to confirm the prognostic utility of bcl-2, but it may prove to be a useful marker for routine practice in the future.

A new and controversial putative prognostic marker for breast cancer is cyclin E. Cyclin E exists as multiple functional low molecular weight isoforms in addition to its complete form. The low molecular weight isoforms of cyclin E induce genetic instability and produce increased resistance to hormonal treatment in vitro. These low molecular weight isoforms have also been reported to have adverse prognostic significance. In a recent paper from the New England Journal of Medicine, overexpression of cyclin E was reported to be “the most powerful prognostic marker for breast cancer that has been identified to date.” Among 114 patients with stage I breast cancer, none of the 102 patients with low cyclin E isoform levels died of breast cancer during the 5 years following the date of diagnosis. In contrast, all of the 12 patients with a high level of cyclin E isoforms died of breast cancer during this time period. These results need to be confirmed, preferably in prospective studies, and verified by independent investigators before cyclin E is adopted as a routine prognostic marker. If the low molecular weight isoforms are more important than the complete protein, Western blotting may be necessary for their identification.

Multiple additional prognostic markers, including DNA ploidy/S-phase, p53, cyclin D, cathepsin D, EGFR, and E-cadherin have been reported to have clinical utility, but each has problems with reproducibility and/or assay standardization, and none of these is currently recommended by the College of American Pathologists for routine use as a prognostic marker for breast cancer.

### Multi-Gene Predictors in Breast Cancer

Gene expression profiling is a method of evaluating hundreds or thousands of genes in tumor cells by extracting the RNA and quantifying the expression of genes relative to so-called housekeeping genes that are expressed at a relatively constant level, regardless of experimental conditions. Gene expression profiling studies have identified a so-called basal-like subgroup of invasive breast carcinomas, in addition to a subgroup that overexpresses genes related to HER2.

Both of these subgroups have been reported to have adverse prognostic significance.

Another subgroup expressing a “70-gene prognosis signature” is reported to have adverse prognostic significance. A commercial assay to detect this signature (Mammaprint) is being tested in lymph-node-negative patients in a prospective randomized study in Europe. The study is comparing the 70-gene signature with common clinical-pathological criteria for selecting patients to receive adjuvant chemotherapy. The assay currently requires fresh frozen tumor tissue.

Another commercially available molecular test that is becoming more popular in the USA is the Oncotype Dx assay, which involves quantitation of 21 genes by real-time PCR. This assay provides a so-called recurrence score that correlates inversely with the likelihood of response to tamoxifen in lymph-node-negative breast cancer patients. This assay can be performed on paraffin tumor tissue. It is currently being evaluated in a large clinical trial involving over 10,000 patients at 900 sites in the USA and Canada.

Both keratin-positive tumor cells in bone marrow and circulating tumor cells in the blood are also reported to be associated with patient outcome. The independent prognostic and predictive value of these tests is still being evaluated.

### Conclusion

Traditional prognostic markers in breast cancer are based on the histologic assessment of the primary tumor and regional lymph nodes. These include histologic type, tumor grade, presence or absence of lymphovascular invasion, tumor size, and lymph node status. Clinically useful biomarkers should provide additional independent prognostic or predictive information. Tumor margin status and pathologic complete response are important prognostic markers following breast conservative surgery and neoadjuvant chemotherapy. Assays for hormone receptors and HER2 are routinely performed as predictive markers for response to endocrine therapy and anti-HER2 therapy, respectively. A variety of additional prognostic markers have been proposed but require further validation. These include assays based on gene expression profiling and RT-PCR, as well as the detection of

keratin-positive cells in bone marrow and circulating tumor cells.

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## Breast Cancer Prognostic Biomarkers

Boon-Huat Bay and George Wai-Cheong Yip  
Department of Anatomy, National University of Singapore, Singapore, Singapore

## Definition

Biomarkers are distinctive and relatively specific biological indicators (in the form of altered gene, protein, carbohydrate, or lipid expression) of physiological or disease processes. ► **Clinical cancer biomarkers** have been broadly categorized into prognostic biomarkers which aid in determining the disease outcome (prognosis) or predictive markers which predict response to therapy. Identification of prognostic biomarkers would enhance the management of ► **breast cancer** patients by helping clinicians make better decisions with regard to the mode of treatment for each patient, such as which group of patients would benefit from chemotherapy after surgical excision of the tumor. Prognostic biomarkers also form the basis for the development of effective targeted therapy against ► **breast cancer**.

## Characteristics

### Clinical Prognostic Indicators

Standard prognostic factors for breast malignancy takes into account clinical and pathological criteria, such as a patient's age, and the morphological features of the cancer, such as its stage and histological grade. Tumor stage involves measuring the size of the tumor and determining if the tumor has invaded into surrounding structures and draining lymph nodes as well as spread distally to other organs (metastasis). There are two main commonly used systems for staging of tumors: the TNM system (T, tumor; N, lymph node status; M, metastasis) and the American Joint Cancer Committee (AJCC) staging. Histological grade is assessed by morphological examination of the tissues under a light microscope. Tumors are classified as Histological Grade 1 (low grade, where the tissue has more resemblance to normal tissue in terms of parameters such as variability of the size of the nucleus and mitosis), Grade 2 (moderately differentiated), and Grade 3 (poorly differentiated) tumors.

These parameters provide the basis for prognostic algorithms, such as the Nottingham Prognostic Indicator which is a reliable predictor of long-term survival of breast cancer patients. However, there are limitations in the use of conventional prognostic tools for predicting patient outcome. Herein lies the importance of the continuous search for clinically useful biomarkers that can provide additional prognostic information.

### Traditional Prognostic Markers

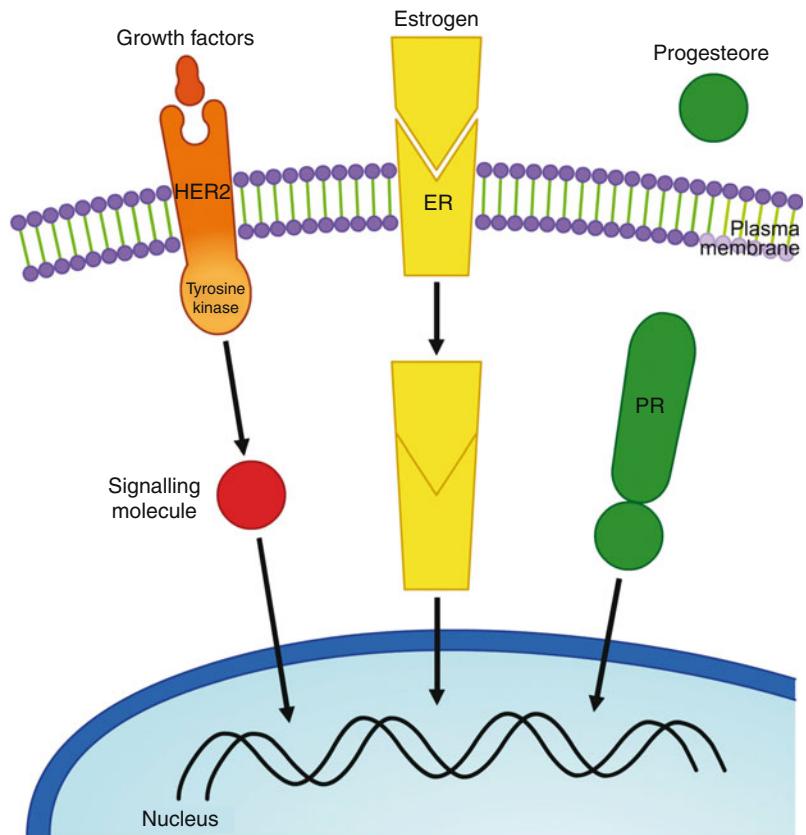
Well-established traditional prognostic markers include ► **estrogen receptor** (ER) status, ► **progesterone receptor** (PR) status, ► **HER-2/neu** (*synonym neu or erbB2*) positivity, and ► **Ki-67** cell proliferation marker.

### Hormone Receptors

Estrogen receptor (ER) is a 65 kDa nuclear molecule, and binds to 17 $\beta$ -► **estradiol** as its principal ligand. Two ER subtypes, ER $\alpha$  and ER $\beta$ , have been described, with the former being present in approximately 70% of breast cancers. Binding of estrogen to ER leads to either homo- or hetero-dimerization of the receptor, which then interacts with hormone response elements to induce ► **transcription** of genes which regulate cellular activity (Fig. 1).

**Breast Cancer Prognostic Biomarkers.**

**Fig. 1** Diagrammatic representation of ER, PR, and HER-2 pathways (Courtesy of S.L. Bay, National University of Singapore)



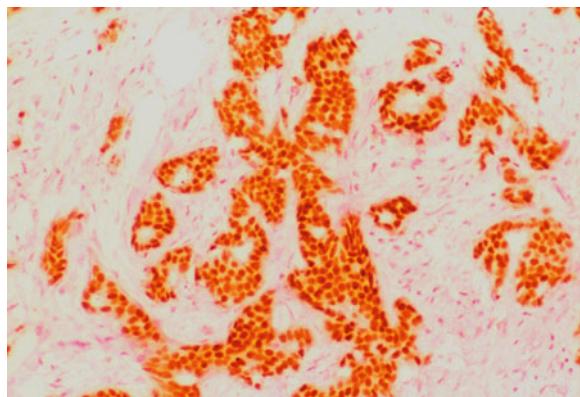
This process can be deactivated by blocking the activity of the receptor or depriving the receptor of the estrogen hormone. Patients with ER-negative breast tumors are more likely to have a higher histological grade and decreased overall survival, whereas the prognosis in ER-positive tumors is relatively better. The presence of ER has been used to guide the use of ► **endocrine therapy**. Drugs such as ► **tamoxifen** target and block the ER receptor and therefore possess anti-carcinogenic properties. They are able to reduce tumor cell proliferation and significantly reduce the risk of recurrence within 5 years by 40% and overall breast specific mortality by 31%. ► **Aromatase inhibitors** like anastrozole and letrozole inhibit the conversion of precursor molecules to estradiol. Patients need to be assessed of their tumor status for the ER marker (endocrine responsiveness) to qualify for either of the treatments. Furthermore, the presence of ER receptor is associated with fewer benefits from ► **chemotherapy**.

Like estrogen, progesterone is a steroid hormone and expression of the progesterone receptor (PR) is

known to be strongly dependent on ER activity. Therefore, PR-positive breast cancers have a more favorable prognosis than PR-negative tumors.

The ER and PR status of breast cancer tissues is determined by ► **immunohistochemistry** (IHC), a technique which uses an ► **antibody** to detect the receptors (Fig. 2).

**Human Epidermal Growth Factor Receptor-2 (HER-2)**  
**Human epidermal growth factor receptor-2 (HER-2 or ERBB2)** is a member of the family of epidermal growth factor receptors. The HER-2 gene is located on chromosome 17q21 and encodes a 185 kDa ► **tyrosine kinase** ► **glycoprotein** (Fig. 1). HER-2 regulates cell ► **differentiation**, ► **adhesion**, and ► **motility**. The status of HER-2 can be determined by immunohistochemistry or more sophisticated ► **fluorescence in situ hybridization** techniques. HER2 expression is estimated to be amplified in approximately 20% of breast tumors. Most clinical studies have shown that ► **amplification** of the HER-2 gene or overexpression of the HER-2 protein is associated with higher-grade tumors,



**Breast Cancer Prognostic Biomarkers.** Fig. 2 Positive estrogen receptor-immunostaining (Immunohistochemistry) in breast cancer tissue with strong reactivity present in the cell nuclei which are stained brown (Courtesy of P.H. Tan, Singapore General Hospital, Singapore)

increased rate of recurrence, lower survival, and a poorer prognosis. The identification of HER-2 as a prognostic biomarker has led to the clinical development of ► **trastuzumab**, a humanized monoclonal antibody against HER-2 protein (► **Monoclonal Antibody Therapy**). ► **Targeted therapy** using trastuzumab in combination with chemotherapy in either a first line or ► **adjuvant** (► **Adjuvant Therapy**) setting has demonstrated survival benefits in breast cancer patients with elevated HER-2 expression.

#### Ki-67 Proliferation Marker

The ► **Ki-67** gene, located on chromosome 10q25, codes for a nuclear non-histone protein. Two protein isoforms (359 kDa and 320 kDa) can be formed by alternative splicing. Ki-67 is found in proliferating cells, where its expression increases during disruption of the nuclear membrane during early mitosis. Elevated expression of Ki-67 is a marker of poor prognosis and increased risk of recurrent disease.

#### Emerging Prognostic Markers

Breast cancer is a heterogeneous disease resulting from the accumulation of multiple gene mutations. Numerous studies have been carried out over the years to understand the different molecular mechanisms involved in breast cancer as well as to obtain prognostic markers to improve diagnosis, therapeutic approaches, and patient management. The development of expression profiling technologies has also

accelerated the rate of discovery of novel potential markers for breast cancer.

#### Genomic Markers

The BRCA1 gene (► **Breast Cancer Genes BRCA1 and BRCA2**) is located on chromosome 17q21 and encodes an 1,863-amino acid nuclear protein that regulates transcriptional activation, cell-cycle ► **checkpoint control**, ► **DNA repair**, chromosomal remodeling, and ► **apoptosis**. BRCA1 is a ► **tumor suppressor gene**, mutations of which relate to the progression of ► **familial breast cancer** (► **BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk**). Loss of BRCA1 in sporadic tumors results in reduced BRCA1 expression or incorrect subcellular localization of the encoded protein. This well-studied gene is associated with high-grade and larger-sized tumors, advanced lymph node stages, vascular invasion, negative estrogen receptor, progesterone receptor and ► **androgen receptor** (AR) and ► **E-Cadherin** expression, and basal-like type of breast carcinoma. Alterations in BRCA1 gene expression result in poor patient survival.

#### Proliferation Markers

Increased proliferative activity is one of the hallmarks of cancer. Besides Ki-67, which is an established proliferation marker, proteins associated with cell proliferation include the cyclins which are involved in regulation of the cell cycle and growth factor receptors such as insulin-like growth factor receptor 1. Other examples of breast cancer proliferation markers are FOXM1 (► **Forkhead Box M1**; a member of the forkhead box superfamily of transcription factors), metallothionein (a metal-binding protein; ► **Metallothionein Enzymes**), ► **securin** (a regulatory protein), and YB-1 (a member of the cold shock domain DNA- and RNA-binding protein superfamily).

#### Anti-apoptosis Markers

► **BCL2** is a mitochondrial protein that inhibits chemotherapy-induced ► **apoptosis** (► **Mitochondria Apoptosis Pathway**). Its expression level is inversely correlated with that of oncogenic Ki-67. Patients with BCL2-negative breast cancer are more likely to respond to chemotherapy. However, overexpression of BCL2 is also correlated with increased survival rates, and this may be due to the presence of concurrent estrogen receptor expression.

Mutation of the p53 tumor suppressor gene (► [TP53](#); ► [P53 Family](#)) has long been implicated in the evasion of apoptosis in human tumors and associated with more aggressive breast cancers.

#### Structural Proteins

► [Cytokeratins](#) (CKs) are a family of major structural proteins present in the cytoplasm of ► [epithelial cells](#). Their molecular weights range from 40 to 68 kDa. In the human breast, CKs are mainly expressed in basally located myoepithelial cells. Basic CK5 (58 kDa) as well as acidic CK14 (50 kDa) and CK17 (46 kDa) are associated with high-grade basal-like breast carcinoma, early tumor recurrence and poor prognosis. Furthermore, expression of these three CKs has been significantly correlated with BRCA1-expressing tumors.

#### Angiogenesis-Associated Markers

Another hallmark of cancer is the formation of new blood vessels (► [Angiogenesis](#)) to help nourish the tumor for its growth. Angiogenic factors include growth factors such as members of the ► [vascular endothelial growth factor](#) family, ► [fibroblast growth factor 2](#), and hepatocyte growth factor (*synonym* ► [Scatter Factor](#)) as well as members of the angiopoietin family. Serum levels of vascular endothelial growth factor (VEGF) may be a useful prognostic factor in breast cancer, as they have been observed to be elevated in malignant breast tumors and predict overall survival and local recurrence. Expression of VEGF has been correlated with estradiol in tumors and may promote cancerous spread by regulating ► [chemokine receptor CXCR4](#). However, a recent report has shown that contrary to expectation, angiogenic factors and receptors were downregulated in primary breast tumors. An intact uterus in postmenopausal women appears to protective females against distal spread of breast cancer by lowering serum VEGF levels.

#### Plasminogen Activators and Inhibitors

Cancer cells make use of proteolytic enzymes to assist in invading surrounding tissues and distant metastasis. ► [Urokinase-type plasminogen activator](#) (u-PA) is a serine protease that degrades ► [extracellular matrix](#) thus easing cancer progression. Elevated expression of u-PA and plasminogen activator inhibitor (PAI1; ► [Plasminogen-Activating System](#)) is associated with

higher recurrence risk and poorer survival in patients with node-negative breast cancer. These patients have also been reported to derive greater benefits from chemotherapy.

#### Glycosaminoglycans and Proteoglycans

► [Glycosaminoglycans](#) (GAGs) and ► [proteoglycans](#) (PGs) play vital roles in cancer progression. GAGs are long, unbranched polysaccharides that are formed by repeating disaccharides of an uronic acid residue alternating with an amino sugar. Four major classes of GAGs have been described, namely, ► [heparan sulfate](#) (HS), chondroitin sulfate/dermatan sulfate, keratan sulfate, and ► [hyaluronan](#). ► [Syndecans](#) are transmembrane HSPGs and consist of four family members. Overexpression of syndecan-1 in breast cancer is linked to poor survival outcome and is predictive of response to ► [neoadjuvant](#) chemotherapy. Through its interactions with heparin-binding growth factors and ► [integrin](#), syndecan-1 regulates cancer progression and tumor-associated ► [angiogenesis](#). Syndecan-4 has also been found to be associated with aggressive ER-negative breast cancer. ► [Glypican 1](#), an HSPG, has been found overexpressed in high-grade breast cancer tissues.

#### Multigene Arrays

Genomics technologies have lead to the development of ► [multigene arrays](#) known to provide prognostic information such as the Oncotype DX assay, an array of 21 genes (comprising 16 outcome-related genes and 5 reference genes) and the MammaPrint 70-gene signature. The MammaPrint has been given approval by the U.S. Federal Drug Administration (FDA) for application as a prognostic tool when used in combination with other clinicopathological parameters.

#### MicroRNA

► [MicroRNAs](#) (miRNAs) are small noncoding RNAs that originate from genes transcribed by RNA polymerase II. Recent studies have demonstrated associations between miRNAs and cancer progression, and stimulated interest in identifying the involved miRNAs. By suppressing the translation of mRNA or cleaving mRNA, miRNAs can regulate cellular proliferation, apoptosis, and differentiation. Alterations of miRNA expression have been reported to be associated with breast cancer development, ► [invasion](#), and ► [metastasis](#).

## Future Directions

Well-established biomarkers of breast cancer are routinely used in medical practice to guide clinical management decisions and to aid in predicting disease outcome. Simultaneous analysis of several molecules has led to the identification of basal-like (also known as triple-negative) breast cancer. This subset of breast cancer is negative for ER, PR, and HER-2, and displays a more aggressive behavior and possesses a poorer prognosis compared with luminal (ER-positive) and HER-2-like (ER-negative, HER-2 positive) breast tumors. Technological advances in genomics, proteomics, and glycomics have led to the discovery of novel predictive and prognostic factors. Efforts are now required to validate the clinical usefulness of these molecules and to determine if they will contribute to personalized breast cancer treatment.

