Philadelphia Chromosome–Positive Leukemias: From Basic Mechanisms to Molecular Therapeutics

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The Philadelphia chromosome translocation (t(9;22)) results in the molecular juxtaposition of two genes, *BCR* and *ABL*, to form an aberrant *BCR-ABL* gene on chromosome 22. *BCR-ABL* is critical to the pathogenesis of chronic myelogenous leukemia and a subset of acute leukemias. The chimeric Bcr-Abl protein has constitutively elevated tyrosine phosphokinase activity. This abnormal enzymatic activation is critical to the oncogenic potential of Bcr-Abl. Initially, protein kinases were thought to be poor therapeutic targets because of their ubiquitous nature and crucial role in many normal physiologic processes. However, the advent of imatinib mesylate (Gleevec, Novartis Pharmaceuticals, Basel, Switzerland), formerly known as STI571 and CGP57148B, demonstrated that designer kinase inhibitors could be specific. This agent has shown striking activity in chronic myelogenous leukemia. It also inhibits phosphorylation of Kit (stem-cell factor receptor) and platelet-derived growth factor receptor. In addition, it has shown similar impressive responses, with little host toxicity, in gastrointestinal stromal tumors, which harbor activating Kit mutations, and in tumors with activated platelet-derived growth factor receptor. The studies of imatinib mesylate provide proof-of-principle for using aberrant kinases as a therapeutic target and are a model for the promise of molecular therapeutics. This paper reviews the current knowledge on the function of Bcr-Abl and its normal counterparts (Bcr and Abl), as well as the impact of this knowledge on the development of a remarkably successful targeted therapy approach.

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For author affiliations, see end of text.
For definitions of terms, see Glossary on page 820.

The discovery of the molecular defect *BCR-ABL*, which results from the Philadelphia chromosome translocation, has laid the foundation for a targeted treatment approach to Philadelphia chromosome–positive leukemias. These leukemias are now considered a paradigm for the emerging field of molecular therapeutics (1, 2). For this review, we searched MEDLINE from 1966 to March 2003 by using the terms *Bcr-Abl*, *Philadelphia chromosome*, *chronic myelogenous leukemia*, *STI571*, and *imatinib mesylate*. We present a comprehensive overview of the molecular genetics of the Philadelphia chromosome translocation and the current state of the art of gene-directed therapy in Philadelphia chromosome–positive leukemias.

The Philadelphia Chromosome

The Philadelphia chromosome was the first consistent chromosome abnormality identified in cancer (3, 4). It is a shortened chromosome 22 that results from the reciprocal exchange of DNA between the long arms of chromosomes 9 and 22; breaks occur at positions q34 and q11 (t(q;22)(q34;q11)).

Phenotypic Heterogeneity of Philadelphia Chromosome–Positive Disease: Clinical Hints at Molecular Subtypes

The Philadelphia chromosome translocation is found in more than 90% of patients with chronic myelogenous leukemia (CML) and some persons with acute leukemia (**Table 1**). About 50% of the cases of Philadelphia chromosome–positive acute leukemia in adults or fewer are characterized by a molecular abnormality that cannot be distinguished from the abnormality found in CML. These patients may be experiencing CML blast crisis with a clinically asymptomatic and therefore undiagnosed chronic phase; the remaining patients harbor a molecular aberration that differs subtly from that in CML (5, 7, 11).

Clinical Features of Philadelphia Chromosome–Positive Leukemias

Chronic Myelogenous Leukemia

The natural history of CML involves inevitable evolution from the chronic phase to an accelerated and then blast phase (13, 18). The chronic phase is characterized by neutrophilic leukocytosis and is easily managed. Its control, however, does not prevent the relentless march toward blast crisis. The blast phase resembles aggressive acute leukemia (myeloid in two thirds of patients and lymphoid in one third of patients); patients generally die within 6 to 12 months.