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## Breast Cancer Rationally Designed Therapies

Angelika M. Burger and Patricia M. LoRusso  
Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

## Synonyms

Breast cancer targeted therapy

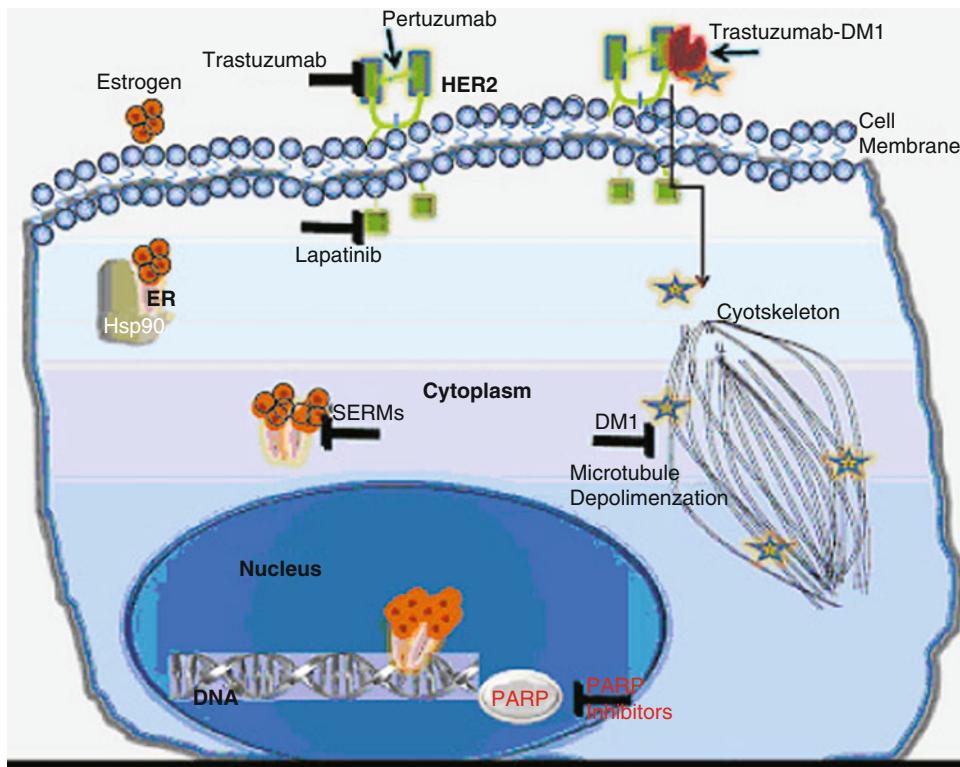
## Definition

Rationally designed therapy, also referred to as ► **targeted therapy**, is directed toward specific, molecular changes that distinguish tumor from normal tissue. Thus, compared to standard ► **chemotherapy**, which kills rapidly dividing cells indiscriminately, targeted therapies should theoretically have fewer side effects. A targeted therapy is rationally designed to attack a hallmark of cancer, a process essential to a particular pathological tumor type or subtype against which the drug is developed. The target should be credentialed and detectable in clinical specimens. The quantitative or qualitative analysis of a molecular target in either tumor or surrogate tissue should correlate with clinical outcome after the targeted therapy is administered.

## Characteristics

► **Breast cancer** is among the best understood malignant diseases. Histological and genetic heterogeneity have been recognized several decades ago. Breast cancer was also one of the first diseases for which diagnostic and ► **prognostic molecular markers** were established and used to guide drug development and treatment decisions. When transcriptional ► **gene expression profiling** became available, breast cancer was the first tumor type for which a molecular classification by gene clustering was introduced, and this has evolved to improve personalized treatment. The ► **Oncotype DX® Recurrence Score®** (Genomic Health, Inc.) and ► **MammaPrint®** (Agendia, Amsterdam, The Netherlands) genomic profiling tests are now available to aid patient-tailored therapy [1]. The wealth of molecular knowledge about disease progression in breast cancer has led to a translation into specific, rationally designed therapies.

The first truly targeted agent in the field of cancer pharmacology was ► **tamoxifen**. Tamoxifen is an almost “ideal” targeted drug: It is directed against the ► **estrogen receptor** to block the binding of estrogen and hence estrogen signaling [2]. It is given as a daily oral dose with little side effects in the therapeutic dose range. Tamoxifen has revolutionized breast cancer treatment since the 1980s for women with estrogen receptor-positive breast cancer. Other breast cancer targeted agents followed soon such as ► **aromatase inhibitors**, human epidermal growth factor receptor



### Breast Cancer Rationally Designed Therapies.

**Fig. 1** Schematic presentation of a breast cancer cell showing current molecular targets and their inhibitors. ► HER2, human epidermal growth factor receptor 2, a receptor with an extracellular and intracellular plasma membrane domain forming homodimers (shown) and heterodimers (not shown); HER2 signaling can be blocked by ► Trastuzumab, an antibody against the extracellular domain of the monomeric receptor; HER2 dimer formation can be inhibited by the antibody ► Pertuzumab, the intracellular domain of HER2 can be blocked by ► Lapatinib. Trastuzumab conjugated with the mitotic spindle poison DM1, termed ► Trastuzumab-DM1 (T-DM1), can block the HER2 receptor and concomitantly after receptor turnover following endocytic trafficking, DM1 is released in the breast

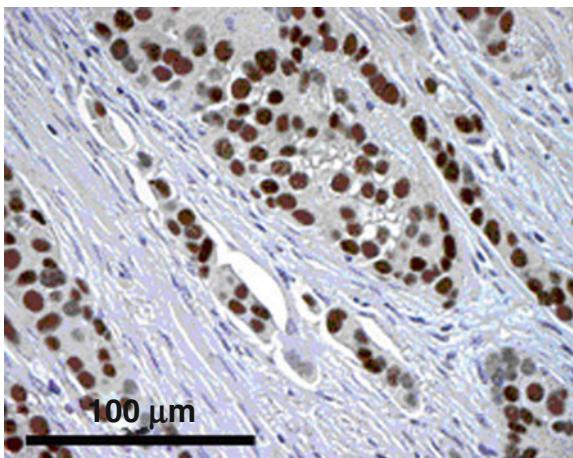
(► HER2/neu) signaling inhibitors, and more recently ► Poly(ADP-Ribose) Polymerase (PARP) inhibitors (Fig. 1).

### Estrogen Receptor

Two ► estrogen receptors encoded by different genes exist, ER-alpha (ER $\alpha$ ) and ER-beta (ER $\beta$ ). In breast cancer, ER $\alpha$  is the most important form and is synonymously used for ER. ER belongs to the family of nuclear hormone receptors (Fig. 2). Nuclear hormone receptor proteins form a class of ligand-activated proteins that, when bound to specific sequences of DNA, serve as on-off switches for ► transcription.

cancer cell causing ► microtubule depolymerization and G2/M ► cell cycle arrest. ► Estrogen receptor (ER) is a ► transcription regulator of breast cancer growth, upon binding of estrogen, ER is released from the ► chaperone heat shock protein 90 (► Hsp90) and translocated into the nucleus. ► Selective estrogen receptor modulators (SERMs) prevent binding of estrogen to ER via mostly antagonistic effects (tamoxifen, raloxifene), estrogen synthesis inhibitors act not only by rapid competitive inhibition but also by inactivation of the enzyme. This effect is long lasting or irreversible (► Aromatase Inhibitors ► Letrozole, ► Anastrozole). ► PARP is a nuclear enzyme responsible for DNA base excision repair. Thus, PARP inhibitors block single-strand-break ► DNA repair, which can lead to synthetic lethality in ► BRCA-mutant breast cancers

Estrogen synthesis (via ► aromatase) and action (via ER) are exceptional targets for the treatment and ► chemoprevention of breast cancer, because of the importance of the ER and its ligand estrogen as a stimulus to the development and progression of breast cancer. The simple concepts of targeting and blocking estrogen action to treat breast cancer with ► tamoxifen, or the idea of blocking the estrogen synthetase (aromatase) enzymes, were proposed and successfully developed and brought into the clinic by Craig V. Jordan and Angela H. Brodie respectively over 30 years ago and have led to valuable drugs. However, the field of selective estrogen receptor



#### Breast Cancer Rationally Designed Therapies.

**Fig. 2** Immunoperoxidase staining for ► estrogen receptor in an invasive breast cancer. Positive ER expression is indicated by strong brown nuclear staining of tumor, but not stromal cells. The anti-ER antibody clone 6F11 was used as it is done in routine pathology laboratories to determine ER status that will qualify a breast cancer patient for anti► hormonal therapy

modulators (SERMs) has become multifaceted with many layers of complexity that are being explored to enhance tissue selectively, address intrinsic resistance, and block the development of acquired antihormonal resistance.

**Estrogen Receptor Modulators** Compounds that target the ER comprise full agonists (agonistic in all tissues) such as the natural endogenous hormone estrogen ( $17\beta$ -► estradiol), mixed agonists/antagonists (agonistic in some tissues while antagonist in others) such as tamoxifen or raloxifene (also termed ► selective estrogen receptor modulators, SERMs), and pure antagonists (antagonistic in all tissues) such as fulvestrant (Fig. 3).

#### Tamoxifen

► Tamoxifen (Nolvadex) blocks the binding of ► estradiol to the ► estrogen receptor ER (Figs. 1 and 3c). It is given orally and at a daily dose of 20 mg. It is the “gold standard” for treatment of ER + breast cancers and has established the principles of tumor targeting as well as treatment strategies to aid survivorship. Analyses of accumulative randomized worldwide clinical trials allow the following conclusions for tamoxifen therapy to be made:

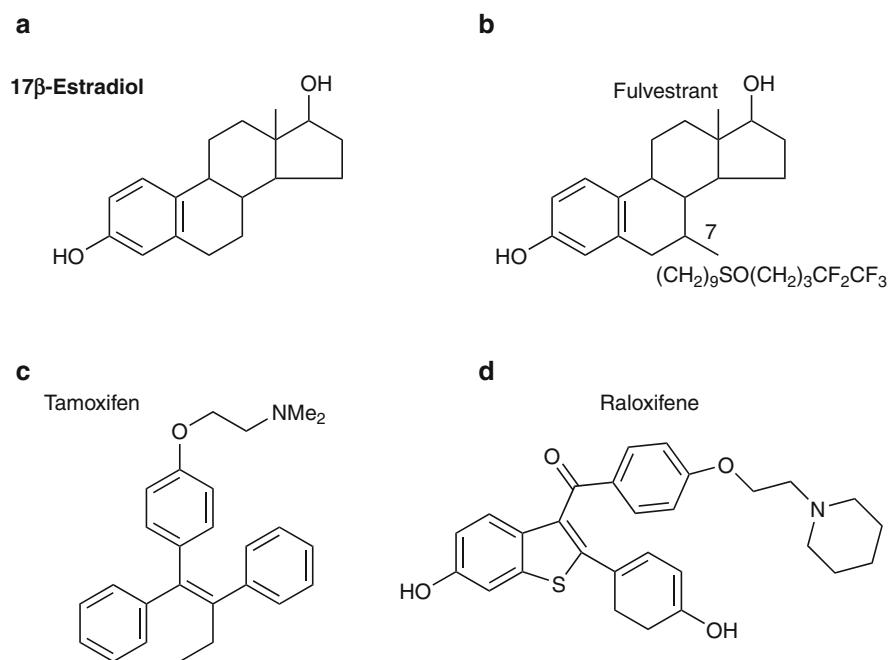
1. Five years of adjuvant tamoxifen enhances disease-free survival.
2. There is a 50% decrease in recurrences observed in ER-positive patients 15 years after diagnosis.
3. Five years of ► adjuvant tamoxifen enhances survival with a decrease in mortality 15 years after diagnosis.
4. Adjuvant tamoxifen does not provide an increase in disease-free or overall survival in ER negative breast cancer.
5. Five years of adjuvant tamoxifen alone is effective in premenopausal women with ER.
6. The benefits of tamoxifen in lives saved from breast cancer, far outweighs concerns about an increased incidence of endometrial cancer in postmenopausal women.
7. Tamoxifen does not increase the incidence of second cancers other than endometrial cancer.
8. No non-cancer-related overall survival advantage is noted with tamoxifen when given as adjuvant therapy.

#### Raloxifene

Raloxifene (EVISTA, Fig. 3d) is an oral ► SERM that has estrogenic actions on bone and antiestrogenic actions on the uterus and breast. Raloxifene is approved by regulatory agencies such as the FDA for the treatment and prevention of osteoporosis in postmenopausal women. The Study of Tamoxifen and Raloxifene, known as STAR trial, has compared raloxifene with the drug tamoxifen in reducing the incidence of breast cancer in postmenopausal women who are at increased risk of the disease. Initial results of STAR in 2006 showed that raloxifene is as effective as tamoxifen in reducing the breast cancer risk of the women on the trial. In STAR, both drugs reduced the risk of developing invasive breast cancer by about 50%. In addition, a 4-year follow-up suggested that women taking raloxifene had 36% fewer ► uterine cancers and 29% fewer blood clots than the women who were on tamoxifen, reducing the serious side effects associated with tamoxifen treatment. Furthermore, the ► CYP2day enzyme is not needed to activate raloxifene. About 10% of people have an abnormal CYP2day enzyme, which alters patients’ drug levels and keeps them from getting the full benefit of tamoxifen. Raloxifene is an alternative to tamoxifen for reducing the risk of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women at high risk for invasive breast cancer.

**Breast Cancer Rationally Designed Therapies.**

**Fig. 3** Molecular structures of ► **estradiol**, ► **fulvestrant**, and the ► **SERMs** ► **tamoxifen** and raloxifene



**Fulvestrant**

► **Fulvestrant** (Faslodex) is an estrogen receptor antagonist that downregulates the ► **estrogen receptor** (ER) and has no agonistic effects. It is a structural analog of 17 $\beta$ -► **estradiol**, unlike the nonsteroidal compounds ► **Tamoxifen** and ► **Raloxifene** (Fig. 3). Fulvestrant has a binding affinity for the ER that is 89% that of estradiol and much higher than that of tamoxifen, which is 2.5% that of estradiol. Fulvestrant–ER binding impairs receptor dimerization, and energy-dependent nucleocytoplasmic shuttling, thereby blocking nuclear localization of the receptor. Moreover, any fulvestrant–ER complex that enters the nucleus is ► **transcription** inactive and unstable, resulting in accelerated degradation of the ER protein, compared with estradiol- or tamoxifen-bound ER. Thus, fulvestrant binds, blocks, and accelerates degradation of ER protein, leading to complete inhibition of estrogen signaling. The biological and antitumor effects of fulvestrant have been evaluated in several clinical trials enrolling postmenopausal women with primary breast cancer. The effects of daily i.m. injections of short-acting fulvestrant for 7 days prior to surgery for primary breast cancer were compared with no pretreatment controls. In patients with ER-positive (ER+) tumors, fulvestrant caused a significant reduction in the median ER index and also abolished

► **progesterone receptor** expression in ER + tumors. This reduction in cellular ER protein caused a significant reduction in tumor proliferation as determined by the proliferation marker Ki67.

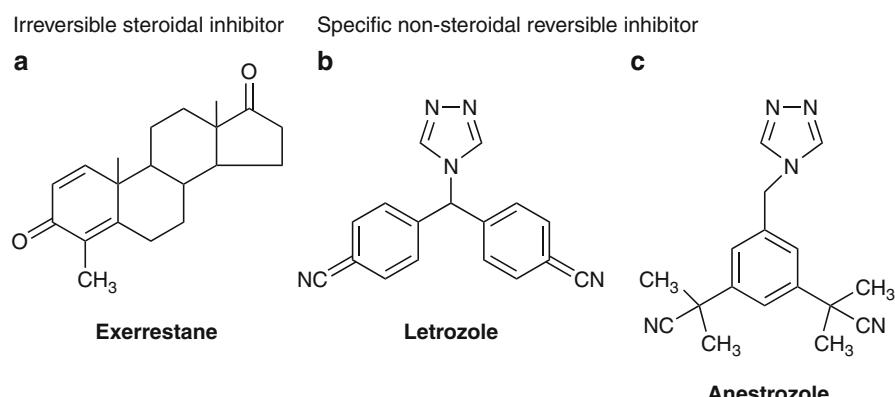
In another study that compared the effects of a single dose of long-acting fulvestrant to continuous daily tamoxifen, or placebo for 14–21 days in patients with primary breast tumors, all fulvestrant doses produced statistically significant reductions in ER expression compared with placebo. At the higher 250 mg dose, fulvestrant-induced reduction was significantly greater than that observed with tamoxifen. Fulvestrant represents a valuable second-line treatment option for postmenopausal women with hormone-sensitive advanced breast cancer, who have progressed on prior tamoxifen therapy.

**Aromatase Inhibitors**

► **Aromatase** belongs to the group of ► **cytochrome P450** enzymes and catalyzes the rate-limiting step in estrogen biosynthesis. It mediates the conversion of the steroid C-19 androgens to C-18 estrogens, thus aromatase inhibitors (AIs) block estrogen synthesis. Aromatase is found in estrogen producing cells of the adrenal glands, ovaries, placenta, and adipose tissue. However, AIs do not block estrogen production by the ovaries, but they can block other tissues from making

### Breast Cancer Rationally Designed Therapies.

**Fig. 4** Molecular structures of ► **aromatase inhibitors** (AIs) that are in clinical use



this hormone. AIs are therefore used in postmenopausal women, when the ovaries are no longer producing estrogen. Aromatase inhibitors can be classified into steroidal and nonsteroidal inhibitors (Fig. 4).

#### Exemestane

Exemestane (Aromasin) is approved to treat advanced ► **breast cancer** in the ► **adjuvant** setting and to prevent recurrent ER-positive breast cancer in postmenopausal patients in the metastatic setting. Exemestane can be used in these women who already have been treated with ► **Tamoxifen** or another ► **Selective Estrogen Receptor Modulator** (SERM). In clinical studies, exemestane was significantly more effective than tamoxifen as first-line therapy in postmenopausal women with advanced breast cancer.

#### Anastrozole

Anastrozole (Arimidex) is the ► **aromatase inhibitor** for which most clinical data are available. Two randomized, double-blind studies demonstrated that anastrozole (1 mg daily) was slightly more effective than ► **Tamoxifen** (20 mg daily) as first-line therapy in postmenopausal women with advanced ► **breast cancer**. Among those with ► **estrogen receptor** positive (ER+) tumors, the benefit was significant in terms of partial and complete responses including stable disease as well as time to progression (TTP). The “Arimidex,” Tamoxifen Alone or in Combination (ATAC) trial demonstrated that anastrozole was significantly better than tamoxifen – or the combination of tamoxifen and anastrozole – at preventing a recurrence of breast cancer in postmenopausal women whose early stage tumors were hormone sensitive. The ATAC trial also showed a significantly greater incidence of ischemic

cerebrovascular events and venous thromboembolic events with tamoxifen, compared with anastrozole.

#### Letrozole

► **Letrozole** (Femara) is approved to treat breast cancer in postmenopausal women. In a multicenter, randomized, double-blind study in advanced breast cancer, letrozole was significantly better than ► **Tamoxifen** in response rate, clinical benefit, time to progression, and time to treatment failure. In addition, a randomized, phase III, double-blind trial (BIG 1–98) of the Breast International Group is comparing several ► **adjuvant** ► **endocrine therapies** in postmenopausal women with ► **estrogen receptor** positive (ER+) breast cancer. Letrozole versus ► **Tamoxifen** treatment was compared in the first analysis of the monotherapy arms of the BIG1–98 study. After a median follow-up of 25.8 months, adjuvant treatment with letrozole was found to reduce the risk of recurrences significantly compared to tamoxifen. Overall, ► **aromatase inhibitors** tend to cause fewer serious side effects than tamoxifen, such as blood clots, stroke, and ► **endometrial cancer**. But aromatase inhibitors can cause more heart problems and more bone loss (osteoporosis). The latter requires treatment with ► **bisphosphonates**. Based on data from multiple, large randomized trials, it was recommended by the American Society of Clinical Oncology (ASCO) technology assessment panel that optimal adjuvant hormonal therapy for a postmenopausal woman with receptor-positive breast cancer includes an aromatase inhibitor as initial therapy or after treatment with tamoxifen. Current treatment strategies for estrogen receptor (ER)-positive breast cancer switch to an aromatase inhibitor (AI) after

tamoxifen for 2–3 years (for a total of 5 years of ► **hormonal therapy**), which offers more benefits than 5 years of tamoxifen; or give an AI for 5 years after taking tamoxifen for 5 years. The latter concept has shown to continue to reduce the risk of the cancer coming back, compared to no treatment after tamoxifen.

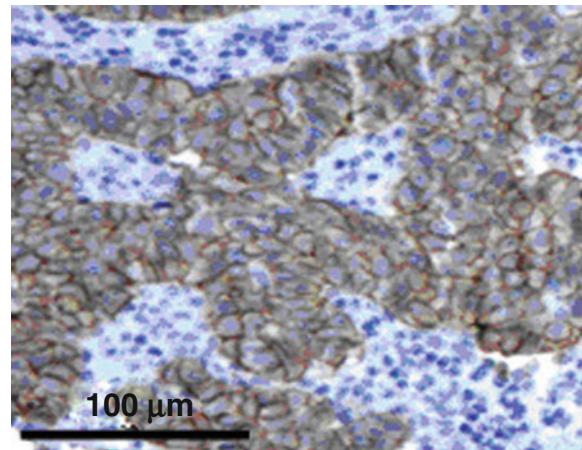
### Human Epidermal Growth Factor Receptor 2

Human epidermal growth factor receptor 2 (► **HER2/neu**, also termed ERBB2) is a member of the epidermal growth factor receptor family of transmembrane ► **receptor tyrosine kinases**. In human breast cancer, ► **amplification** of HER2/neu has been associated with rapid progression, anchorage-independent cell growth, cell ► **migration**, ► **angiogenesis**, tumorigenicity, ► **invasion**, metastatic potential, increased ► **aromatase** activity, and resistance to ► **apoptosis**.