The clinical heterogeneity of CML remains an enigma. For example, the median survival in recent years (before the advent of imatinib mesylate) has been about 5 to 6 years. Some patients, however, have an aggressive course from the outset and die within a year of diagnosis; other patients survive for 20 or more years. It is presumed that secondary genetic events supplant the effect of the Philadelphia chromosome translocation and drive blast crisis, but it is not known whether the tempo of these events is predetermined or random.

Acute Leukemia

The Philadelphia chromosome is discerned in a subset of patients with acute lymphoblastic leukemia (ALL) and, although rare, in patients with acute myelogenous leukemia (AML). Acute lymphoblastic leukemia is characterized by uncontrolled growth of immature lymphoid cells in bone marrow, blood, lymphoid organs, and extramedullary sites, as well as by pancytopenia. The presence of the Philadelphia chromosome predicts a high incidence of induction failure and relapse after chemotherapy.

Glossary

Apoptosis: Programmed cell death.
Arg: Abl-related gene.
ATM: The gene characterizing ataxia telangiectasia, a disease in which there is susceptibility to damage by gamma radiation because of a DNA repair defect.
Exon: A segment of DNA that is represented in the messenger RNA product.
G protein: Guanosine triphosphate–binding protein that functions in intracellular signaling.
Guanosine triphosphate–activating proteins (GAPs): inactive G proteins involved in intracellular signaling.
Guanine nucleotide exchange factors (GEFs): activate G proteins involved in intracellular signaling.
Kinase: An enzyme that transfers the terminal phosphate from adenosine triphosphate to a substrate; kinases regulate diverse cellular processes.
Kit: Stem-cell factor receptor.
Meiosis: A special type of cell division by which eggs and sperm cells are produced.
Phosphorylation: Adding a phosphate group; phosphorylation often serves to activate a substrate.
SH2 domain: Src homology-2 domains; highly conserved noncatalytic regions of about 100 amino acids that bind SH2-binding sites consisting of 3 to 5 amino acids, including a phosphotyrosine.
Splicing: Process of excision followed by rejoining.
Transcription: Process by which DNA is turned into RNA.
Transformation: Alteration in a cell that results in cancer-like features.
Xeroderma pigmentosum B (XPB): A disorder characterized by a defect in DNA repair after ultraviolet light exposure.

Molecular Basis of the Philadelphia Chromosome Translocation

The molecular genetics of the Philadelphia chromosome translocation is now known (**Table 2**) (**Figure 1**) (7, 8, 19–25). The t(9;22) anomaly leads to an exchange of DNA between chromosomes 9 and 22. The 3′ part of the *ABL* gene (3′ is the end of the gene, where transcription [synthesis of RNA] ceases) is moved from chromosome 9 (its normal position) to chromosome 22 and is juxtaposed to the proximal segment of the disrupted *BCR* gene on chromosome 22. The result is a chimeric *BCR-ABL* gene.

The breaks in the *BCR* gene on chromosome 22 vary. In CML, they most often occur centrally, that is, between exons 12 and 16 (also known as exons b1 to b5), in a region designated as the major breakpoint cluster region (M-bcr). Exons are segments of genes that are represented in the messenger RNA product. However, in a small subset of patients, a more distal region (between exons 19 and 20; the micro-bcr) is disrupted. In contrast, in ALL, about 50% of patients with Philadelphia chromosome–positive disease have breaks within the central M-bcr; the remainder have more proximal breaks, just distal to the first exon of *BCR* (in the minor-bcr [m-bcr]). As a result of these variable breakpoints as well as promiscuous alternative splicing (excising and rejoining) between *BCR* and *ABL* exons, different amounts of DNA from *BCR* are joined to *ABL* exons 2 to 11 (20) (**Figure 1**). Therefore, breaks in m-bcr join only the first exon of *BCR* to the entire *ABL* gene from exon 2 to the end of the gene (e1–a2 junction), breaks in M-bcr join all of *BCR* up to exons 13 or 14 (also known as exon b2 or b3 of M-Bcr) to *ABL* (again, the entire gene from exon 2 to the end) (b2–a2 or b3–a2 junction), and breaks in micro-bcr join all of *BCR* up to exon 19 to *ABL* (exons 2 to 11) (e19–a2 junction). As a result, Bcr-Abl proteins are sized at 190, 210, and 230 kDa, respectively (**Figure 1**). Hence, the smallest Bcr-Abl protein ($p190^{Bcr\text{-}Abl}$) contains less of Bcr than does the larger Bcr-Abl protein $p210^{Bcr\text{-}Abl}$; $p230^{Bcr\text{-}Abl}$ contains a still larger segment of Bcr. All harbor the same amount of Abl. Subtle differences in the biological effects of the various Bcr-Abl proteins may be crucial to disease phenotype.