HER2/neu is amplified in 25–30% of breast cancers, and in these cases the encoded protein is present in abnormally high levels in the malignant cells. In the clinic, overexpression of HER2/neu is an independent ► **predictive biomarker** for responses to multiple systemic therapies including relative resistance to ► **endocrine therapies** particularly ► **Tamoxifen** and response to HER2/neu-targeted therapies such as ► **Trastuzumab** and ► **Lapatinib**.

### Trastuzumab

► **Trastuzumab** (► **Herceptin**) is a recombinant IgG1 kappa, humanized ► **monoclonal antibody** that selectively binds with high affinity in a cell-based assay ( $K_d = 5 \text{ nM}$ ) to the extracellular domain segment IV of the human epidermal growth factor receptor 2 protein, ► **HER2**. Trastuzumab is produced by recombinant DNA technology in Chinese Hamster Ovary (CHO) cell cultures. Its chemical formula is C6470H10012N1726O2013S42. Trastuzumab acts on both the extracellular and the intracellular domain of the HER2 receptor. When trastuzumab is bound to the HER2 receptor, it induces immune cells to kill the cancer cell via a mechanism termed ► **anti-body-dependent cellular cytotoxicity**. Trastuzumab also blocks downstream HER2 signaling, thus inhibiting cell proliferation. Detection of HER2 protein overexpression is necessary for selection of patients appropriate for trastuzumab therapy, because these are the only patients studied and for whom benefit has been shown. Several FDA-approved commercial



### Breast Cancer Rationally Designed Therapies.

**Fig. 5** Immunoperoxidase staining for HER2 by HercepTest (Dako) in an invasive breast cancer. Positive HER2 expression is indicated by *strong brown* plasma membrane staining of tumor, but not stromal cells. Nuclei are counterstained with hematoxylin (blue)

assays are available to aid in the selection of patients for trastuzumab therapy. These include HercepTest™ and Pathway® HER-2/neu (► **Immunohistochemistry assays**) and PathVysion® and HER2 ► **FISH** pharmDx™ (FISH assays). **Figure 5** shows an example of the HercepTest of a patient eligible for trastuzumab treatment.

Trastuzumab is approved for the treatment of HER2 overexpressing node positive or node negative (estrogen receptor/progesterone receptor negative or with one high risk factor) breast cancer in an adjuvant setting as part of combination regimens or as single agent following multimodality-based chemotherapy. It is also approved for the treatment of metastatic breast cancer in combination with ► **Paclitaxel** for first-line treatment or as a single agent for treatment of HER2-overexpressing breast cancer in patients who have received one or more chemotherapy regimens for metastatic disease.

Phase 1 ► **clinical trials** of trastuzumab showed that the antibody is safe and confined to the tumor. Subsequent phase 2 trials demonstrated that many women with HER2-positive metastatic disease who had relapsed after chemotherapy had a response to trastuzumab; trastuzumab when given with chemotherapy was superior to its effectiveness when used alone. The results of a phase 3 trial in which women with cancers that overexpressed HER2 who had not

previously received chemotherapy for metastatic disease were randomly assigned to receive either chemotherapy alone or chemotherapy plus trastuzumab demonstrated that the addition of trastuzumab to chemotherapy was associated with a longer time to disease progression, a higher rate of objective response, a longer duration of response, a lower rate of death at 1 year, longer survival, and a 20% reduction in the risk of death.

Four large multicenter randomized trials studying the adjuvant use of trastuzumab accrued thousands of patients, and reported interim outcome analyses in 2005: NSABP B-31, NCCTG N9831, HERA, and BCIRG 006. The B-31 and N9831 trials were analyzed together because of the similarities in their design and patient populations. HER2+ breast cancer were all treated with a standard North American adjuvant chemotherapy regimen of four cycles of ► doxorubicin and ► cyclophosphamide (AC) followed by ► paclitaxel (T); half of these patients were randomized to additionally receive trastuzumab therapy for 1 year. At a median follow-up of 2 years there was a significant improvement in disease-free survival for patients receiving trastuzumab ( $P = 0.0001$ ). In addition, there was a 33% reduction in the risk of death in this group ( $P = 0.015$ ).

The European multicenter trial HERA was evaluating the use of trastuzumab after chemotherapy in over 5,000 HER2+ patients. The investigators randomized patients to either 1 or 2 years of trastuzumab versus observation after chemotherapy. Patients were required to have received a minimum of four cycles of predefined ► adjuvant and/or ► neoadjuvant therapy. The first interim analysis demonstrated a statistical improvement in disease-free survival among patients receiving 1 year of trastuzumab compared with observation ( $P = 0.0001$ ). The second interim analysis with a median follow-up of 23.5 months demonstrated a statistically significant 34% reduction in the risk of death ( $P = 0.0115$ ).

The BCIRG 006 study randomized 3222 HER2+ patients to 3 arms: ► chemotherapy with doxorubicin and cyclophosphamide (AC) for four cycles followed by ► docetaxel for four cycles; AC for four cycles followed by docetaxel for four cycles plus trastuzumab for 1 year; and a third novel arm lacking an ► anthracycline, of docetaxel plus ► carboplatin and trastuzumab, with trastuzumab continuing for 1 year. At an interim analysis performed at 36 months of

follow-up, the results demonstrated a significant improvement in disease-free and overall survival for both trastuzumab containing arms compared to chemotherapy alone. All results were statistically significant. Most importantly, the incidence of grade 3/4 cardiac toxicity with the novel non-anthracycline arm was similar to the control arm and less than that of the anthracycline plus trastuzumab arm. All four trials confirmed a significant reduction in the risk of recurrence with the addition of trastuzumab to chemotherapy for HER2+ early stage breast cancer.

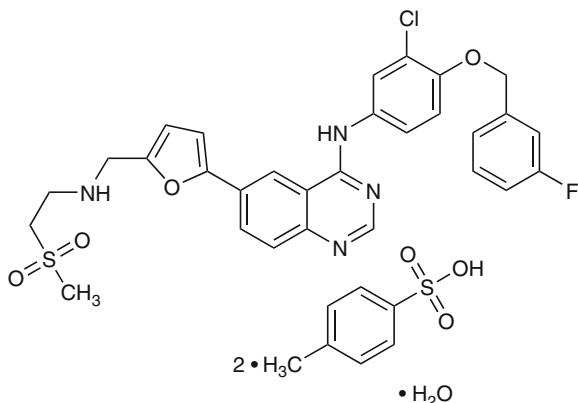
### Lapatinib

► Lapatinib (Tykerb) is a small molecule of the 4-anilinoquinazoline class of ► tyrosine kinase inhibitors. It is used as the monohydrate of the ditosylate salt, with chemical name *N*-(3-chloro-4-[(3-fluorophenyl)methyl]oxy)phenyl)-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furanyl]-4-quinazolinamine bis (4-methylbenzenesulfonate) monohydrate (Fig. 6).

The registration trial enrolled 399 subjects with HER2 overexpressing locally advanced or metastatic breast cancer, progressing after prior treatment that included ► anthracyclines, ► taxanes, and ► trastuzumab. Subjects were randomized to receive either lapatinib once daily (continuously) plus ► capecitabine, or to receive capecitabine alone. The primary endpoint was time to progression (TTP) defined as time from randomization to tumor progression or death related to breast cancer. The median TTP was 23.9 weeks for the combination treatment versus 18.3 weeks for capecitabine alone, for a response rate of 31.8% versus 17.4%, respectively.

### Pertuzumab

► Pertuzumab (Omnitarg) is an investigational humanized ► monoclonal antibody that binds to ► HER2 near the center of domain II as compared to ► trastuzumab that binds to the domain IV of the extracellular segment of the HER2 receptor. Pertuzumab binding is predicted to sterically block the region necessary for HER2 dimerization with other members of the epidermal growth factor receptor family specifically ► HER3. Because trastuzumab does not prevent heterodimerization, the formation of ligand-induced HER2-containing heterodimers might still be possible, and hence pertuzumab represents a distinct entity for targeting HER2-expressing breast cancers. In a phase II study of pertuzumab plus trastuzumab, 66 patients



#### Breast Cancer Rationally Designed Therapies.

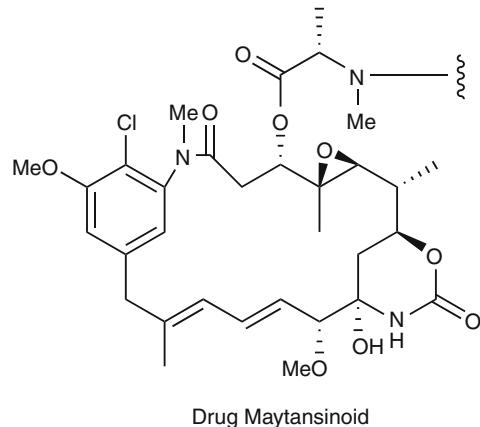
**Fig. 6** Molecular structure of lapatinib. Lapatinib is a reversible small-molecule tyrosine kinase inhibitor which targets the intracellular tyrosine kinase domains of both HER2 and EGFR tyrosine kinases and has the advantage that it can be administered orally. Lapatinib is approved in combination with capecitabine, a prodrug, that is enzymatically converted to 5-fluorouracil in the tumor where it inhibits DNA synthesis

with HER2+ metastatic breast cancer progressing on trastuzumab were enrolled and treated with 3-weekly pertuzumab and weekly or 3-weekly trastuzumab. Interim results revealed a response rate of 24.2%, with a clinical benefit rate of 50%. The most frequently encountered toxicities were diarrhea, fatigue, nausea, rash, and headache. Currently, a double-blind multicenter phase III study, CLEOPATRA, is randomizing patients to ► docetaxel plus trastuzumab versus docetaxel plus trastuzumab and pertuzumab as first-line treatment for HER2+ advanced breast cancer.

#### Trastuzumab-DM1

T-DM1, direct covalent coupling of cytotoxic agents to ► monoclonal antibodies, is an alternative to naked antibody-targeted therapy. Because ► HER2 is highly expressed on breast tumor cells (1–2 million copies per cell) compared with normal epithelial cells, HER2 represents an ideal target for antibody-drug conjugate therapy. Trastuzumab-DM1 is an antibody-cytotoxic drug conjugate whereby trastuzumab is coupled with the cytotoxic drug maytansinoid (DM). Maytansinoids (Fig. 7) are derivatives of the antimicrotubules in a manner similar to the ► Vinca alkaloids.

T-DM1 has demonstrated antitumor activity in trastuzumab-sensitive and trastuzumab-resistant



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**Fig. 7** Molecular structure of mytosanoid attached to trastuzumab to form trastuzumab-DM1

preclinical models of HER2+ breast cancer. It is one of most promising new investigational drugs for the treatment of HER + metastatic breast cancer.

A phase I study evaluated 3-weekly dosing of T-DM1 in patients with HER2+ metastatic breast cancer progressing on trastuzumab. Tumor responses were seen in five of nine patients (44%) at the maximally tolerated dose (MTD). A rapidly reversible ► thrombocytopenia was the dose-limiting toxicity. There was no evidence of deleterious cardiac effects.

Subsequently a weekly dosing schedule showed partial remissions in 9 of 15 evaluable patients (53%). Interim results of a phase II study of 3-weekly T-DM1 as third-line therapy and beyond for patients with HER2+ metastatic breast cancer progressing on prior trastuzumab (some of which had also received lapatinib), evaluated 107 patients for efficacy with a median follow-up of 4.4 months. There was no grade 3 or 4 cardiac dysfunction reported. The confirmed outside reviewer response rate in evaluable patients was 27.1%. Further phase II studies are now underway, including a randomized comparison of T-DM1 versus the combination of trastuzumab and docetaxel as first-line therapy for HER2+ and a randomized phase III trial of T-DM1 versus capecitabine plus lapatinib in metastatic breast cancer.

Overall, HER2-targeted therapies have redefined the natural history of HER2-overexpressing breast cancers and have contributed to dramatic improvement in the management of metastatic breast cancer.

### Poly(ADP-Ribose) Polymerase (PARP)

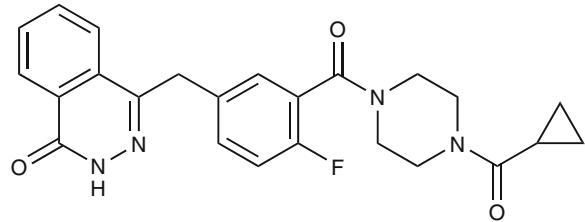
The inhibition of ► Poly(ADP-Ribose) Polymerase (PARP) has emerged as a promising molecular target in breast cancer patients that are carriers of a ► BRCA1 or BRCA2 mutation and for which only few treatment options exist. The concept of inhibiting PARP1 as a therapeutic strategy in patients with mutant BRCA status is based on the so-called synthetic lethal approach. PARP1 is activated by DNA single-strand breaks (SSBs). SSBs can arise endogenously through spontaneous base loss, base damage, sugar damage, or abortive ► topoisomerase-1 activity. Once PARP1 has bound these breaks, it becomes activated and modifies itself and other proteins with long polymers of ADP-ribose required for the repair of SSBs. PARP1 is involved in base excision repair (BER), but not ► nucleotide excision repair. PARP1 loosens chromatin and recruits ► DNA repair proteins. If SSBs are not repaired, they form DNA ► double-strand breaks (DSBs) upon DNA ► replication. The latter are then repaired via ► homologous recombination repair (HR). Important proteins in HR DSB DNA damage signaling and repair are ► Histone 2AX (H2AX), which is phosphorylated within minutes after double-strand breaks occur; the ► tumor suppressor proteins ► BRCA1 and BRCA2, together with their partner proteins ► Rad50 and ► Rad51, respectively; as well as the excision repair enzyme ERCC1.

It is believed that BRCA-defective cells have high levels of endogenous single-strand breaks (SSBs) and thus, their dependency on ► homologous recombination repair HR renders BRCA-defective cells sensitive to PARP inhibitors. The principle of combining single defects (base excision repair (BER) inhibition and HR deficiency) that would not be lethal alone, but only together, is known as the synthetic lethal approach to total tumor cell kill.

Several PARP inhibitors are currently in early phase clinical studies that aim to exploit synthetic lethality. In particular, the drug olaparib has shown promising single agent activity in BRCA-mutant breast cancers; other inhibitors such as ABT-888 are currently being tested for their efficacy in breast cancer in combination regimens.

#### Olaparib

Olaparib (AZD2281, KU-0059436), is a second-generation PARP inhibitor, that has oral bioavailability (Fig. 8).



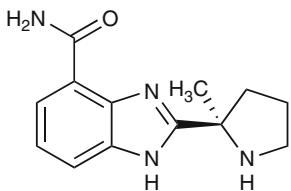
**Breast Cancer Rationally Designed Therapies.**  
**Fig. 8** Molecular structure of olaparib

Olaparib phase I results were reported. The dose-limiting toxicities were mood alteration, fatigue/somnolence, and ► thrombocytopenia. Low-grade toxicities were nausea, vomiting, taste alteration, anorexia, and anemia. Initially, the phase I study was open to patients with unselected tumors resistant to prior therapies; however, the only responses seen were in the patients with BRCA mutations. Overall 9 of 23 patients with BRCA mutations (BRCA deficient) responded, including one complete response. The latter was a breast cancer patient with a BRCA2 mutation, who had pulmonary and lymph-node metastases and had previously had disease progression while receiving ► anthracycline-based ► chemotherapy.

Subsequently, patients with BRCA-mutant breast cancer resistant to prior multiple therapies were enrolled on a phase II study of olaparib 400 mg twice daily (cohort 1), the maximum tolerated dose from the phase I study. As enrollment was robust, the study was amended to allow a second, sequential group of patients (cohort 2) to receive olaparib 100 mg twice daily, which was thought to be the biologically active dose. Fifty percent of patients in cohort 1 and 64% of patients in cohort 2 had also ► triple negative breast cancer (estrogen, progesterone, and HER2 receptor negative). The overall response (OR) was 41% and progression-free survival was 5.7 months for patients receiving olaparib 400 mg twice daily; they were 22% and 3.8 months, respectively, for patients receiving the 100 mg twice daily dose. The toxicity was similar to that reported in the phase I study. Olaparib is a very promising new agent for the treatment of BRCA-mutant breast cancer and could, in the future transform the treatment of this disease type.

#### ABT-888

ABT-888 (Fig. 9) is another potent inhibitor of ► PARP activity. It inhibits both, PARP-1 and PARP-2 with K(i)s of 5.2 and 2.9 nmol/L, respectively.



### Breast Cancer Rationally Designed Therapies.

**Fig. 9** Molecular structure of ABT-888

The compound is orally bioavailable and crosses the blood–brain barrier. In the ► **BRCA1 and BRCA2 mutant** ► **breast cancer** ► **xenograft** ► **mouse model**, ABT-888 potentiated ► **Cisplatin**, ► **Carboplatin**, and ► **Cyclophosphamide**, causing regression of established tumors, but single agent ABT-888 showed only modest tumor growth inhibition.

ABT-888 was initially evaluated clinically in a nontherapeutic phase 0 study, intended to demonstrate the feasibility of phase 0 trials for the rapid development of new targeted agents by the United States National Cancer Institute. Patients were treated with a single dose of ABT-888 at five different dose levels. Blood samples were collected for ► **pharmacokinetics** (PK) and ► **pharmacodynamic** (PD) analyses (► **Lead Optimization**), and for studies of peripheral blood leukocyte. Paired tumor ► **biopsies** to evaluate the effect of ABT-888 on PARP activity and ► **DNA damage** signaling within the tumor were also obtained. The authors concluded that this study design was feasible, providing PK and PD data within 5 months. Additionally, they were able to suggest an appropriate phase I starting dose, 10 mg twice daily, based on the measured levels of PARP inhibition between paired blood and tumor specimens. A large number of trials utilizing ABT-888 are currently ongoing including a combination with ► **Temozolomide** in breast cancers (NCT01009788) and ABT-888 in combination with ► **Irinotecan** in ► **BRCA-mutant** or ► **triple negative breast cancers**.

### Conclusion

Breast cancer was one of the first malignant diseases against which molecularly targeted agents were developed in the early 1980s and for which individualized treatment is becoming a reality. Today, breast cancer is treated by molecular classification rather

than histological type. We have now targeted agents available for most of the major categories of breast cancers that require systemic treatment and these agents have significantly improved outcome and overall survival. Patients with estrogen receptor positive tumors receive ER-directed therapies, HER2-positive cancers are treated with HER2 inhibitors, and patients with BRCA-mutant tumors appear to benefit from PARP inhibitors. Thus, breast cancer can be considered an example of the importance of understanding the underlying biology of a disease in order to design and tailor effective treatments that can turn an acute disease into one that is chronic and significantly more manageable.

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### Breast Cancer Resistance Protein

#### Definition

The breast cancer resistance protein is a plasma membrane transporter and member 2 of the subfamily G of ATP-binding cassette transporters. It is predominantly localized in the epithelium of small and large intestine, in hepatocyte canalicular membranes, in ducts and lobules of the breast, on the placental syncytiotrophoblast, and in the plasma membrane of stem cells. Physiological substrates include sulfated steroids. Transported xenobiotics are, e.g., several dietary carcinogens, the chlorophyll breakdown product

pheophorbide and the receptor tyrosine kinase inhibitor imatinib. The breast cancer resistance protein is involved in conferring multidrug resistance.

#### ► Membrane Transporters

## Breast Cancer Stem Cells

Pranele Rameshwari

Medicine-Hematology/Oncology, UMDNJ-New Jersey Medical School, Newark, NJ, USA

### Synonyms

Breast cancer-initiating cell (breast CIC); Breast tumor-initiating cell (breast TIC)

### Definition

► **Breast cancer** stem cells are self-renewing cells that form new tumor cells. They represent a small fraction of cells that are resistant to ► **chemotherapy** and ► **radiation therapy**. Chemotherapeutic agents with some effect on breast cancer stem cells include 5-> **fluorouracil**, platinum-based agents (► **Platinum Complexes**), ► **taxol**, and ► **doxorubicin**. The frequency of tumor stem cells varies, depending on tumor type. However, <5% of tumor cells may exhibit stem-like phenotype with enhanced tumorigenicity as compared with the molecularly heterogeneous population without stem-like property.

### Characteristics

#### Origins of the Breast Cancer Stem Cells

Tumors of many solid organs contain a population of cells with stem-like phenotype. Much evidence has accumulated on breast cancer stem cells, including expression of ► **stem cell markers** in heterogeneous populations of breast cancer cells. Nestin, an intermediate filament protein characteristic of neural stem cells, has been found in breast cancer. It is unclear whether breast cancer stem cells are derived from

dedifferentiation of mammary progenitors and differentiated epithelia, or from malignant transformation of resident stem cells in the breast. Stem-like cells from mammary epithelia have been shown to carry a similar gene expression profile as malignant cells. Particular subtypes of breast cancer have been correlated with stem cell signatures. The complexity of the origins of breast cancer stem cells is high because there may be many subtypes of breast cancer stem cells. Nonetheless, this idea is not universally accepted and research efforts are underway.

#### Characterization of Breast Cancer Stem Cells

Breast cancer stem cells express high levels of ► **ATP-binding cassette transporters**, which likely accounts for their ► **drug resistance**. The particular gene expression signature of ► **mammosphere**-derived cells includes numerous drug efflux channels, regulators of ► **apoptosis** and the ► **cell cycle**, transport molecules, and signaling molecules. In addition, aldehyde dehydrogenase (ALDH1; ► **Aldehyde Dehydrogenases**), a ► **detoxification** enzyme expressed by stem cells, has been found in breast cancer stem cells. ALDH1 expression has been associated with resistance to ► **taxol**- and ► **epirubicin**-based chemotherapy regimens.

Breast cancer stem cells have the ability to generate tumors in vivo after serial transplantation as ► **xenograft** in immunocompromised NOD/SCID mice (► **Mouse Models**). A few as 100 human cells generate xenograft tumors in NOD/SCID mice, as compared with the requirement for more than one million cells of a heterogeneous phenotype to generate a tumor. ► **CD44+** breast cancer cells are resistant to apoptosis.

#### Signal Transduction Pathways

There is no consensus on a signaling pathway for self-renewal of breast cancer stem cell and their survival. The ► **Wnt Signaling** pathway, found in stem cells, is dysregulated in breast cancer, and signaling through this pathway favors cell ► **motility** in highly aggressive breast cancer cell lines. ► **Notch/Jagged Signaling** and TGF-beta (► **Transforming Growth Factor beta**) signaling, which are vital in embryonic development, are important for the ► **progression** of breast cancer. The TGF-beta superfamily member ► **Nodal** binds to its receptor ► **Cripto-1** to promote breast cancer. These similarities between embryological development and

breast malignancies support the idea that breast cancer may have a stem cell basis.

### Breast Cancer Metastasis to Bone Marrow

► **Metastasis** of breast cancer demonstrates organotropism for the brain, lungs, liver, and bone marrow. Epithelial cancers in particular have a predilection for bone marrow. Metastatic seeding of breast carcinoma to the bone marrow appears to occur years prior to clinical detection, an event that confers poor prognosis in the long-term. Current investigations seek to detect disseminated tumor cells (DTCs) in the bone marrow and circulating tumor cells (CTCs) in the blood. Early identification of DTCs and CTCs may assist in early intervention with the goal of eliminating micrometastatic foci.

### Prospects on Treatment

Currently, no therapy is available that specifically targets breast cancer stem cells. However, many scientists have suggested methods that may offer hope. Intraoperative fluorescent imaging may be a method that can detect breast cancer stem cells. ALDH1 expression has been suggested as an independent predictor of chemotherapy response in the clinic, but the current evidence is unclear due to mixed data. Efforts to target breast cancer stem cell have been met with little success. Most importantly, targeting breast cancer stem cells could be a major challenge since the current information show similarities with other endogenous stem cells.

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## Breast Cancer Susceptibility Genes

Lisa Wiesmüller

Department of Obstetrics and Gynaecology of the University of Ulm, Ulm, Germany

### Definition

► **Breast cancer** susceptibility genes are inherited factors that cause a genetic predisposition to breast cancer, meaning that mutations in these genes increase the risk of developing breast cancer. Several breast cancer susceptibility genes such as the well-known *BRCA1* and *BRCA2* genes have been identified. Predisposing factors are divided into three classes according to the associated breast cancer risk: high-, intermediate-, and low-► **penetrance** (► **Modifier Loci**) genes in addition to a few genes with uncertain penetrance. Dysfunction in multiple low-penetrance genes can also accumulate to a higher predisposition. Most of these genes contribute to the genome stability either as structural or regulatory components of ► **DNA double-strand break repair**. The breast cancer susceptibility genes known so far together do not explain the incidence of breast cancer that clusters in families. Thus, >70% of the risk of familial breast cancer remains unsettled.

### Characteristics

#### High Penetrance

##### *BRCA1* and *BRCA2*

The *BRCA1* gene with a size of around 80 kbp is located on the long (q) arm of chromosome 17 at band 21.31. The 1,863 amino acid long *BRCA1* protein contains a Zinc finger (► **Zinc-Finger Proteins**) within the N-terminal ► **RING finger domain**, C3HC4 type domain, and the *BRCA1* C Terminus domain (► **BRCT Domain**). *BRCA1* forms the central scaffold for the assembly of a multicomponent complex involved in DNA double-strand break repair and ► **DNA damage** signaling. The *BRCA2* gene with a size of around 84 kbp is located on the long arm of chromosome 13 at band 12.3. The *BRCA2* protein is 3,418 amino acids long and contains eight repeats of

the 40 residue BRC motifs. Several out of these motifs in human BRCA2 can directly bind to ► **RAD51** a protein that mediates strand exchange, i.e., the central step in ► **homologous recombination repair**. Thus, both BRCA1 and BRCA2 play important roles in the maintenance of genomic stability by facilitating repair of DNA double-strand breaks, particularly *via* homologous recombination. ► **Breast Cancer Genes BRCA1 and BRCA2** More than 2,600 mutations have been found on chromosome 17 in BRCA1 and on chromosome 13 in BRCA2, causing a greater than tenfold relative risk of breast cancer ► **(BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk)**. Moreover, they are also associated with an elevated risk of ► **ovarian cancer**. ► **Biallelic mutations** in BRCA2 result in ► **Fanconi anemia**, complementation group D1 (FA-D1), with an incidence of 5–10 per million. Fanconi anemia is a ► **chromosomal instability** disease associated with severe familial precancerosis. Patients with this rare syndrome suffer from early onset cancer of different types, including ► **breast cancer**, ► **ovarian cancer** and ► **brain tumors**, soft-tissue sarcomas ► **(Non-Rhabdomyosarcoma Soft Tissue Sarcomas)**, leukemias (► **Hematological Malignancies, Leukemias and Lymphomas**), ► **lung cancer**, ► **laryngeal carcinoma**, and ► **adrenocortical cancer**. Biallelic mutations in BRCA1 have never been reported by now (Fig. 1).

#### TP53

The ► **TP53** gene with a size of around 20 kbp is located at the short arm of the chromosome 17 at band 13.1. Functionally distinct regions identified in the 393 amino acid long p53 are the acidic N-terminal region, containing an acidic ► **transcription** ► **transactivation** domain, a proline-rich domain required for interaction with various ► **proapoptotic proteins**, the DNA-binding domain localized in the core region of the protein, and the basic C-terminal region involved in tetramerization and regulation of p53 activity. Germline as well as somatic mutations have been identified; in fact, it is one of the most mutated genes in the human genome. Most of the mutations are found in the central DNA-binding region. TP53 plays a central role in the regulation of ► **apoptosis**, ► **DNA repair**, ► **cell cycle** control and ► **senescence**. Somatic mutations causing inactivation and disruption of p53 protein are found in almost all

human cancers. Inactivating germline mutations cause the ► **Li-Fraumeni syndrome**. This syndrome is rare and mutations of TP53 are uncommon in non-Li-Fraumeni breast cancer families. Thus, the attributable risk of TP53 mutations to familial breast cancer is very low. Nevertheless, 20–35% of all breast tumors bear a TP53 mutation, some tumor subtypes being more often mutated. Over 1,400 TP53 mutations have been described in breast cancers.

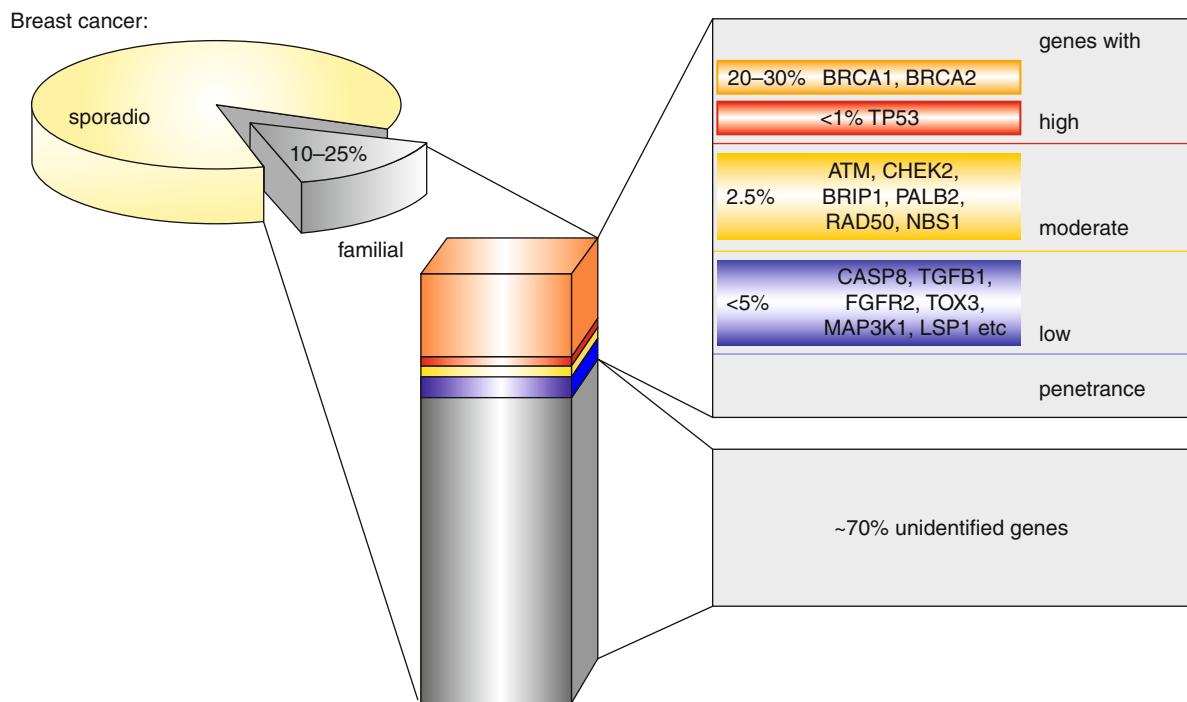
#### Intermediate Penetrance

##### ATM

The *ATM* gene with a size of around 15 kbp is located at the long arm on chromosome 11 at band 22.3 and consists of 66 exons, 62 of which encode a protein of 3,056 amino acids. The ► **ATM protein** belongs to a protein family known as the PI3K ► **(PI3K Signaling)-related** ► **protein kinases** (PIKK), and is involved in the response to DNA double-strand breaks, as it initiates a signaling cascade by phosphorylation of many DNA repair proteins like ► **p53** ► **(P53 Family)**, ► **Nibrin**, BRCA1, and ► **checkpoint kinase** ► **CHK2**. Heterozygous carriers of ► **Ataxia Telangiectasia** causing mutations in the ATM gene have approximately twofold higher risk of breast cancer. Over 300 distinct mutations have been reported of which >80% are base substitutions or insertions/deletions that generate premature termination codons ► **(Nonsense Mutation)** or ► **splicing** abnormalities. A possible link between Ataxia Telangiectasia (A-T), the syndrome caused by ATM disruption, and breast cancer was first found in the 1980s when it was observed that the relatives of A-T patients had an increased risk of breast cancer. A-T is a neurodegenerative, autosomal ► **recessive** disorder characterized by high sensitivity to ► **ionizing radiation**, immune deficiency, and cancer predisposition.

##### CHEK2

The CHEK2 gene with a size of about 50 kbp is located on the long arm of chromosome 22 at band 11. Chk2, the ► **protein kinase** encoded by CHEK2, comprises 514 amino acids and, like ATM, is a central mediator of the ► **DNA damage response**. To regulate DNA double-strand break repair Chk2 phosphorylates p53 and BRCA1. The role of CHEK2 in breast cancer susceptibility was suggested by the identification of a truncating mutation in exon 10 that abolishes Chk2



**Breast Cancer Susceptibility Genes. Fig. 1** Contribution of breast cancer predisposing genes to familial breast cancer

kinase activity (1100delC). A population carrier frequency of ~0.7% has been found for this mutation in Northern and Western European populations, which leads to an approximately twofold increased risk in breast cancer to female heterozygous carriers.

#### BRIP1

BRCA1-interacting protein C-terminal helicase 1; synonym ► **BACH1 Helicase**. The *BRIP1* gene with a size of around 180 kbp is located on the long arm of chromosome 17 at band 22. *BRIP1* protein codes for a ► **helicase**, also called BACH1 or FANCJ, which was first identified as a 1,249 amino acid long BRCA1 binding protein. Direct interaction involves the ► **BRCT domain** of BRCA1, which is critical for DNA repair and ► **tumor suppressor** functions of BRCA1. Monoallelic *BRIP1* mutations (diverse small insertions and deletions) confer a twofold increased risk of familial breast cancer. Biallelic mutations in the *BRIP1* gene result in ► **Fanconi anemia** subtype J (FA-J).

#### PALB2

Partner and localizer of BRCA2; the *PALB2* gene with a size of around 38 kbp is located on the short arm of

chromosome 16 at band 12.2. The 1,186 amino acid long *PALB2* protein is known to interact with BRCA2, facilitating BRCA2-mediated DNA repair by promoting the localization and stability of BRCA2. Truncating mutations of *PALB2* were detected in familial breast cancer, and the relative risk of *PALB2* mutations was estimated to be 2.3-fold. Similar to the breast cancer susceptibility genes BRCA2 and *BRIP1*, a biallelic truncation mutation leads to a subtype of Fanconi anemia (FA-N).

#### RAD50

The *RAD50* gene with a size of 87 kbp is located on the long arm of chromosome 13 at band 31. The 1,312 acid long *RAD50* protein is a component of the conserved MRN complex containing ► **MRE11**, *RAD50*, and ► **Nibrin**, which mediates ► **DNA double-strand break repair** through multiple structural and enzymatic functions in DNA end processing (MRE11: nuclease) and alignment (*RAD50*: DNA end tethering), which are regulated by the Nibrin component. In Finland, a truncating mutation in the *RAD50* gene was found to cause a relative risk of 4.3 to familial breast cancer.

## NBN

The chromosomal location of the *NBN* gene with a size of approximately 50 kbp is 8q21. In the *NBN* gene, which encodes the 754 amino acid long ► **Nibrin** protein (formerly called ► **NBS1** or p95), a rare protein-truncating allele first identified in Polish breast cancer patients is associated with an approximately two-fold increased risk. NBN mutations are underlying the so-called ► **Nijmegen breakage syndrome** (NBS) characterized by microcephaly, immune deficiency, growth retardation, bird-like face, ► **genomic instability** and cancer predisposition.

## Uncertain Penetrance

### PTEN

The ► **PTEN** ► **(Phosphatase and Tensin Homolog Deleted on Chromosome 10)** gene with a size of around 105 kbp is located on the long arm of chromosome 10 at band 23.3. It encodes a 403 amino acid long lipid phosphatase that functions as a ► **tumor suppressor**, containing a tensin-like domain as well as a catalytic domain. There exist links between this lipid phosphatase and the pathways involving ► **p53**, Ras (► **RAS Genes**), and ► **TOR**. In addition, it plays an essential role in the chromosomal stability through induction of ► **RAD51** and ► **DNA double-strand break repair**. Mutations in PTEN cause the ► **Cowden syndrome**, known as multiple ► **hamartoma** syndrome, which is associated with benign and malignant tumors of the breast.

### RAD51C

Most recently, highly penetrant mutations were identified in the gene *RAD51C* in families with both breast and ovarian cancer (1.3%), however, not with breast cancer only. The *RAD51C* gene has a size of about 57 kbp and is localized to a region of chromosome 17q23. The encoded protein encompasses 376 amino acids and plays a role in damage signaling, recruitment of RAD51 to the sites of damage, and processing homologous recombination repair intermediates in different complexes with products of other members of the RAD51 family of related genes (*RAD51B*, *RAD51D*, *XRCC2*, *XRCC3*). As with *BRCA2*, *BRIP1*, and *PALB2*, biallelic mutations in the *RAD51C* gene cause Fanconi anemia (subtype O).

## STK11

Serine/threonine protein kinase 11; the *STK11* gene with a size of around 23 kbp is located on the short arm of chromosome 19 at band 13.3. It codes for a 433 amino acid long serine/threonine kinase inhibiting cellular proliferation, controlling cell polarity, and interacting with the ► **TOR** pathway. *STK11* is the causative gene of the ► **Peutz–Jeghers syndrome** which is characterized by hamartomatous intestinal polyps, mucocutaneous pigmentation, and an elevated risk for several cancers, including breast cancer.

### CDH1

The *CDH1* gene with a size of around 100 kbp is located on the long arm of chromosome 16 at band 22.1. It encodes the 647 amino acid long protein ► **E-cadherin**, which plays a critical role in the stability of the cell polarity as a transmembrane protein. Mutations in this gene are related to ► **Hereditary Diffuse Gastric Cancer** as well as lobular breast carcinoma. The risks associated with the mutations remain unclear.

## Low Penetrance

Data from the HapMap project and from genome-wide association studies identified several candidate genes and low-penetrance breast cancer susceptibility genes, respectively. The increased risk with these genes is small (approximately 40%) and together accounts for less than 5% of the familial risk. The gene products are not involved in DNA repair but in ► **apoptosis** (► **Caspase-8**), mammary gland development (Transforming Growth Factor beta1), ► **inflammation** (► **Tumor Necrosis Factor**), and control of cell proliferation (► **Fibroblast Growth Factor** Receptor 2, *MAP3K1* ► **(MAP Kinase)**).

## Role in DNA Repair and Damage Signaling as the Common Denominator

There is increasing evidence that development of breast cancer is associated with dysfunctions in DNA repair pathways. Germline loss-of-function mutations affecting one allele of the *BRCA1*, *BRCA2*, or *TP53* genes predispose to breast cancer with high, i.e., up to tenfold, risk. The more recently established susceptibility genes *ATM*, *CHEK2*, *BRIP1*, *PALB2*, *NBN*, and *RAD50* confer a twofold increase in breast cancer risk. These 9 mentioned breast cancer susceptibility genes

are all directly or indirectly linked to ► DNA double-strand break repair, where they play important roles in the two major repair pathways ► non-homologous end joining and ► homologous recombination repair.

### Clinical Impact

Knowing, understanding, and detecting the genetic factors is highly relevant for risk estimation and clinical management, for prevention as well as for targeted therapy of breast cancer. In unaffected women with a positive family history of BRCA1 or BRCA2 mutation the risk can be determined by identifying the high-risk mutations. Noncarriers can be calmed, and risk reducing therapies can be offered to carriers. These include surgical (prophylactic mastectomy and prophylactic oophorectomy) and medical (chemoprevention) options as well as intensive care programs. The breast cancer risk reductions associated with these options range from a 90% risk reduction associated with prophylactic mastectomy to approximately 50% with oophorectomy or ► tamoxifen treatment. Currently, risk estimation in BRCA-negative breast cancer families is empirically based upon the family history of cancer. To discriminate between family members with a higher versus lower risk, it might be useful to provide genotyping of multiple genetic predisposition factors identified as breast cancer susceptibility genes. In the general population individuals with increased risk or predisposition to breast cancer can as well be identified by genotyping. In the future, detection of defective DNA double-strand break repair activities may be useful in order to extend the limits of genotyping. Functional analysis may, thus, serve as an additional marker for breast cancer risk assessment and may also predict responsiveness to targeted therapies such as to inhibitors of ► poly(ADP-ribose) polymerase (PARP). Inhibition of PARP, an enzyme involved in ► base excision repair, leads to an accumulation of DNA lesions normally repaired by homologous recombination which requires breast cancer susceptibility gene products such as BRCA1 and BRCA2. BRCA1 and BRCA2 defective cells are very sensitive to the inhibition of PARP, which provides a new targeted therapy option.

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## Breast Cancer Targeted Therapies

Metzger Otto, De Azambuja Evandro and

Piccart-Gebhart Martine

Institut Jules Bordet, Université Libre de Bruxelles, Belgium

### Definition

A number of signaling pathways have been identified participating in the transition of normal cells to fully fledged ► breast cancer. The therapeutic targeting of these deregulated pathways has a great likelihood of success if associated with acceptable levels of toxicity for patients. However, although positive study results have led to the approval of a number of new targeted agents, it is important to highlight the drawbacks of such therapies before discussing their applications. The lack of engagement of a specific target in the oncogenic process can be responsible for clinical study failures. The concept of “oncogenic addiction” (► Oncogene Addiction) explains how some cancers with multiple genetic and epigenetic abnormalities are dependent on, or “addicted” to, a reduced group of genes for the maintenance of their malignant phenotype and cancer cell survival. This oncogenic addiction can render a cell target exceptionally susceptible to a particular drug, such as a ► kinase inhibitor. However, the plasticity of signaling pathways in cancer cells represents an important challenge for the development of new targeted agents. Cross-talk between

different signaling pathways can explain treatment failures, such as the disappointing results of mTOR (► [mammalian target of rapamycin](#)) inhibition in metastatic breast cancer. A more in-depth understanding of deregulated signaling pathways is imperative for bringing targeted therapy to clinical practice and for better selecting treatments for individual patients in this era of treatment tailoring and personalized therapy.