Implications of Molecular Studies in Philadelphia Chromosome–Negative CML and Acute Leukemia

Approximately 5% to 10% of patients with CML lack the Philadelphia chromosome translocation. However, subchromosomal analysis has shown that the molecular fingerprint of CML (*BCR-ABL*) is present in about half of these patients (10, 14, 16, 17). Similarly, about 10% of patients with Philadelphia chromosome–negative ALL harbor the *BCR-ABL* gene (15). Patients with CML who are *BCR-ABL* positive have an identical clinical course and response to therapy, regardless of the presence of the Philadelphia chromosome (16, 17). Some patients with CML are *BCR-ABL* negative, but their clinical course is distinct from that of their *BCR-ABL*–positive counterparts, mostly because progression of disease is rarely manifested by blast crisis (10, 14).

Table 1. **Frequency of the Philadelphia Chromosome, $p210^{Bcr\text{-}Abl}$, and $p190^{Bcr\text{-}Abl}$ in Leukemia***

Disorder	Ph Chromosome–Positive Patients	Ph Chromosome–Positive Patients Expressing $p210^{Bcr\text{-}Abl}$	Ph Chromosome–Positive Patients Expressing $p190^{Bcr\text{-}Abl}$	Ph Chromosome–Negative, Bcr-Abl–Positive Patients	Ph Chromosome–Negative, Bcr-Abl–Positive Patients Expressing $p210^{Bcr\text{-}Abl}$	Ph Chromosome–Negative, Bcr-Abl–Positive Patients Expressing $p190^{Bcr\text{-}Abl}$
	← % →					
CML†	90–95	>99	<1	5	100	Rare
Adult ALL	20	50–80	20–50	10	~50	~50
Pediatric ALL	5	10	90	Not known	Not known	Not known
AML	2	50	50	Rare	Not known	Not known

* Percentages are approximate. ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; CML = chronic myelogenous leukemia; Ph = Philadelphia. The information contained in this table is summarized from references 5 to 17.
† Although rare, some patients express both $p210^{Bcr\text{-}Abl}$ and $p190^{Bcr\text{-}Abl}$ (12).

Table 2. **Molecular Features of *BCR*, *ABL*, and *BCR-ABL****

Feature	*BCR*†	*ABL*‡	*BCR-ABL*
Chromosomal location	22q11	9q34	22q11
Gene size, *kb*	130	230	Variable
Exons, *n*	23	11	Variable
messenger RNA, *kb*	4.5 and 7.0	6.0 and 7.0	7.0, 8.5, and 10
Molecular weight of major proteins, *kDa*	130 000 and 160 000	145 000	190 000, 210 000, and 230 000

* kb = kilobase; kDa = kilodalton.
† Also contains alternative exons 1′ and 2′ within the first intron.
‡ Two alternative first exons exist (exons 1a and 1b).

Is the Philadelphia Chromosome Translocation a Random Event?

Exposure to ionizing radiation is a risk factor for CML, and *BCR-ABL* fusion transcripts can be induced in hematopoietic cells by exposure to radiation in vitro (26). However, the occurrence of the translocation may not be a totally stochastic event. Indeed, the physical distance between the *BCR* and *ABL* genes in human lymphocytes and $CD34^+$ progenitor cells is shorter than might be expected by chance (27), and such proximity may favor a translocation event. The physiologic function of this proximity is not known.