## Characteristics

### Endocrine Therapy

#### Hormone Receptor–Positive Breast Cancer and Targeted Therapy

Hormone receptor–positive breast cancers are characterized by the expression of ► [estrogen receptor](#) (ER) and/or ► [progesterone receptor](#) (PR) and represent one of the most important targets ever established in clinical oncology. The importance of endocrine manipulation in breast cancer dates from 1896, when Sir George Beatson documented the first tumor response following ► [oophorectomy](#). Subsequently, surgical approaches were replaced by high-dose ► [estrogen](#) treatment, the first effective medical ► [endocrine therapy](#) for women with breast cancer. High-dose estrogen therapy was then substituted by ► [Tamoxifen](#) in both premenopausal and postmenopausal women due to its more favorable safety profile. Estrogen depletion with ► [aromatase inhibitors](#) (AIs) has proven, on average, more effective than Tamoxifen alone in postmenopausal patients with advanced disease.

Tamoxifen acts as a competitive antagonist of estrogen for binding to the ► [estrogen receptor](#). It also has partial agonist effects, which can be beneficial for protecting postmenopausal women from bone demineralization, but can also be detrimental, since it is associated with increased risk of ► [uterus cancer](#) and thromboembolism. The third generation aromatase inhibitors ► [Anastrozole](#), ► [Letrozole](#), and Exemestane are now available in clinical practice. These agents suppress estrogen levels in postmenopausal women, inhibiting or inactivating ► [aromatase](#), an enzyme responsible for the synthesis of estrogens from androgenic substrates. Unlike Tamoxifen, no agonist effect is observed with aromatase inhibitors, and the estrogen deprivation is associated with musculoskeletal pain, osteoporosis, and vaginal dryness.

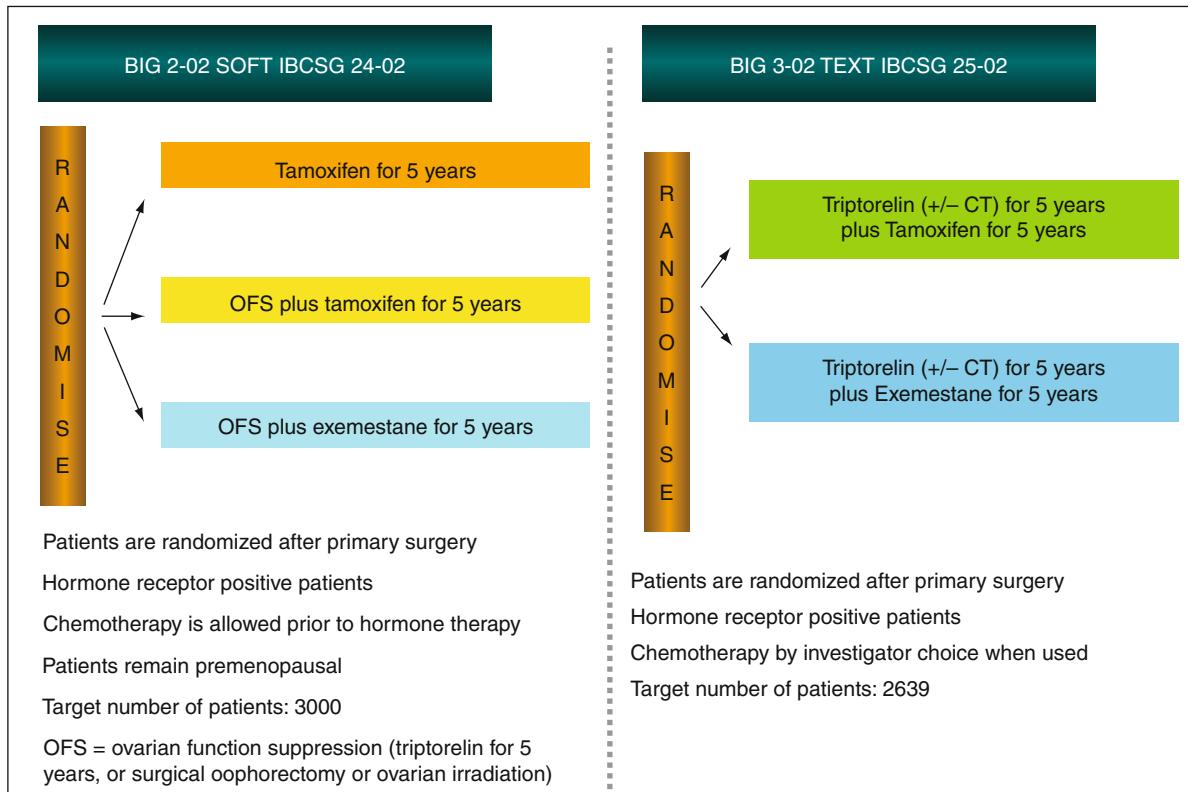
The choice of endocrine therapies in breast cancer is mainly based on menopausal status, status of disease (early or advanced), and risk of early progression.

#### Endocrine Therapy in Premenopausal Metastatic Breast Cancer Patients

In general, up to 60% of patients with hormone receptor–positive advanced breast cancer respond to ► [hormonal therapy](#). Premenopausal patients can be treated with ► [Tamoxifen](#), ovarian ► [ablation](#)/suppression, or the combined strategy (tamoxifen and ovarian suppression/ablation), when used as first-line therapy. Tamoxifen is associated with a response rate (RR) in the range of 50%, and median time to progression (TTP) of 12–18 months can be observed. In a ► [meta-analysis](#) of four randomized clinical trials (n = 506 patients) and a median follow-up of 6.8 years, the combined strategy was superior in terms of response rate, time to progression, and overall survival (OS). Ovarian suppression/ablation is a therapeutic strategy for patients who progress on tamoxifen treatment. Although the combination of ovarian suppression with aromatase inhibitors is sometimes prescribed, no randomized clinical trial addressing this treatment combination has yet been presented, and this strategy should be restricted to clinical trials. Such trials are being run under the umbrella of the Breast International Group (Fig. 1) in the ► [adjuvant](#) setting.

#### Endocrine Therapy in Postmenopausal Metastatic Breast Cancer Patients

For postmenopausal patients, ► [aromatase inhibitors](#) are usually preferred as the first-line ► [hormonal therapy](#) option. In a combined analysis of two randomized clinical trials (n = 1,021 patients), aromatase inhibitors demonstrated a superior time to progression when compared with ► [Tamoxifen](#) (median time to progression: 10.7 versus 6.4 months, respectively; P = 0.022). In a ► [meta-analysis](#) of 23 clinical trials, superior response rate, time to progression, and overall survival favored aromatase inhibitors over Tamoxifen for the first-line treatment of metastatic breast cancer. The therapeutic options for patients progressing on aromatase inhibitors are ► [Tamoxifen](#) and ► [Fulvestrant](#). Fulvestrant is an estrogen ► [receptor antagonist](#) that downregulates the estrogen receptor and is given as intramuscular monthly injections. ► [Fulvestrant](#) and Exemestane, a steroid aromatase inhibitor, were



**Breast Cancer Targeted Therapies.** **Fig. 1** Ongoing adjuvant clinical trials evaluating the role of ovarian function suppression in early breast cancer. Abbreviations: *BIG* Breast International Group [1], *IBCSG* International Breast Cancer

Study Group [2], *SOFT* Suppression of Ovarian Function Trial, *TEXT* Tamoxifen and Exemestane Trial, *OFS* Ovarian Function Suppression, *CT* Chemotherapy

equally effective in a randomized clinical trial involving patients who were previously treated with a non-steroidal aromatase inhibitor.

The second-line therapeutic options for patients who have received first-line Tamoxifen treatment are Fulvestrant or an aromatase inhibitor. Fulvestrant was compared to ► **Anastrozole** in two randomized clinical trials including patients previously treated with Tamoxifen. No superiority to Fulvestrant could be demonstrated, but an unplanned non-inferiority analysis showed similar efficacy.

**Endocrine Therapy in Early Stage Breast Cancer Patients**  
Following primary treatment of early breast cancer, ► **adjuvant** ► **hormonal therapy** is indicated for the subset of patients whose tumors express hormone receptors. ► **Tamoxifen**, when administered for 5 years, reduces the annual risk of recurrence by 47% and death by 26%. The benefit of Tamoxifen continues beyond treatment, and women treated for 5 years have

a lower rate of recurrence and mortality up to 15 years after surgery. Despite this high magnitude of benefit observed with Tamoxifen, up to 50% of relapses cannot be prevented.

Several randomized trials evaluated the role of aromatase inhibitors in the adjuvant setting for hormone receptor-positive postmenopausal patients. Treatment with an aromatase inhibitor for 5 years, an early switch to an aromatase inhibitor after 2 or 3 years of Tamoxifen (up to 5 years) and sequential treatment (5 years of aromatase inhibitor after 5 years of Tamoxifen) have demonstrated superior disease-free survival (DFS) when compared to Tamoxifen alone. Overall survival has been demonstrated up to now in a subpopulation of node-positive patients that received ► **Letrozole** after 5 years of Tamoxifen, and in the latest follow-up of the ARNO and ABCSG8 trials, where patients received anastrozole for 3 years after 2 years of tamoxifen.

For premenopausal women, the standard endocrine treatment consists of the use of Tamoxifen for 5 years,

but newer strategies (e.g., ovarian ► **ablation** plus Tamoxifen, or ovarian ablation plus an aromatase inhibitor) are currently being explored. According to the European Society of Medical Oncology and American Society of Clinical Oncology recommendations, aromatase inhibitors should be included in the adjuvant treatment of postmenopausal women, but an ideal strategy cannot yet be recommended based on the available clinical evidence.

### Hormone Receptor–Positive Breast Cancer and Newer Targeted Therapies

Although substantial gains have been achieved with endocrine targeted agents, approximately 40% of patients with hormone receptor–positive breast cancer tumors do not respond to first-line endocrine therapy and a substantial number of patients receiving endocrine therapy will experience disease progression as the result of acquired resistance (► **Breast Cancer Anti-estrogen Resistance**). The better understanding of ► **estrogen receptor** signaling has revealed deregulated signaling pathways that could be potentially targeted to prevent endocrine resistance. Among these, the ► **epidermal growth factor receptor** (EGFR) family of ► **receptor tyrosine kinases** and the ► **insulin-like growth factors**, including the activation of their downstream signaling pathways (Ras/Raf/MEK/MAPK and PI3K/AKT), deserve special attention. Preclinical and retrospective clinical data indicate that cross-talk between estrogen and EGFR pathways is implicated in endocrine resistance. The synergistic effect of an anti-EGFR and anti-estrogen combination prevents the occurrence of resistant clones in breast cancer cell lines. The EGFR family includes four different ► **receptor tyrosine kinases**: HER1 or EGFR, ► **HER2**, HER3, and HER4. Each receptor is composed of an extracellular ligand-binding domain, a single hydrophobic transmembrane domain and a cytoplasmic tyrosine kinase containing domain. Following growth factor binding, EGFR forms homo- or heterodimers leading to the activation of kinases and phosphorylation of cytoplasmic tyrosine residues. The expression of EGFR is variable, ranging from 6% to 13%, depending on the tumor samples evaluated, the method of assessment, and cutoff used. Although pre-clinical data show a beneficial effect of the dual blockade of hormone and EGFR signaling pathways, this has not resulted in substantial clinical benefit in clinical trials. For example, ► **Gefitinib**, an oral EGFR tyrosine

kinase inhibitor, when combined with ► **Tamoxifen** or anastrozole did not translate into increased therapeutic efficacy, neither in the ► **neoadjuvant** nor the advanced setting (Table 1).

The phosphatidylinositol 3-kinase (► **PI3K Signaling**) pathway is one of the most important signaling pathways in clinical oncology. Its activation usually occurs through a receptor tyrosine kinase, such as EGFR or IGF-1R. The activated PI3K catalyzes the phosphorylation of PIP2 (phosphatidylinositol-4,5-biphosphate) to PIP3. ► **PTEN** (phosphatase and tensin homolog deleted from chromosome 10) works as a negative regulator with the ability to dephosphorylate PIP3, forming the inactive PIP2. PIP3 acts as a docking site for ► **AKT**, which is a central mediator of the PI3K pathway. AKT induces cell survival by blocking ► **apoptosis**, cell proliferation, and increased cell energy due to the modulation of glucose metabolism. The mutation rate of the PI3K/AKT pathway in breast cancer ranges from 24% to 46%, with higher mutation rates observed in the lobular histology subtype (40–46%). The relationship between PI3K mutation and breast cancer prognosis is not uniformly represented in different series of breast tumors. In a retrospective analysis of 250 breast tumors, PI3K mutation was positively correlated with larger tumor size, hormone receptor positivity and worse overall survival. Subsequently, three retrospective series with a total of 1,048 patients independently demonstrated that the presence of PI3K mutation was associated with a better prognosis. Common to all series is a positive correlation between PI3K mutations with hormone receptor positivity.

Most of the attempts to block the PI3K/AKT signaling pathway in breast cancer have been carried out on the downstream pathway target called ► **mammalian target of rapamycin** (mTOR). ► **Rapamycin** was the first mTOR inhibitor developed, followed by rapamycin ester analogs with improved therapeutic properties, such as ► **everolimus** and ► **temsirolimus**. The development of mTOR inhibitors in breast cancer dates from an era in which there were no doubts about the poor prognosis that PI3K/AKT activation implied. The dual blockade of hormone and PI3K pathways with ► **aromatase inhibitors** and mTOR inhibitors failed to demonstrate benefit in advanced and early breast cancer (Table 2). The fact that a blockade of mTOR can abrogate negative feedback on the IGF1 signaling pathway can, in part, explain the failure of an

**Breast Cancer Targeted Therapies. Table 1** Phase II randomized trials of targeted based therapy in combination with endocrine therapy in metastatic breast cancer

	Treatment	Line	Pts	RR (%)	TTP (months)	OS (months)	Comments
Polychronis et al.	Gefitinib + anastrozole vs. gefitinib + placebo	Preoperative	56	50 vs. 54	NR	NR	Primary endpoint: biologic change in proliferation (ki67): 5.6% difference p = 0.0054
Smith et al.	Gefitinib + anastrozole vs. anastrozole + placebo	Preoperative	206	61 vs. 48	NR	NR	Primary endpoint: biologic change in proliferation (ki67): not achieved
Cristofanili et al.	Gefitinib + anastrozole vs. anastrozole + placebo	First	94	49 vs. 34	14.5 vs. 8.2 HR: 0.55 (CI 0.32–0.94; p = NR)	NR	Enrollment was stopped early, limiting statistical analysis
Osborne et al.	Gefitinib + tamoxifen vs. tamoxifen + placebo	First and second	206	14.9 vs. 12.4	10.9 vs. 8.8 HR: 0.84 (CI 0.59–1.18; p = 0.31)	NR	Second stratum: treatment after progression to AI (84pts): no significant benefit
Carpenter et al.	Letrozole vs. letrozole + daily temsirolimus vs. letrozole + intermittent temsirolimus	First	92	NR	NR	NR	Percentage of patients who experienced PR or CR was similar between arms
Chow et al.	Letrozole + temsirolimus vs. letrozole + placebo	First	992	24 vs. 24	9.2 vs. 9.2	NR	Negative phase III clinical trial
Baselga et al.	Everolimus + letrozole vs. letrozole	Preoperative	270	68.1vs. 59.1 p = 0.062	NR	NR	Significance threshold, one-sided p < 0.10
Johnston et al.	Tipifarnib + letrozole vs. tipifarnib + placebo	Second	113	30 vs. 38	10.8 vs. 5.6	27.6 vs. NR	No gain observed with the addition of tipifarnib

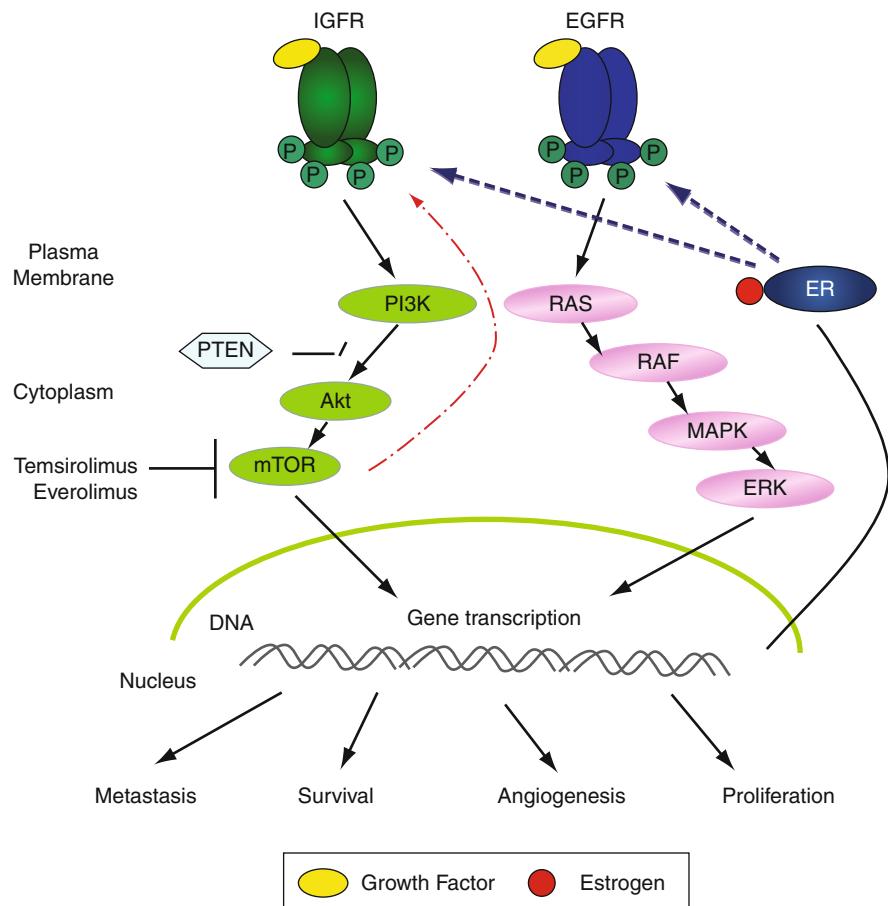
Abbreviations: *Pts* patients, *RR* response rate, *TTP* time to progression, *OS* overall survival, *NR* not reported

**Breast Cancer Targeted Therapies. Table 2** Development of new therapeutic agents targeting HER2 – Phase II results in metastatic breast cancer

	Drug	Mechanism of action	Study phase	Treatment	Pts	Response rate
Gelmon et. al.	Pertuzumab	Blocks HER2 dimerization by binding on domain II of HER2	II	Trastuzumab + pertuzumab	66	CR 7.6% PR 16.7%
Burstein et.al.	Neratinib (HKI 272)	Irreversibly inhibits the tyrosine kinase receptors EGFR and HER2	II	Neratinib	124	26% with prior exposure to trastuzumab, 51% without exposure to trastuzumab
Vukelja et. al.	T-DM1	Trastuzumab acts as specific delivery agent for a anti-microtubule agent – DM1 (mytansine)	II	T-DM1	31	30% (planned accrual to 100 patients)
Modi et. al.	Tanespimycin	Chaperone HSP-90 inhibition – degradation of clients like HER, AKT, RAF1, BCR-ABL	II	Trastuzumab + tanespimycin	21	24%

Abbreviations: *Pts* patients, *CR* complete response, *PR* partial response, *HPS-90* Heat Shock Protein 90

**Breast Cancer Targeted Therapies.** Fig. 2 Cross-talk between IGFR, EGFR, and endocrine signaling pathways. Endocrine-resistant breast cancer tumors are able to escape to anti-hormonal treatment throughout activation of IGFR and EGFR, as demonstrated with the dotted arrows. Blockade of the ► PI3K signaling pathway at the ► mTOR level, as demonstrated with ► everolimus and ► temsiroliimus, has the potential to leave unopposed a negative feedback loop able to reactivate the IGFR receptor, as demonstrated in the red dotted arrow. Abbreviations: *IGFR* ► insulin-growth factor receptor, *EGFR* ► epidermal growth factor receptor, *ER* ► estrogen receptor



mTOR blockade in breast cancer. Signaling through IGF1-R activates PI3K and AKT, counteracting the blockade imposed by the mTOR inhibitors (Fig. 2).