BCR-ABL Can Be Detected in Normal Persons: Implications for Leukemogenesis

Recent intriguing studies have discerned *BCR-ABL* fusion transcripts at very low frequency in the blood of many healthy persons (28). The corollary to this observation is that the presence of the *BCR-ABL* translocation alone may not be sufficient to cause leukemia. Possible pathogenic cofactors include a defect in immunosurveillance or a second genetic aberration. Alternatively, the stage of differentiation of the cell harboring the *BCR-ABL* may be critical to the development of disease. Furthermore, the presence of *BCR-ABL* in normal persons raises critical questions about the assessment of minimal residual disease in leukemic patients with this aberration.

The *ABL* Gene

Forms of Abl

Viral and Cellular ABL

The cellular *ABL* gene is the human homologue of the viral *ABL* (v-*ABL*) oncogene carried by the Abelson murine leukemia virus (29, 30). Viral *ABL* originates from cellular *ABL* (c-*ABL*). Presumably, at some point in evolution, the Abelson murine leukemia virus incorporated the mammalian *ABL* gene (30).

Abl Protein

Human Abl is a ubiquitously expressed 145-kDa protein with two isoforms (30). In hematopoietic cells, steady-state levels of Abl decrease with myeloid maturation (31). Abl functions as a nonreceptor tyrosine kinase enzyme (see Glossary) with protean biological effects (**Figure 1**) (32–47).

Subcellular Location of Abl

While Bcr-Abl is found exclusively in cytoplasm, surprisingly (for a tyrosine kinase enzyme), Abl can shuttle between the nucleus, where it can bind DNA, and the cytoplasm, where it binds the actin cytoskeleton (42). In primary human hematopoietic cells (31) and neurons (48), Abl is more cytoplasmic than nuclear.

Biology of Abl

Cytoplasmic functions of Abl include signaling and cytoskeletal molding; nuclear Abl has been implicated in regulation of the cell cycle (42) and in genotoxicity (49). Abl also has DNA binding capacity of uncertain significance.