Until now, the available clinical evidence has not been sufficient to allow the use of new targeted agents for the treatment of hormone receptor-positive tumors. However, substantial therapeutic benefit has been achieved in the subgroup of hormone receptor-positive tumors that also express the HER2 protein.

### Targeting the HER2 Subtype of Breast Cancer

The identification of the epidermal growth factor receptor 2 (► HER2), and its subsequent targeted treatment, the anti-HER2 ► monoclonal antibody ► trastuzumab, represents a milestone in the era of targeted therapy. HER2 is overexpressed in 15–20% of breast cancer patients, and its overexpression is associated with worse prognosis. HER2 activation occurs throughout interactions between other tyrosine kinase family members (EGFR, HER3, and HER4).

Ligand binding to EGFR, HER3, and HER4 leads to receptor homo- or heterodimerization, and the subsequent activation of the intracellular signaling cascade. HER2 has no ligand identified, but its conformation makes it the preferred heterodimerization partner of the other HER family members. The assessment of HER2 status must be performed in all diagnosed breast cancer patients in accordance with the American Society of Clinical Oncology and the College of American Pathologists recommendations (Fig. 3).

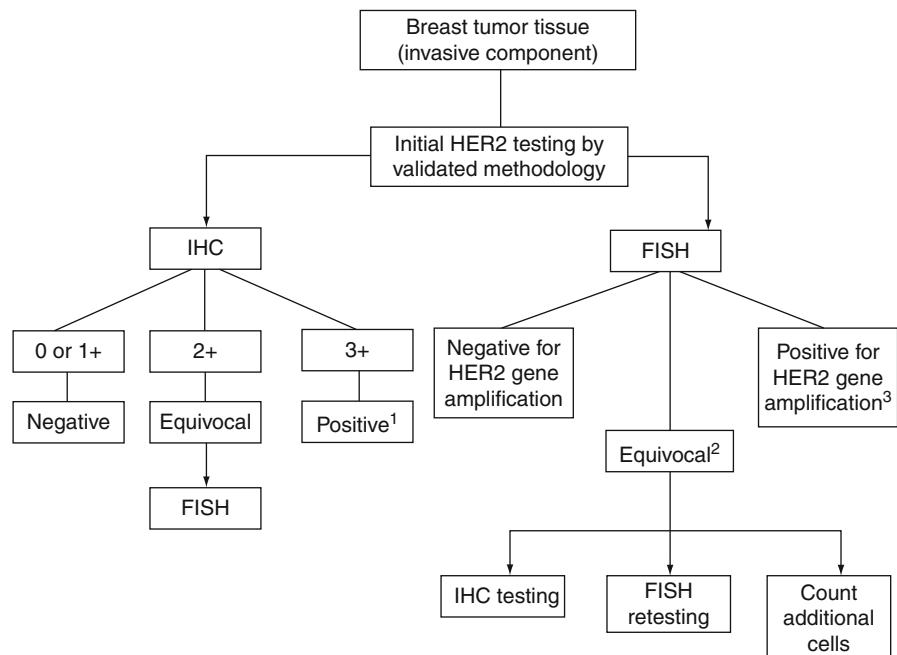
### Targeted Anti-HER2 Therapy in Metastatic Breast Cancer Patients

► **Trastuzumab** is a recombinant humanized monoclonal antibody directed at the extracellular domain of the HER2 protein. Besides blocking HER2 receptor activation, trastuzumab is also able to activate the immune system in a process called ► **antibody-dependent cellular cytotoxicity** (ADCC). In a retrospective

**Breast Cancer Targeted Therapies.** **Fig. 3** Algorithm for ► HER2 testing. (1)

Positivity defined as uniform intense membrane staining of >30% of invasive tumor cells; (2) HER2/chromosome 17 ratio of 1.8–2.2 or HER2 gene copy number of >4 and <6 per nucleus; (3) positivity defined as HER2/chromosome 17 ratio >2.2 or >6 HER2 gene copies per nucleus, *FISH*

► fluorescence in situ hybridization, *IHC*  
► immunohistochemistry



analysis of 54 patients, the probability of response to trastuzumab was correlated with the immune system activation. The addition of trastuzumab to chemotherapy in the first-line treatment of metastatic breast cancer improved RR, TTP, and OS, and is approved as a first-line therapy. Despite the great achievements of trastuzumab therapy, disease progression is commonly observed. Therefore, understanding the underlying mechanisms of resistance has formed the basis for subsequent therapy development.

► **Lapatinib** is an oral tyrosine kinase inhibitor of EGFR and HER2 approved in combination with chemotherapy for the second-line treatment of patients with metastatic breast cancer previously treated with ► **Anthracycline**, ► **Taxane**, and ► **Trastuzumab**. Lapatinib can theoretically overcome trastuzumab mechanisms of resistance such as the truncated form of HER2 receptor (p95) and mutated PTEN. Lapatinib also shows some modest antitumor activity in patients with metastasis to the central nervous system and seems to be less cardiotoxic than Trastuzumab. A randomized clinical trial compared Lapatinib plus ► **Capecitabine** versus Capecitabine alone in HER2-positive metastatic breast cancer patients previously exposed to Trastuzumab. The addition of Lapatinib increased TTP, but no statistical gain in overall survival was observed. A subsequent study evaluated the combination of Lapatinib plus ► **Paclitaxel** regardless

of HER2 status and demonstrated a positive TTP benefit only in the subset of HER2-positive metastatic breast cancer patients, while no benefit was observed in the HER2-negative population. Therefore, Lapatinib should be used in HER2-positive patients only.

The benefit demonstrated with Trastuzumab in metastatic HER2-positive breast cancer patients, coupled with its favorable toxicity profile, led many physicians to continue prescribing the targeted treatment, in combination with other chemotherapy agents, at the moment of disease progression. This strategy was endorsed by different research groups, and positive results were reported in a retrospective analysis. A possible explanation for Trastuzumab benefit beyond disease progression could be related to its chemotherapy-sensitizing properties. Antibody-dependent cell-mediated cytotoxicity (ADCC) could also be responsible for the maintenance of Trastuzumab activity. The benefit of continuing Trastuzumab beyond disease progression was evaluated in a randomized phase III trial, in which patients progressing on Trastuzumab were randomized to receive Capecitabine or Capecitabine plus Trastuzumab. Although prematurely stopped due to low accrual, a benefit of increased RR and TTP was observed. That the blockade of HER2 continues with trastuzumab use reiterates the importance of HER2 signaling pathway, even in the advanced disease setting.

The blockade of the HER2 signaling pathway with different targeted agents used concomitantly has the potential to increase therapeutic efficacy and diminish resistance to such therapy. Preclinical models have demonstrated that Trastuzumab combined with Lapatinib can increase the ► apoptosis rate. To evaluate the synergy between Lapatinib and Trastuzumab, a phase III clinical trial with 296 metastatic breast cancer patients was conducted. Patients with previous exposure to Trastuzumab, Anthracycline, and Taxanes were randomized to receive Lapatinib monotherapy or Lapatinib combined with Trastuzumab. Better ► progression-free survival was observed with the combined therapy (8.4 versus 12 months HR = 0.77; 95% CI 0.6–1.0; p = 0.029).

#### Targeted Anti-HER2 Therapy Combined with Hormone Therapy in Metastatic Breast Cancer Patients

Overexpression of the ► HER2 oncogene is linked to intrinsic resistance to ► endocrine therapy in preclinical models. In a population of 241 patients with hormone receptor-positive breast cancer patients treated with ► Tamoxifen, HER2 overexpression was associated with lower overall response and time to disease progression (38% versus 56%, P = 0.02; 4.1 months versus 8.7 months, p < 0.001; respectively). In the ► adjuvant “► Arimidex, ► Tamoxifen, Alone or in Combination” (ATAC) trial, patients were treated with Tamoxifen, Anastrozole, or combination hormonal therapy. Initial results demonstrated greater benefit of Anastrozole in the subset of HER2-positive tumors, but after central pathology review, it was found that the results were inferior in the HER2 subgroup regardless of Anastrozole or Tamoxifen treatment. Resistance to hormone therapy can be mediated at least in part by HER2 expression, and the opposite is also true, with preclinical evidence demonstrating resistance to HER2 targeted therapy through estrogen receptor activation.

The possibility of blocking the competing signaling pathways (HER2 and hormone receptor) was evaluated as a promising strategy to eliminate the cross-talk between signaling pathways and increase therapeutic benefit. A phase III trial evaluated the combination of Trastuzumab and Anastrozole compared to Anastrozole in 208 HER2-positive, ► estrogen receptor-positive metastatic breast cancer patients. The combination therapy led to an increase in progression-free survival (PFS; 4.8 versus 2.4 months;

p = 0.0016) compared to the control arm. The improved progression-free survival (4.8 months) in that trial was less impressive in comparison with the PFS obtained when Trastuzumab was combined with chemotherapy (7.4 months); however, any cross-trial comparison should be viewed cautiously.

The availability of ► Lapatinib and its broader spectrum of activity have led to its evaluation in combination with hormone therapy. A phase III trial compared ► Lapatinib plus ► Letrozole versus letrozole monotherapy as first-line therapy for hormone receptor-positive metastatic breast cancer patients. Median PFS in the HER2-positive and hormone receptor (ER)-positive subset increased from 3 to 8.2 months with combined therapy (HR = 0.71; 0.53–0.96; p = 0.019). The dual blockade of HER2 and estrogen receptor with Lapatinib and Letrozole can represent a good alternative to block the cross-talk between the ER and HER2 signaling pathways.

Significant advances have been achieved in the field of targeted therapy for the HER2 subgroup of breast cancer patients. Trastuzumab combined with chemotherapy (non-anthracycline) is approved for the first-line treatment of metastatic breast cancer. Lapatinib plus Capecitabine is the approved second-line agent for patients previously treated with ► Anthracycline, ► Taxanes, and ► Trastuzumab. In patients using anti-HER2 therapies, cardiac monitoring should be performed every three months during treatment. Also, the avoidance of drugs interfering with ► CYP3A4 is indicated during Lapatinib treatment. Important for daily clinical practice, blocking the HER2 pathway with an anti-HER2 therapy in metastatic breast cancer patients should be maintained unless severe toxicity occurs.

#### Targeted Anti-HER2 Therapy in Early Breast Cancer Patients

The positive results of ► Trastuzumab in the advanced setting were subsequently reproduced in early breast cancer studies. ► Adjuvant trastuzumab given for 1 year halved the risk of recurrence in women with ► HER2-positive breast cancer, regardless of age, tumor size, nodal status, hormone receptor status, and type of adjuvant chemotherapy used. A positive impact on overall survival was also demonstrated. At present, Trastuzumab is recommended for the adjuvant treatment of patients with HER2-positive breast cancer greater than 1 cm or with node-positive disease for

the duration of 1 year, based on the results of large, randomized well-conducted clinical trials. Due to the potential cardiotoxicity of Trastuzumab, particularly when used after ► **anthracycline**, cardiac function assessment is recommended before and during trastuzumab treatment.

The updated analysis of the HERA trial in 2009 at 4 years of median follow-up demonstrates the maintained benefit of Trastuzumab in terms of disease-free survival (HR = 0.76; 0.66–0.87; p < 0.0001), but not overall survival (HR = 0.85; 0.70–1.04; p = 0.10). There were a nonnegligible number of patients (~50%) originally randomized to the observation arm to whom Trastuzumab was offered after the release of the trial's first results in 2005. In the intention-to-treat analysis presented in 2009, the overall survival results may give a false impression of diminished efficacy of Trastuzumab over time. However, these results should be carefully interpreted due to the likely positive impact of late Trastuzumab initiation in patients originally randomized to observation. Crossover has rendered any future comparison with the observation arm much more challenging since a “pure” observation arm no longer exists.

► **Lapatinib** is currently being tested as an ► **adjuvant therapy** in two large randomized trials. The ALTTO trial represents a tremendous global effort designed to explore the potential benefits of dual HER2 receptor targeting in early disease. The four-arm design of the ALTTO trial includes Trastuzumab for 1 year, Lapatinib for 1 year, Trastuzumab followed by Lapatinib for a total duration of 1 year, and Lapatinib in combination with Trastuzumab for 1 year. This 8,000 patient trial is almost completed, and its results may change current practice in HER2-positive breast cancer treatment. The TEACH trials aims to evaluate the benefit of Lapatinib in early breast cancer for HER2-positive patients who have not received Trastuzumab, even if introduced many years after diagnosis. This trial ended accrual after the randomization of 3,000 patients, and its results are eagerly awaited.

### Triple Negative Breast Cancer and Targeted Therapy

► **Triple negative breast cancer** accounts for 15% of all diagnosed breast cancer patients and is clinically represented by ► **estrogen receptor**, ► **progesterone receptor**, and ► **HER2** negative tumors. Triple

negative breast cancer is a group of highly proliferative tumors associated with aggressive clinical behavior. The identification of targets such as EGFR and the possibility to target the ► **DNA damage repair** (► **DNA Repair**) machinery have opened new avenues for drug development. Targeting the tumor supply by blocking ► **angiogenesis** is also a strategy evaluated for this disease subclass.

The prognostic importance of EGFR (► **HER2/neu**) expression was retrospectively evaluated in a series of 930 patients with a follow-up of 17 years. The expression of EGFR, considered positive if present at any level, could be demonstrated in 13% of 614 interpretable samples. Among patients with basal cytokeratin expression (CK5/CK6), a higher expression of EGFR could be observed (54%). In the subgroup of patients expressing EGFR, worse survival was observed independently of nodal status and tumor size. In metaplastic breast cancer, which accounts for approximately 1% of all breast cancers, up to 80% EGFR expression is observed.

One of the main pitfalls for targeting EGFR pathways is the presence of activating mutations in the signaling cascade (Ras/RAF/MEK/MAPK; ► **Raf Kinase**) downstream of EGFR dimerization. ► **K-RAS** mutation is a common event in ► **colorectal cancer** and in ► **lung cancer** tumorigenesis, but mutations in Ras and ► **B-Raf** genes have been rarely identified in human primary breast cancer, although they were identified at considerable frequency in breast cancer cell lines, which indicates a possibility for acquired mutations at advanced stages.

► **Cetuximab**, a chimeric ► **monoclonal antibody** targeting EGFR was evaluated in two randomized phase II trials. Cetuximab as a single agent was associated with a low response rate (6%). When combined with chemotherapy, Cetuximab increased response rate, but no gain in progression-free survival was observed (Table 3). The results presented until now raise concern regarding the role of EGFR as an important target in triple negative breast cancer, and the results of ongoing phase III trials with Cetuximab are awaited.

Targeting mechanisms of ► **DNA damage repair** is an innovative approach being developed for ► **triple negative breast cancer**. Cancer cells acquire DNA mutations over time, and failures in the mechanisms of DNA repair favor ► **genetic instability** and tumorigenesis. The remaining DNA repair mechanisms

**Breast Cancer Targeted Therapies. Table 3** Randomized trials of targeted therapy plus chemotherapy versus chemotherapy – triple negative breast cancer patients

Study	Treatment	Line	Study		RR (%)	TTP (months)	OS (months)
			phase	Pts			
Carey et al.	Cetuximab vs. Cetuximab plus carboplatin	1-2-3	II	102	6 vs. 18	NR vs. 2	NR
O'Shaughnessy et al.	Irinotecan + carboplatin + Cetuximab vs. irinotecan + carboplatin	1-2	II	154	33 vs. 28	4.7 vs. 4.5	12.6 vs. 12.3
O'Shaughnessy et al.	BSI-201 + gemcitabine + carboplatin vs. gemcitabine + carboplatin	1-2-3	II	123	48 vs. 16 (p = 0.002)	6.9 vs. 3.3 (p < 0.0001)	9.2 vs. 5.7 (p = 0.0005)

Abbreviations: Pts patients, RR response rate, TTP time to progression, OS overall survival

(those that are not lost during tumor progression), are upregulated and involved in resistance to DNA-damaging agents.

► DNA repair mechanisms can be classified into categories repairing either single- or double-stranded breaks. When one DNA strand is affected and the complementary strand is intact, direct repair, ► base excision repair (BER), ► nucleotide excision repair, and ► mismatch repair are activated to correct it. For damage in both DNA strands, two main repair pathways are available: ► nonhomologous end joining (NHEJ) and error-free homologous repair. Homologous repair can be subsequently divided into gene conversion (GC) and single strand-annealing (SSA). The different DNA repair pathways are organized to maintain stability and integrity of the genome.

Deficiencies in the ► BRCA1 gene pathway are important for understanding the sensitivity to drugs targeting DNA repair in triple negative breast cancer. The BRCA1 gene plays a key role in maintaining genomic stability by promoting repair of ► DNA double-strand breaks. The majority of breast tumors arising in ► BRCA1 germline mutation carriers display a triple negative phenotype determined by ► immunohistochemistry or by genomic techniques. Although the majority of triple negative tumors are sporadic and lack BRCA1 mutations, the integrity of the BRCA1 pathway seems also to be compromised in these tumors. Therefore, drugs blocking single-strand repair could be selectively lethal to cells lacking functional BRCA1 (dysfunctional double-strand repair) (Fig. 4).

► Poly-(adenosine diphosphate (ADP)-ribose) polymerases (PARPs) are a large family of multifunctional enzymes, with PARP1 as the most abundant. PARP1 is involved in the mechanism of single-strand DNA

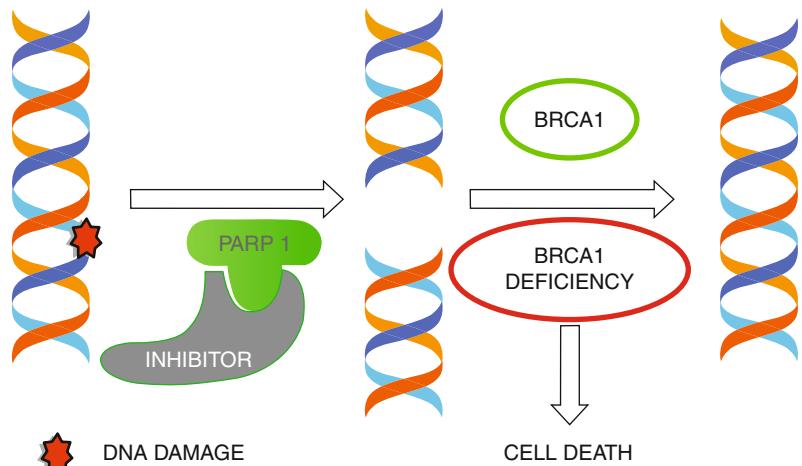
repair called ► base excision repair (BER). BER involves the sensing of the lesion followed by the recruitment of a number of other proteins. PARP-1 senses and binds to DNA nicks and breaks, and activates catalytic activity, causing poly-(ADP) ribosylation of PARP-1 itself. This modification signals the recruitment of other components of ► DNA repair pathways. The activity of a single agent PARP inhibitor was evaluated in a phase II trial of ► BRCA1/BRCA2 carriers with breast cancer. ► Olaparib, an oral PARP inhibitor, as a single agent was associated with a 38% response rate and manageable toxicity profile. The role of PARP inhibition was also evaluated in patients with triple negative phenotype regardless of BRCA mutation state. In a randomized phase II trial, the addition of a PARP inhibitor (BSI-201) to chemotherapy (► Carboplatin and ► Gemcitabine) increased the response rate (RR)(48 vs. 16%, p = 0.002), time to progression (TTP)(6.9 vs. 3.3, p < 0.0001) and overall survival (OS) (9.2 vs. 5.7, p = 0.0005) (Table 3). The combination of classic ► chemotherapy drugs known to cause DNA damage, such as ► platinum, with drugs designed to block DNA repair is under evaluation in a phase III trial.

#### Anti-angiogenic Therapy

Abnormal ► angiogenesis is a hallmark of cancer and is characterized by an increase in the number of proliferating endothelial cells in the vicinity of the tumor as well as altered morphology of the tumor vasculature. The ability of solid tumors to produce pro-angiogenic factors and recruit new blood vessels allows nutrients and oxygen delivery and subsequently tumor growth and survival. The hope behind blocking angiogenesis is that it will restrict tumor growth. ► Vascular endothelial growth factor (VEGF) is

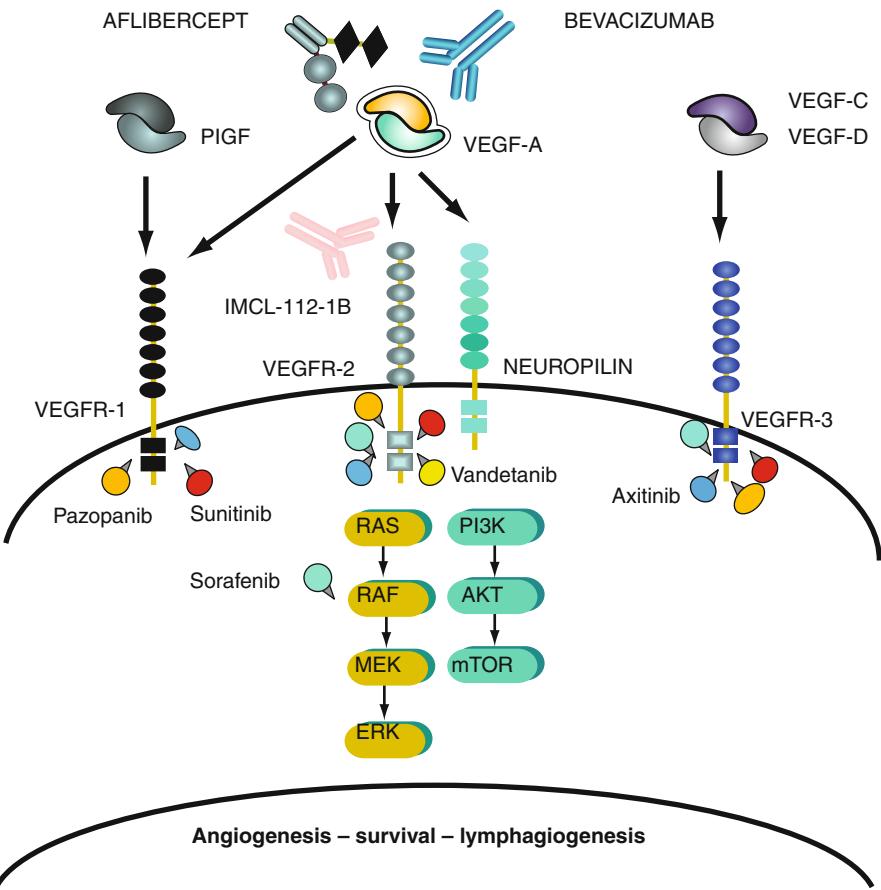
### Breast Cancer Targeted Therapies.

**Fig. 4** Mechanism of action of ▶ PARP inhibitors. The ▶ BRCA1 protein promotes DNA stability by correcting DNA double-strand breaks. PARP inhibitors act by inhibiting the correction of single-strand breaks. The combination of BRCA1 gene mutation or malfunction and PARP inhibitor can be selectively lethal to breast cancer cells



### Breast Cancer Targeted Therapies.

**Fig. 5** Mechanisms of action of ▶ antiangiogenesis drugs. ▶ Bevacizumab and IMC-112-1B – ▶ monoclonal antibodies; ▶ Aflibercept – VEGF trap; ▶ Pazopanib, ▶ Sunitinib, ▶ Sorafenib, Vandetanib, and Axitinib are examples of anti-angiogenic ▶ tyrosine kinase inhibitors



one of the most important pro-angiogenic factors. The VEGF family comprises five VEGF glycoproteins referred to as VEGF-A (also called VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor

(PIGF). The VEGF ligands bind and activate three tyrosine kinase receptors called VEGFR-1 (also known as FLT1), VEGFR-2 (also known as KDR), and VEGFR-3 (also known as FLT4). VEGFR-2 is

**Breast Cancer Targeted Therapies. Table 4** Randomized trials of anti-angiogenic therapy plus chemotherapy versus chemotherapy

Study	Treatment	Study			RR (%)	PFS (months)	OS (months)
		Line	phase	Pts			
Miller et al.	Capecitabine + bevacizumab vs. capecitabine	1,2,3	III	462	19.8 vs. 9.1 p = 0.01	4.86 vs 4.17 HR: 0.98 (CI 0.77–1.25; p = 0.857)	15.1 vs.14.1 HR: NR p NR
Miller et al.	Paclitaxel + bevacizumab vs. paclitaxel	1	III	722	36.9 vs. 21.2 p <0.001	5.9 vs. 11.8 HR:0.6 (CI 0.51–0.70; p < 0.001)	26.7 vs. 25.2 HR: 0.88 (CI NR; p = 0.16)
Milles et al. (AVADO)	Docetaxel + bevacizumab (7.5 mg/Kg) vs. docetaxel + placebo	1	III	736	55.2 vs. 44.4 p =0.0295	8.7 vs. 8.0 HR: 0.79 (CI 0.63–0.98; p = 0.0318)	NR
	Docetaxel + bevacizumab (15 mg/kg) vs. docetaxel + placebo				63.1 vs. 44 p = 0.0001	8.8 vs. 8.0 HR: 0.72 (CI 0.57–0.90; p = 0.0099)	NR
Robert el al. (RIBBON-1)	Capecitabine plus bevacizumab	1	III	1,237	35.4 vs. 23.6 P = 0.0097	9.8 vs. 6.2 HR: 0.68 (CI 0.54–0.86; p = 0.0011)	NR
	Taxane or Anthra plus bevacizumab				51.3 vs. 37.1 P = 0.0055	10.7 vs. 8.3 HR: 0.77 (0.69–0.99 p = 0.04)	NR
Boer et al.	Vandetanib + docetaxel vs. vandetanib	1	II	64	NR	8.7 vs. 6.0	NR
Rugo et al.	Axitinib + docetaxel vs. docetaxel	1	II	168	40 vs. 23 (p = 0.038)	8.2 vs. 7.0 HR: 0.73 (p = 0.052)	NR
Slamon et al.	Lapatinib + pazopanib vs. lapatinib	1	II	141	36.2 vs. 22.2	NR	NR
Baselga et al.	Sorafenib + capecitabine vs. capecitabine	1,2	II	229	31 vs. 38 p = NR	4.1 vs. 6.4 HR: 0.57 (CI 0.41–0.80; p = 0.0006)	NR

Abbreviations: HR + hormone receptor positive, PFS progression-free survival, OS overall survival, MBC metastatic breast cancer, Pts patients, HR hazard ratio, NR not reported

expressed in endothelial cells involved in angiogenesis, and in circulating bone marrow-derived endothelial progenitor cells. VEGFR-2 is a key mediator of angiogenesis upon activation by VEGF-A. VEGFR-3 is expressed mainly on lymphatic cells and preferentially binds to VEGF-C and VEGF-D. VEGFR-1 is expressed in vasculature and in other cell types. Its exact role is not known, but it probably works as a negative regulator of angiogenesis due to its high affinity to VEGF-A and its diminished activation of intracellular signaling. A class of non-tyrosine kinase receptors called ► **neuropilins** is also important for the VEGF mediated signaling, and can act as co-receptors for VEGFR-2.

VEGF is usually secreted by cancer cells, tumor-associated stromal cells, and from various host cells, such as platelets and muscle cells. The interaction of VEGF with other signaling pathways and growth factors mediates proliferation, survival, and vascular

permeability of ► **endothelial cells**. VEGF also has a direct effect on mediating ► **invasion** mechanisms, and is also considered to be an immune modulator, allowing an ► **immune escape** from tumors.

Numerous agents that target the VEGF pathway are in clinical development, including agents targeting the VEGF ligand and agents targeting the VEGF receptors (VEGFRs) (Fig. 4). ► **Bevacizumab** is a humanized recombinant ► **monoclonal antibody** designed to block VEGF-A. In a randomized clinical trial including patients with metastatic breast cancer and the ► **HER2**-negative phenotype, the addition of Bevacizumab to ► **Paclitaxel** increased RR and doubled progression-free survival, but no benefit of overall survival could be observed. The observed benefit in progression-free survival was similar in hormone receptor-positive and triple negative breast cancer in the performed sub-analysis. The addition of Bevacizumab to other types of chemotherapy in

**Breast Cancer Targeted Therapies. Table 5** Targeted therapy in clinical development in breast cancer

Target	Drug	Study phase	Description of pathway
FTI	Tipifarnib	II	Covalent attachment of a farnesyl group to substrates of signaling pathways, such as RAS. The phase II results of tipifarnib are described in Table 2. AZD3409 is a geranyl and farnesyl transferase inhibitor.
	Lonafarnib	I	
	AZD3409	I	
HDAC		II	HDAC inhibition results in acetylation of histones and also transcription factors such as p53, GATA-1 and estrogen receptor-alpha.
MEK 1/2	AZD6244	I	Inhibition of MEK, a component of RAS/RAF signaling pathway. Demonstrated efficacy in patients with mutant K-RAS.
PKC $\beta$	Enzaustarin	I	PKC family consists of serine/threonine intracellular kinases involved in intracellular signaling. PKC family is involved in EGFR, VEGF, PI3K/AKT, and RAS/RAF/MAPK signaling
Src	Dasatinib	I	Non-receptor protein kinases known to interact with EGFR and HER2 pathways. Src is able to activate RAS/RAF/MAPK and PI3K/AKT pathways, and inhibit ABL.
	AZD0530	I	
	Bosutinib	I	
Apoptosis-involved proteins	TRAIL	II	TRAIL is an apoptosis inducer and is targeted with agonist monoclonal antibodies against TRAIL receptors 1 and 2.
	Oblimersen	I	G3139 is an antisense oligonucleotides able to downregulate Bcl2, which is a anti-apoptotic protein
	G3139		
	LY2181308	I	Antisense molecule directed against survivin, which is a strong anti-apoptotic agent
CDKs	Bortezomib	II	Inactivation of nuclear factor-kappa beta (NF-K $\beta$ ) by proteasome inhibitor leads to accumulation of pro-apoptotic proteins.
	Flavopiridol	I	Inhibition of cell cycle with cyclin-dependent kinase inhibitors.
p53	Ad5CMV-p53	II	Intratumoral administration of a non-replicating adenoviral vector that contains the human wild type p53.

Abbreviations: FTI farnesyl transferase inhibitors, HDAC histone deacetylase inhibitor, TRAIL tumor necrosis factor-related apoptosis-inducing ligand, CDKs cyclin-dependent kinases

first-line treatment of HER2-negative metastatic breast cancer was also positive in two subsequent trials. Although generally well tolerated, Bevacizumab may be associated with increased hypertension, proteinuria, neurotoxicity, and left ventricular dysfunction.

The possibility of blocking angiogenesis with ► **tyrosine kinase inhibitors** is being evaluated with different drugs (Fig. 5 and Table 4). ► **Sunitinib** is a multi-target tyrosine kinase inhibitor, with the ability to block VEGFR-1, -2, -3, among other receptors. In a phase II trial of metastatic breast cancer patients previously exposed to ► **Anthracyclines** and ► **Taxanes**, sunitinib monotherapy was associated with an 11% response rate. Two randomized phase III trials with ► **Sunitinib** were prematurely stopped. Sunitinib combined with Paclitaxel was not superior to Sunitinib combined with Bevacizumab. Sunitinib monotherapy was not superior to ► **Capecitabine** monotherapy. The benefit of adding Sunitinib to ► **Docetaxel** is being

evaluated in a randomized clinical trial, and the results are also pending. Sunitinib is associated with hypertension, left ventricular dysfunction, and proteinuria. In other disease settings cases of hypothyroidism were reported and seemed to correlate with treatment duration.

► **Sorafenib**, an oral multi-target inhibitor of ► **RAF kinase** and the receptor tyrosine kinase family (VEGFR-1, -2, and -3, PDGFR- $\beta$ , c-KIT, and FLT3), was evaluated in a randomized phase II trial including patients with advanced breast cancer. A total of 229 patients were randomized to receive ► **Capecitabine** with or without the addition of ► **Sorafenib** as first- or second-line therapy. The addition of Sorafenib to Capecitabine was able to increase progression-free survival from 4.1 months to 6.4 months (HR = 0.57, 95% CI 0.41–0.80,  $p = 0.0006$ ).

Despite the benefits observed with Bevacizumab in the first-line treatment of metastatic breast cancer,

intrinsic and acquired resistance to anti-angiogenic therapy is commonly observed (Table 5).

## Conclusions

Targeted therapies for treating breast cancer are evolving rapidly, but the complexity and flexibility of signaling pathways imposes a huge challenge for drug development plans. To address this problem, mathematical kinetic models are now being proposed with which to perform computer simulations in order to evaluate whether a given targeted drug is likely to be promising for a given type of breast cancer. While such models involve much needed dynamicity, the current common practice involving the analysis of single tumor biopsies is not sufficiently informative for optimal development of targeted drugs. The neoadjuvant model offers an elegant way to address this deficiency and holds promise for a more rational targeted therapy development in some tumor types such as breast cancer. Initial biopsies can be compared to biopsies taken during targeted treatment, and correlations between target expression and blockade can be easily obtained.

For the subgroup of breast cancer patients expressing hormone receptors, long-term gains have been achieved with hormone treatment, but the combination of hormone therapy with novel targeted drugs has not yet provided survival benefits for patients with advanced breast cancer. Great achievements have been obtained for the HER2 breast cancer subtype. More recently, an innovative strategy was evaluated in the subset of triple negative breast cancer patients. The blockade of single-strand DNA repair pathways with PARP inhibitors added lethality to triple negative breast cancer cells, in which the double-strand repair pathway is intrinsically dysfunctional.

New strategies are being evaluated, such as farnesyl transferase inhibitors, histone deacetylase inhibitors, and SRC inhibitors. A better knowledge of signaling pathways and the cross-talk taking place between them as well as a closer collaboration between clinicians and scientists should result in an accelerated path toward improved anticancer treatment “tailoring.”

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## Breast Conservation

### Definition

Surgical removal of cancer from the breast while preserving the remainder of the breast tissue.

► [Oncoplastic Surgery](#)

## Breast Imaging Reporting and Data System

### Definition

BIRADS; <http://www.birads.at/index.html>

## Breast Implant

### Definition

Device placed into the body to enlarge or reconstruct the breast after removal.

► [Oncoplastic Surgery](#)

## Breast Reconstruction

### Definition

Surgical procedure to simulate the appearance of the breast after it has been removed for the treatment of cancer.

- Oncoplastic Surgery
- 

## Breast Reduction

### Definition

Surgery to reduce the size of the breast.

- Oncoplastic Surgery
- 

## Breast Regressing Protein 39 Kd

- Serum Biomarkers
- 

## Breast Stem Cells

### Definition

Self-renewing cells in the breast that are capable of producing all of the breast epithelial cell types and likely play an important role in breast cancer.

- Basal-Like Breast Cancer
- 

## Breast Surgery

### Definition

Surgical procedures on the breast used to treat congenital deformities or to remove cancerous tissue.

- Oncoplastic Surgery
- 

## Breast Transillumination

- Optical Mammography
- 

## Breslow Depth

### Definition

Referring to ► [cutaneous desmoplastic melanoma](#) tumor thickness (according to *Breslow*). The single most important factor in predicting survival for patients with stage I/II melanoma. Is measured from the top of the granular layer (for non-ulcerated lesion) or from the ulcer base overlying the deepest point of invasion (for ulcerated lesions) to the deepest extension of the tumor using an ocular micrometer. According to the AJCC-criteria for malignant melanoma, tumor thickness and the presence or absence of ulceration are the primary criteria for the tumor classification.

- Desmoplasia
  - Desmoplastic Melanoma
- 

## BRG- and BRM-associated Factor, 47 kDa

- [hSNF5/INI1/SMARCB1 Tumor Suppressor Gene](#)
- 

## Brilliant Yellow S

- Curcumin
- 

## BRIP1

### Definition

BRCA1 interacting protein C-terminal helicase 1; ► [BACH1 Helicase](#) (synonym). The BRIP1 gene with a size of around 180 kbp is located on the long arm of chromosome 17 at band 22. BRIP1 protein

codes for a ► **helicase**, also called BACH1 or FANCJ, which was first identified as a 1249 amino acid long BRCA1 binding protein. Direct interaction involves the ► **BRCT domain** of BRCA1, which is critical for DNA repair and ► **tumor suppressor** functions of BRCA1. Monoallelic BRIP1 mutations (diverse small insertions and deletions) confer a twofold increased risk of familial ► **breast cancer**. Biallelic mutations in the BRIP1 gene result in ► **Fanconi anemia** subtype J (FA-J).

## BRIT1 Gene

Deborah Jackson-Bernitsas<sup>1</sup>, Shiaw-Yih Lin<sup>1</sup> and Kaiyi Li<sup>2</sup>

<sup>1</sup>Department of Systems Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

<sup>2</sup>Department of Surgery, Baylor College of Medicine, Houston, TX, USA

## Synonyms

MCPH1; Microcephalin

## Definition

BRCT-repeat inhibitor of hTERT expression.

## Characteristics

### Significance of BRIT1 in the Development of Cancer

BRIT1 was first described through a genetic screen for transcriptional repressors of the catalytic subunit of ► **human telomerase, (hTERT)**. This catalytic subunit, hTERT, is the rate-limiting determinant of and is necessary for telomerase activity and thus is highly significant for cellular immortalization by preventing natural cellular senescence. Therefore, molecules that negatively control hTERT activity, such as BRIT1, directly influence the development of precancerous and cancerous cells.

The BRIT1 amino acid sequence matched a previously identified gene, ► **microcephalin** (► **MCPH1**)

that is implicated as one of the contributing factors in the autosomal recessive neuro-developmental disorder, primary microcephaly. Additional biological roles for BRIT1 in the DNA damage response pathway were suggested by the protein structure. The presence of three ► **BRCA1/BRCA2 germ line mutation** domains within its structure connected BRIT1 with a group of proteins involved in DNA damage repair and checkpoint control such as ► **53BP1**, ► **MDC1**, and BRCA1. Proper cellular response to DNA damage is essential for the maintenance of genomic stability and is consequently crucial in prevention of neoplastic transformation. Depletion of BRIT1 by experimental manipulation abolishes normal DNA damage response and introduces chromosomal and centrosomal abnormalities. The reduction of BRIT1 expression in normal human mammary epithelial cells by experimental RNA interference generated chromosomal breaks, dicentric chromosomes, and telomeric fusions. Additional chromosomal aberrations were introduced when BRIT-deficient cells were submitted to genotoxic insult. The resultant genomic instability generated from the loss of an appropriate BRIT1-mediated checkpoint and DNA repair mechanism may contribute to tumor formation. Functional impairment or loss of proteins, such as BRIT1, may significantly contribute to tumorigenic development by allowing the perpetuation of damaged or mutated genes within a cell, resulting in the inappropriate expression and control of the affected genes.

In addition to the influence on hTERT expression, BRIT1 appears to control the expression of two vital checkpoint-regulating proteins BRCA1 and Chk1. BRCA1 and Chk1 protein levels are dramatically reduced in cells where BRIT1 has been experimentally reduced by RNA interference. A concurrent reduction in mRNA levels of BRCA1 and Chk1 were observed in BRIT1 knockdown cells suggesting that BRIT1 exercises an influence on the transcription of these genes. The significance of BRCA1 in breast cancer has been previously established. Whether BRIT1 functions directly as a specific transcription factor or as a chromatin-modifying factor is unclear at this time; however, as a controller of these key players in the DNA damage checkpoint control network, BRIT1 is extremely important to the maintenance of normal cell function and thus in the prevention of tumorigenesis.

Aberrations of BRIT1 have been identified in various different human cancers. BRIT1 mRNA and protein expression is aberrantly reduced in several breast

cancer cell lines and is reduced in some human ovarian and prostate epithelial tumors as compared to the corresponding normal tissue. Reduction of BRIT1 gene copy number significantly correlated with genomic instability found in the specimens. Additionally, reduced BRIT1 expression correlated with the duration of the relapse-free intervals and with the occurrence of metastases in some breast cancer patients suggesting that BRIT1 deficiency may contribute to the aggressive nature of breast tumors. A mutant form of BRIT1, isolated from one human breast tumor specimen, lacked two C-terminal BRCT-domains of the protein. This shorter form of BRIT1 resulted in a loss of function with respect to DNA damage response when tested experimentally. Therefore, significant evidence exists to directly link defective or reduced BRIT1 protein expression to several forms of cancer and to implicate BRIT1 as a novel tumor suppressor gene.