Abl Tyrosine Kinase Enzymatic Activity

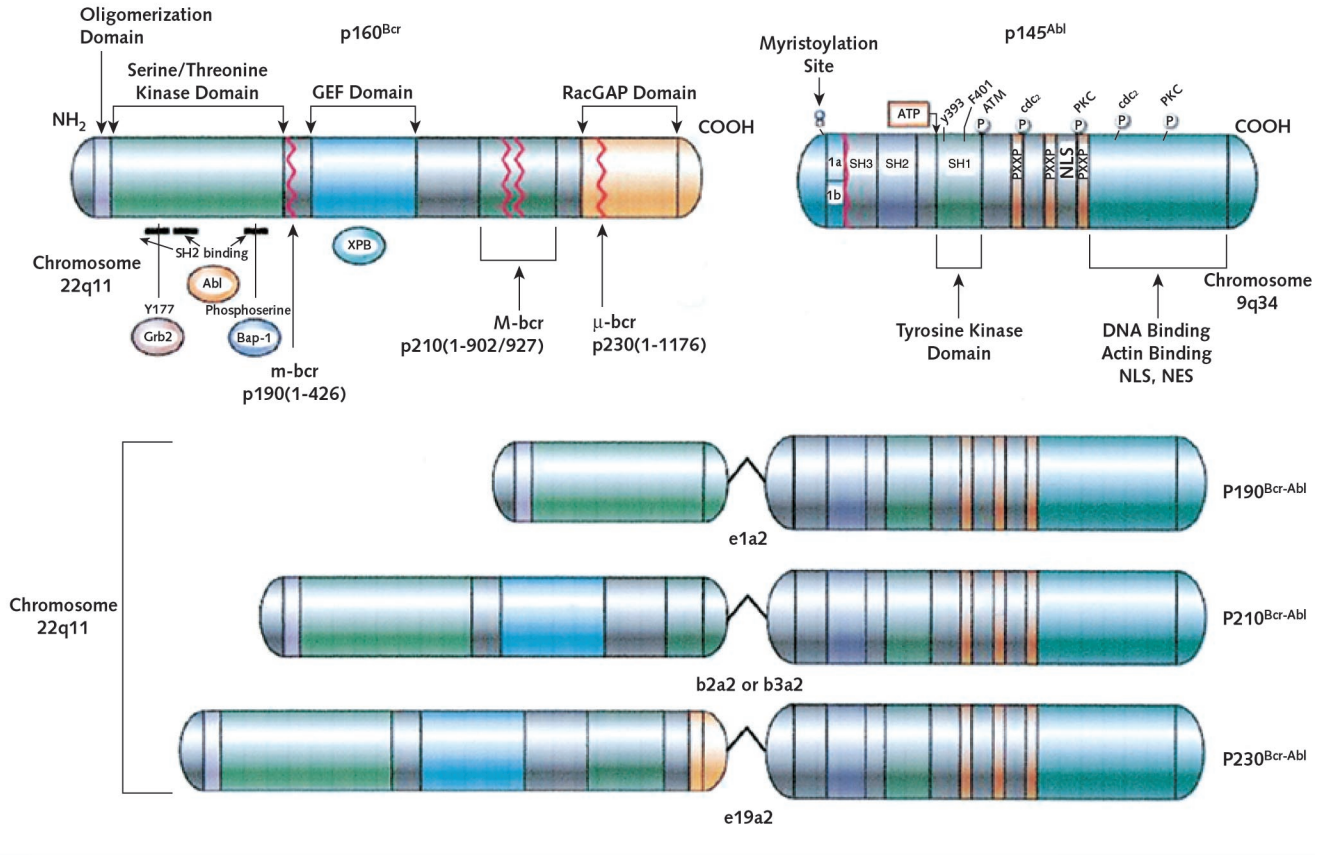
Tyrosine kinases are enzymes that phosphorylate (add a phosphate group) to a tyrosine in a substrate. They have a catalytic domain, which promotes the transfer of the terminal phosphoryl group from adenosine triphosphate (ATP) to a tyrosine amino group acceptor in a substrate (or they may autophosphorylate). Normal Abl phosphorylation (see Glossary) is tightly controlled (42), probably by motifs in the *N*-terminal. Loss of this region (as occurs in the formation of *BCR-ABL*) results in high constitutive kinase enzymatic activity, a key factor in the oncogenic potential of transforming Abl proteins (50–53).

Other Properties: Impact on Cytoskeleton, Cell Cycle, and DNA Repair

Abl influences the cytoskeleton locally, and, in turn, Abl kinase activity is modified by outside-in cellular signals (43, 46, 47, 54). Most cytoplasmic Abl is associated with filamentous actin, a building block of the cellular cytoskeleton (46). Abl also interacts with cell-cycle regulatory genes at several checkpoints, thereby affecting cellular proliferation (41, 42). Both positive and negative regulatory effects have been reported, depending on the cell-cycle phase studied.

Abl has DNA-binding activity, which may be involved in initiating transcription of DNA to RNA, in DNA damage response, and in meiotic processes (see Glossary) (33, 55, 56). A role for Abl in DNA repair has been suggested by its interaction with other molecules involved in this process, such as the ATM gene product. Mutation of the ATM gene product causes ataxia telangiectasia, a disorder characterized by hypersensitivity to radiation damage (57–59).