### BRIT1 Function in DNA Damage Response

In the normal course of events, cellular DNA is subjected to a variety of endogenous and environmental factors that induce damage within its structure. In response to these insults, normal cells activate complex mechanisms to detect, signal the presence of, and subsequently repair DNA damage when possible. Propagation of the DNA damage alarm progresses through a complex signal transduction network that includes the BRIT1 protein. Initially, sensor proteins recognize the location of damaged or altered DNA structure and transmit a signal through mediator molecules to transducer proteins. The transducer proteins transmit the signal to numerous downstream effectors involved in specific pathways (Fig. 1). Two distinct DNA damage repair networks, both requiring BRIT1 activity, have been described. The ► ATM (ataxia telangiectasia mutated) pathway is activated by double-stranded breaks in the DNA observed after exposure to ionizing radiation while the ► ATR (ATM and Rad3-related) pathway is activated by prolonged presence of single-stranded DNA induced by either ultraviolet radiation or stalled DNA replication. ATM and ATR are essential kinases that are responsible for phosphorylating numerous transducer and effector proteins in the DNA damage network. BRIT1 colocalizes with numerous molecules associated with these two signaling networks including ►  $\gamma$ H2AX, MDC1, 53BP, ► NBS1, p-ATM, ATR, p-RAD17,

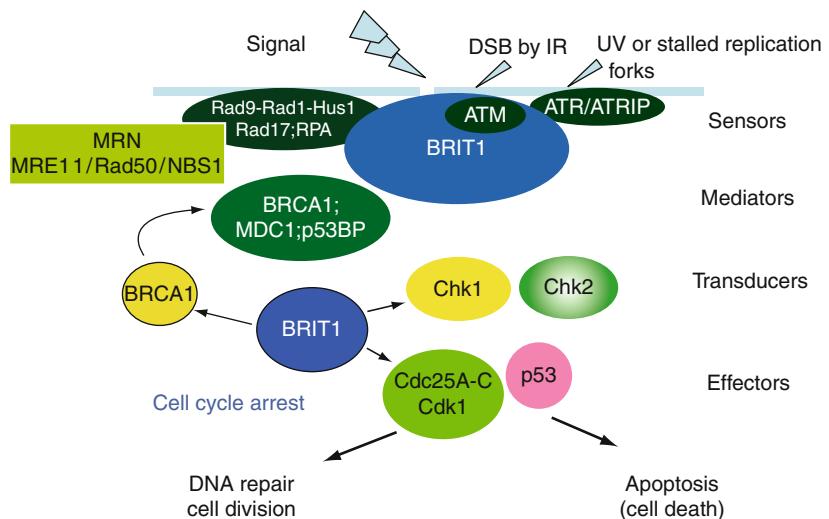
and p-RPA34 after DNA damage is induced. In the absence of functional BRIT1, all of these molecules with the exception of  $\gamma$ H2AX fail to localize to sites of DNA damage strongly suggesting that the BRIT1 molecule is an essential mediator for the subsequent repair processes. However, BRIT1 expression does not influence the chromatin binding of proteins unrelated to DNA damage such as Orc-2 indicating that the BRIT1 function is highly specific. The depletion of BRIT1 inhibits the recruitment of phosphorylated ATM to double-stranded DNA broken ends and subsequently blocks the phosphorylation of multiple down-stream members of the ATM repair pathway including Chk2 and NBS1. Depletion of BRIT1 also abolishes the UV-induced phosphorylation of RPA34 and reduces the levels of phosphorylated RAD17 indicating the importance of BRIT1 in the ATR signaling pathway. Based on these observations, it has been determined that BRIT1 is a pivotal protein in both of the DNA damage signaling networks and for this reason has great significance in the prevention neoplastic transformations of cells.

Figure 1 illustrates a simple model for the function of BRIT1 based on current experimental evidence. After exposure to ionizing radiation, double-stranded breaks in DNA occur resulting in the recruitment the MRE11/RAD50/NBS1 (MRN) complex and MDC1 to the damaged site thus facilitating the recruitment and kinase activity of ATM. Activated p-ATM phosphorylates NBS1, H2AX, and BRCA1 which localize to sites of DNA damage. Increased single-stranded DNA by exposure to ultraviolet radiation induces the coating of RPA on DNA leading to the recruitment of ATRIP and ATR to the sites of DNA damage. Activated ATR then phosphorylates critical downstream molecules such as Rad17 and Chk1 further propagating the DNA damage signal in the cell. BRIT1 appears to regulate the recruitment of NBS1, MDC1, and thus the MRN complex in the ATM pathway. BRIT1 also regulates the recruitment of RPA, which in turn recruits ATRIP/ATR complex initiating the ATR signaling cascade.

### BRIT1 Function in Cell-Cycle Control

Normally, the progression of a cell through the cell division cycle is stalled to allow for its DNA repair and if the damage cannot be repaired, the cell enters programmed cell death (apoptosis). This retardation

**BRIT1 Gene. Fig. 1** BRIT1 function in DNA damage and cell-cycle control



or cell-cycle checkpoint is essential to maintain the integrity of cellular DNA that insures normalcy in consecutive descendant cells. Key molecules involved in cell-cycle arrest, (p53, chk1 and chk2), are all activated when phosphorylated by ATM or ATR. BRIT1 clearly impacts the activity of both ATM and ATR by affecting the association of these molecules to damaged DNA. Activated p53 induces cell-cycle arrest at G1 while p-Chk1 and p-Chk2 negatively regulate Cdc25 phosphatases that promote transition through the cell cycle thereby inducing the execution of G1/S, G2/M and intra-S checkpoints of the cell cycle. BRIT1 is required for the activation of intra-S and G2/M cell-cycle checkpoints after cellular exposure to ionizing radiation. The influence of BRIT1 on control of these cell-cycle checkpoints may result from BRIT1's regulation of three checkpoint regulator proteins, Chk1, BRCA1, and NBS1. In the absence of BRIT1, BRCA1, and Chk1, expression is significantly reduced and NBS1 fails to be phosphorylated. BRCA1 plays a significant role in homologous recombination DNA repair and possibly serves as a scaffold for ATM and ATR thus affecting phosphorylation of many downstream effector proteins. Therefore the regulation of BRCA1 by BRIT1 dramatically affects multiple aspects of cell-cycle control and DNA damage repair.

The normal cellular response to ionizing radiation is to arrest the cell cycle also at G2, allowing for the initiation of DNA repair; however, BRIT1-depleted cells continue to progress through G2 indicating that BRIT1 is essential in the activation of this important

cell-cycle checkpoint. Additionally, BRIT1-depleted cells continue to synthesize DNA and proceed through mitosis unlike normal cells exposed to ionizing radiation. Replication of DNA damaged by ionizing radiation could easily result in the propagation of mutated or disrupted genes and contribute to tumorigenesis.

BRIT1 also controls the cell's entry into mitosis by affecting the stabilization of the cdc25A, a key phosphatase in cell-cycle control. Cells derived from a microcephaly patient (BRIT1 defective) maintained a persistent level of the phosphatase, cdc25A following UV treatment. Cdc25A is targeted for degradation when phosphorylated by Chk1 kinase during normal cell division and its degradation is usually amplified by UV exposure. Degradation of cdc25A abolishes the activation of the Cdk2-cyclin complex inhibiting DNA synthesis. Conversely, inappropriate persistence of cdc25A allows for the continued DNA synthesis despite aberrations or damage in the structure. These BRIT1 mutant cells also harbor reduced levels of phosphorylated Cdk1-cyclin B complex that is essential for mitotic entry. It was proposed that the regulation of mitosis by BRIT1 is both ATR dependent through regulation of cdc25A stability and ATR independent through regulation of Cdk1-cyclin B phosphorylation.

The affect of BRIT1 on cell-cycle control is therefore multifaceted (Fig. 1). Complete loss of the BRIT protein results in reduced protein levels of BRCA1 and Chk1 and impairs the activity of a multitude of vital proteins through their diminished phosphorylation.

Presence of a mutated BRIT1 protein allows for expression of BRCA1 and Chk1 but still blocks proper signal transduction by inhibiting the activities of both ATM and ATR kinases.

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## BRM

### Definition

Biological response modifier.

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## BRMS1

Alexandra C. Silveira<sup>1</sup> and Danny R. Welch<sup>2</sup>

<sup>1</sup>Department of Pathology and Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL, USA

<sup>2</sup>Department of Cancer Biology, Kansas University Cancer Center, Kansas City, KS, USA

### Definition

*BRMS1* (Breast Metastasis Suppressor 1) is a human ► metastasis suppressor gene that, when reexpressed, suppresses metastasis of human breast carcinoma,

ovarian, and melanoma cell lines in immunocompromised mouse models.

## Characteristics

The *BRMS1* gene is located on chromosome 11q13.1-q13.2. It spans over 8.5 kb and is comprised of ten exons, the first exon being untranslated. *BRMS1* cDNA is 1485 base pairs and encodes a 246 amino acid protein (MW ~ 28.5 kD, although it runs more slowly (~35 kDa) by SDS-PAGE). It is highly conserved with the mouse ortholog (*Brms1*) having 95% homology at the amino acid level. Like human *BRMS1*, the mouse ortholog has suppresses metastasis in murine models of breast cancer.

*BRMS1* protein contains two nuclear localization sequences, two coiled-coil motifs and imperfect leucine zippers. It also contains a glutamic acid rich N-terminus and a potential endoplasmic retention signal. *BRMS1* shows almost ubiquitous expression in human tissues; highest expression is in kidney, placenta, peripheral blood lymphocytes, and testis, and lowest expression in brain and lung. Subcellular fractionation and immunofluorescence studies have determined that *BRMS1* protein is predominantly (>90%) nuclear. *BRMS1* protein is stabilized by interaction with the chaperone protein, Hsp90, and is further regulated by proteasomal degradation.

### Cellular and Functional Characteristics

*BRMS1* reestablishes gap junctional cell–cell communication in human breast cancer cell lines. Studies using MDA-MB-435, MDA-MB-231, and the ovarian cancer cell line HO-8910 PM show an inverse effect of *BRMS1* expression on cell motility. Further, overexpression of *BRMS1* in H1299 human lung carcinoma cells and MDA-MB-435 cells results in suppressed growth in soft agar. Additionally, *BRMS1* transfection increases apoptosis in suspended non-small cell lung carcinoma.

The exact mechanism by which *BRMS1* affects these phenotypes is as yet unknown. However, it is known that *BRMS1* interacts with several different proteins and large (megadalton) protein complexes, most notably with class I and class II histone deacetylases (HDACs) and the transcription factor NFκB. *BRMS1* is

specifically a core member of mSin3-HDAC chromatin remodeling/transcriptional repression complexes, but its involvement is implicated in other HDAC complexes. Additionally, *BRMS1* and HDAC1 function as NF $\kappa$ B corepressors; chromatin-bound *BRMS1* facilitates HDAC-1-mediated deacetylation and inactivation of NF $\kappa$ B. Studies also suggest that direct interaction of *BRMS1* and the RelA/p65 subunit of NF $\kappa$ B represses the transactivation potential of NF $\kappa$ B. Further, *BRMS1* leads to a reduction in NF $\kappa$ B translocation by inhibiting the phosphorylation and degradation of the NF $\kappa$ B inhibitor, I $\kappa$ B.

Studies show reduced phosphoinositide signaling in *BRMS1* transfected cells. Decreased phosphoinositide signaling results in decreased mobilization of intracellular calcium, a known regulator of metastasis. *BRMS1* expression also downregulates fascin, an actin-bundling protein associated with cell motility. Further, repression of NF $\kappa$ B results in decreased expression of anti-apoptotic genes and two tumor-metastasis activators, osteopontin and urokinase-type plasminogen activator.

### Clinical Relevance

*BRMS1* is regulated at both the RNA and protein levels. To date, only a single study has examined levels of *BRMS1* protein in patient samples to find a loss of *BRMS1* in nearly 25% of 238 breast cancer cases. Further, the study showed loss of *BRMS1* correlated with disease-free survival when stratified by loss of estrogen receptor, progesterone receptor, or Her2 overexpression. Other clinical studies have studied *BRMS1* mRNA expression in human cancers compared to adjacent noncancerous tissues or regional lymph nodes. Since *BRMS1* is regulated at the protein level, looking exclusively at mRNA may be misleading. Nonetheless, the majority of studies show high levels of *BRMS1* correlates with increased disease-free survival and diminished progression.

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### BRN-5547136

- Temozolomide

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### Broder Histological Classification

#### Definition

Refers to histological classification of differentiation in ► squamous cell carcinoma. Devised by Broder. Grades 1, 2, and 3 denoted ratios of differentiated to undifferentiated cells of 3:1, 1:1, and 1:3, respectively. Grade 4 denoted tumor cells having no tendency toward differentiation.

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### Bromodeoxyuridine

#### Synonyms

- BrdU

#### Definition

A compound that, due to its chemical structure, can substitute for thymidine in DNA.

- Fragile Sites

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## Bromodomain

### Definition

Conserved domain that specifically recognizes and binds to acetylated lysine residues that occur within a protein.

► [Histone Modifications](#)

intestine and in the proximal tubules of the kidney. Microvilli are small projections of the plasma membrane which greatly enlarge the surface area of the cell. Individual microvilli can only be distinguished using an electron microscope; in a light microscope, the microvilli are observed collectively as a fuzzy fringe at the surface of the epithelium which has, therefore, been termed brush border.

► [Membrane Transporters](#)

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## Bronchogenic Carcinoma

► [Lung Cancer](#)

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## Brother of the Regulator of Imprinted Sites

► [BORIS](#)

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## Brown Adipose Tissue

### Definition

BAT is present in many newborn or hibernating mammals, and its primary function is to generate heat.

► [Cachexia](#)

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## Brp-39

► [Serum Biomarkers](#)

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## Brush Border

### Definition

Is formed by the densely packed microvilli of the surface of columnar epithelial cells, e.g., in the

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## Bryostatin-1

Bassel El-Rayes

Karmanos Cancer Institute, Wayne State University,  
Detroit, MI, USA

### Definition

Bryostatins are a class of macrocyclic lactones. Bryostatins are potent modulators of ► [Protein Kinase C \(PKC\)](#). Bryostatin-1 was isolated from the marine invertebrate *Bugula neritina*. Bryostatin-1 is currently not available for commercial use.

### Characteristics

#### Rationale for Targeting the PKC

PKC is a family of homologous serine/threonine protein ► [Kinases](#) that transduce signals linked to diverse cellular processes, including proliferation, differentiation, angiogenesis, and ► [apoptosis](#). The PKC family includes 12 isoforms subdivided into three major classes based on their cofactor requirements for activation. Aberrant regulation of the PKC enzymes activity has been demonstrated in a number of malignancies, including breast, colorectal, pancreatic, and non-small cell lung cancer.

#### Preclinical Activity of Bryostatin-1

Treatment of cancer cell lines with bryostatin-1 results in the activation of PKC. However, prolonged exposure to bryostatin-1 induces PKC inhibition most

probably through ubiquitin-mediated degradation. Inhibition of PKC activity results in cell cycle arrest, apoptosis, cell differentiation, and modulation of chemoresistance.

Bryostatin-1 has been shown to potentiate the effects of several classes of cytotoxic agents, including vincristine in diffuse large cell lymphoma, melphalan in Waldenstrom's macroglobulinemia, gemcitabine in pancreatic and breast cancer, and paclitaxel and mitomycin C in gastric cancer cell lines. The synergism between bryostatin and cytotoxic agents is sequence dependent.

### Single-Agent Activity of Bryostatin-1

Phase I trials of bryostatin-1 used two different schedules. The maximal tolerated doses were  $25 \mu\text{g}/\text{m}^2$  when infused over 24 h and  $120 \mu\text{g}/\text{m}^2$  when infused over 72 h. The most common side effects included ► **myalgia**. Other observed toxicities included headache, phlebitis, and transient ► **thrombocytopenia**.

Single-agent bryostatin-1 has been studied in phase II trials for lymphoma, renal, colorectal, head and neck, sarcoma, and melanoma. Bryostatin-1 did not demonstrate single-agent activity in any of these diseases.

### Bryostatin-Based Combinations

Since PKC activation contributes to chemoresistance, the combinations of bryostatin-1 and cytotoxic agents were tested. In Chronic Lymphocytic Leukemia (CLL) and indolent Non-Hodgkin Lymphoma, bryostatin-1 was evaluated in combination with fludarabine. Patients received fludarabine daily for 5 days and bryostatin-1 over 24 h infusion either before or after fludarabine. The combination was well tolerated. Partial and complete responses were observed in six and two patients (total number 27), respectively. Bryostatin-1 and vincristine was evaluated in patients with refractory B-cell lymphoma. Twenty four patients were enrolled on the study. The bryostatin-1 was well tolerated at a dose of  $50 \mu\text{g}/\text{m}^2$  over 24 h infusion. The regimen had activity with five patients having objective response and five having stable disease.

Bryostatin-1 was also evaluated in combination with cisplatin in two phase I trials. In the first trial, bryostatin-1 ( $30 \mu\text{g}/\text{m}^2$  over 24 h infusion) had no significant activity. In the second trial, bryostatin-1

was administered at a dose of  $15–55 \mu\text{g}/\text{m}^2$  over 72 h. In this study, three responses were reported. A phase I trial of gemcitabine and bryostatin-1 ( $25–35 \mu\text{g}/\text{m}^2$  over 24 h) revealed that the regimen was well tolerated and resulted in stable disease in 8 out of 36 patients. Bryostatin-1 ( $15–50 \mu\text{g}/\text{m}^2$  infused over 24 h) was evaluated in combination with paclitaxel. Partial responses were observed in patients with pancreatic and gastroesophageal cancer. The common finding in these trials is that bryostatin-1 can be combined safely with cytotoxic chemotherapy agents.

Phase II trials evaluating the activity of bryostatin-1 with cisplatin in cervical cancer had disappointing results. Fourteen patients were enrolled on the trial, and there were no treatment responses. Ajani et al. reported on 37 patients with gastroesophageal and gastric cancer treated with bryostatin-1 ( $40 \mu\text{g}/\text{m}^2$  infused over 24 h) and weekly paclitaxel ( $80 \text{ mg}/\text{m}^2$ ). The response rate was 29% which is higher than the previously reported response rates with paclitaxel. In a phase II trial, bryostatin-1 ( $50 \mu\text{g}/\text{m}^2$  infused over 24 h) and weekly paclitaxel ( $90 \text{ mg}/\text{m}^2$ ) were evaluated in patients with non-small cell lung cancer. Of 11 response evaluable patients, stable disease was seen in five patients. Therefore, the bryostatin-1 and paclitaxel combination did not demonstrate significant activity in lung cancer.

### Future Directions

Mixed results have been observed in the trials evaluating bryostatin-1 and cytotoxic chemotherapy agents. The future challenges in the development of bryostatin-1 include the identification of biomarkers that can predict activity and the development of combination therapy with other targeted agents. Another approach for the development of bryostatins is through the modification of the chemical structure in order to identify analogues with better safety or efficacy profiles than bryostatin-1.

### References

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3. Kortmansky J, Schwartz GK (2003) Bryostatin-1: a novel PKC inhibitor in clinical development. *Cancer Invest* 21:924–936

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## BSA

### Definition

Body surface area; the current practice of using body-surface area (calculated using a formula derived from height and weight) in dosing anticancer agents has been implemented in clinical oncology about half a century ago. By correcting for BSA, it was generally assumed that the interindividual variation in the pharmacokinetics of the drug administered would be reduced which would lower the risk of serious adverse effects without reducing the agent's therapeutic effect. Recently, doubt has arisen to this hypothesis, and for many anticancer drugs the rationale for individualization of dosage based on BSA is lacking.

- ▶ [Irinotecan](#)
- ▶ [Pharmacokinetics/Pharmacodynamics](#)

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## B-Scan Ultrasonography

### Definition

Ophthalmologic ultrasound provides a two-dimensional ultrasound image of the echogenicity of the ocular structures providing a cross-sectional view allowing for the diagnosis and characterization of multiple disorders including retinal and choroidal detachments, vitreous hemorrhages, vitritis, and intraocular tumors.

- ▶ [Uveal Melanoma](#)

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## BSF-2

- ▶ [Interleukin-6](#)

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## BTAK

- ▶ [Aurora Kinases](#)

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## BUB1

### Definition

Budding uninhibited by benzimidazoles 1 is a protein that is required for the spindle assembly checkpoint. BUB1 is a protein kinase; it phosphorylates CDC20 and inhibits ubiquitin ligase activity of APC/C. In mammals, BUB1 depletion causes embryonic lethality in mice.

- ▶ [Mitotic Arrest-Deficient Protein 1 \(MAD1\)](#)

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## Bulk Minerals

### Definition

Are mineral nutrients that are typically required to be ingested by humans in amounts of hundreds of milligrams to a few grams per day. This category includes calcium, phosphorus, and magnesium and, along with electrolytes, is sometimes referred to as macrominerals.

- ▶ [Mineral Nutrients](#)

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## Bulky

### Definition

A lymphoma is bulky if a nodal lymphoma mass with largest dimension of 10 cm or greater is present.

- ▶ [Diffuse Large B-Cell Lymphoma](#)

## Burkitt Lymphoma

### Definition

Burkitt lymphoma is caused by ► [Epstein-Barr virus](#) (EBV) and occurs mainly in sub-Saharan Africa.

## Burkitt Lymphoma Cell Lines

### Definition

Burkitt lymphoma cell lines are EBV-infected B cell lines established from Burkitt lymphoma biopsies; these cells are tumorigenic in nude mice.

- [BCL6 Translocations in B-Cell Tumors](#)
- [Epstein-Barr Virus](#)

## BWS

### Definition

- [Beckwith-Wiedemann Syndrome](#).

## Bystander Effect

### Definition

When cells are killed indirectly by virtue of neighboring cells that transfer toxic products to them.

- [HSV-TK/Ganciclovir Mediated Toxicity](#)