Figure 1. **The normal Bcr and Abl proteins and the various aberrant Bcr-Abl counterparts.**



Functional sites in the Bcr protein include a serine and threonine kinase domain in exon 1, a central guanine exchange factor (*GEF*) domain, and a carboxy-terminal guanosine triphosphatase–activating protein (*GAP*) domain. Src homology-2 (*SH2*)–binding sites are also present in exon 1. The Bcr-associated protein (Bap-1) interacts with the more distal of these sites. Growth factor receptor–bound protein 2 (*Grb-2*) associates with the proximal SH2-binding site containing a phosphotyrosine in position 177. Abl interacts with the second and third SH2 binding sites. The GEF domain interacts with the xeroderma pigmentosum B (*XPB*) DNA repair protein. The normal Abl protein contains three SH domains near the *N*-terminal. The tyrosine at position 393 (*Y393*) is the major site of autophosphorylation within the kinase domain. Phenylalanine 401 (*F401*) is highly conserved in protein tyrosine kinases containing SH3 domains. The central area of the protein has proline-rich regions (*PXXP*) capable of binding to SH3 domains and a nuclear localization signal (*NLS*). The carboxy-terminus contains DNA as well as G- and F-actin–binding domains, a nuclear export signal (*NES*), and nuclear localization signals. The phosphorylation sites by *Atm*, *cdc2*, and protein kinase C (*PKC*) are depicted. At the bottom of the figure, various Bcr-Abl proteins and their junction breakpoints are shown. Ragged red lines indicate breakpoints in Bcr and Abl.

THE *BCR* GENE

BCR is situated on the long arm of chromosome 22 (22q11) (**Table 2**). It is translated into two major proteins that have molecular weights of 160 000 and 130 000 kDa (60, 61). Similar to the situation with Abl, Bcr protein levels decrease with myeloid maturation in hematopoietic cells (31).

Subcellular Location of Bcr

Like the Abl protein, the normal Bcr protein resides in both the cytoplasmic and nuclear compartments (31, 62–64). In the nucleus, Bcr associates with condensed DNA in both interphase and metaphase (63).

Biology of Bcr

The *BCR* gene is a complicated molecule with many different functional motifs. It is implicated in the two major signaling pathways in eukaryotes (phosphorylation and guanosine triphosphate [GTP] binding) (65–76).

The first exon of the *BCR* gene is pivotal to oncogenesis. It is the one exon of *BCR* included in all known Bcr-Abl fusion proteins (73, 74). Bcr has serine and threonine kinase enzymatic activity in its first exon. It can phosphorylate itself as well as key substrates and, hence, propagates cellular signals. Several Src homology-2 (SH2)–binding domains are also in the first exon of *BCR.* SH2 domains are highly conserved, noncatalytic regions of 100 amino acids that bind SH2-binding sites consisting of 3 to 5 amino acids, including a phosphotyrosine. This interaction is important in the assembly of signal transduction complexes (75).

Bcr also interacts with or has homology to G proteins (see Glossary) at multiple levels (69, 71). These proteins are essential players in intracellular signaling, cytoskeletal organization, cell growth, and normal development. G proteins cycle between an inactive guanosine diphosphate

(GDP)–bound state and an active GTP-bound state. Homeostasis within this process is regulated by guanosine triphosphatase (GTPase)–activating proteins (which turn off G proteins) and guanine nucleotide exchange factors (which turn on G proteins) (see Glossary). Bcr has both GTPase-activating protein and guanine nucleotide exchange factor functions, suggesting a dichotomous role for this molecule in G protein–associated signaling pathways. Finally, Bcr (and $p210^{Bcr\text{-}Abl}$) interact with the xeroderma pigmentosum gene product (70, 76). Xeroderma pigmentosum is an inherited disorder whose hallmark is increased sensitivity to sunlight coupled with a defect in the DNA damage response process. Therefore, Bcr may also participate in DNA repair.

Association of Bcr with Normal Abl and with Bcr-Abl

Bcr binds to SH2 domains of normal Abl and can form complexes with Bcr-Abl (77). The result of interaction between Bcr and Bcr-Abl may be functional feedback regulation (74).

THE BIOLOGY OF *BCR-ABL*

$p210^{Bcr\text{-}Abl}$ and $p190^{Bcr\text{-}Abl}$ are pleiotropic molecules with many qualitatively similar activities; their differences are still being unraveled. Of interest, current studies suggest that not only is $p210^{Bcr\text{-}Abl}$ critical to the development of the chronic phase of CML, but its effect on the DNA repair process may also be responsible for genomic instability and, hence, disease progression (**Table 3**, **Figures 2** and **3**).

Kinase Activation

Tyrosine kinase enzymatic activity is central to cellular signaling and growth, and constitutively elevated kinase activity has been associated with transformation in several systems. The Abl protein is a nonreceptor tyrosine kinase whose enzymatic activity is under close physiologic control (22). In contrast, Bcr-Abl proteins are constitutively active tyrosine kinases. The degree of transforming activity of Bcr-Abl correlates with the degree of tyrosine kinase activity (90). $p190^{Bcr\text{-}Abl}$, which has higher tyrosine kinase activity, is therefore associated with the development of the more aggressive acute leukemia phenotype, while $p210^{Bcr\text{-}Abl}$ plays a role in the more indolent chronic leukemia phenotype.

Ras Signaling

$p210^{Bcr\text{-}Abl}$ and $p190^{Bcr\text{-}Abl}$ execute their transforming capabilities at least in part via activation of Ras, a vital protein in the intracellular signaling pathway (94, 95). Ras can also be aberrantly activated by mutation, a common event in tumorigenesis. The mechanisms by which Bcr-Abl interact with the Ras pathway are complex and include various adaptor and docking proteins.

Adhesion Molecules

Chronic myelogenous leukemia is clinically characterized by premature bone marrow release of progenitor cells, a phenomenon that may be attributed to defects in the adhesion properties of these cells. The effects of Bcr-Abl on specific intracellular substrates may cause an alteration in cytoskeletal structure, with subsequent inside-to-outside perturbation of adhesion molecules (47, 86, 96).

Table 3. **Biological Properties of $p210^{Bcr\text{-}Abl}$ and $p190^{Bcr\text{-}Abl}$***

$p210^{Bcr\text{-}Abl}$
Has constitutively activated tyrosine kinase enzyme
Can bind cytoskeletal actin
Expression decreases with myeloid differentiation
Complexes with tyrosine phosphoprotein Crk1, an SH2/SH3 adaptor protein
Interacts with docking protein $p62^{Dok}$
Attenuates programmed cell death
Is linked to Jak-Stat signaling pathway (especially Stat5)
Is linked to phosphotidylinositol 3-kinase/Akt signaling pathway
Activates Jun kinase
Interacts with and regulates DNA repair proteins
Has transforming activity partially mediated via Ras signaling pathway
Induces alterations in adhesion properties
Alters expression and phosphorylation of Ship protein (which is involved in myelopoiesis)
Interacts with Kit (stem-cell factor receptor) and IL-3 receptor β(c) subunit
Upregulates production of IL-3
$p190^{Bcr\text{-}Abl}$
Has constitutively activated tyrosine kinase enzyme
Attenuates programmed cell death
Is linked to Jak-Stat signaling pathway (especially Stat5)
Is linked to phosphotidylinositol 3-kinase/Akt signaling pathway
Has transforming activity mediated through Ras signaling pathway
Induces ubiquitin-dependent degradation of the Abl tyrosine kinase inhibitor, Abelson interacting protein
May induce alterations in adhesion properties

* IL-3 = interleukin-3; SH2/SH3 = Src homology-2/-3. The information contained in this table is summarized from references 23, 31, 35, 40, and 78–99.

Programmed Cell Death (Apoptosis)

Bcr-Abl–induced survival enhancement may be mediated by modulating proteins, such as Bcl-2, which suppresses programmed cell death, or Bad, which promotes programmed cell death. Of note, several studies show that *BCR-ABL*–positive cell lines are resistant to programmed cell death induced by DNA damage (98).

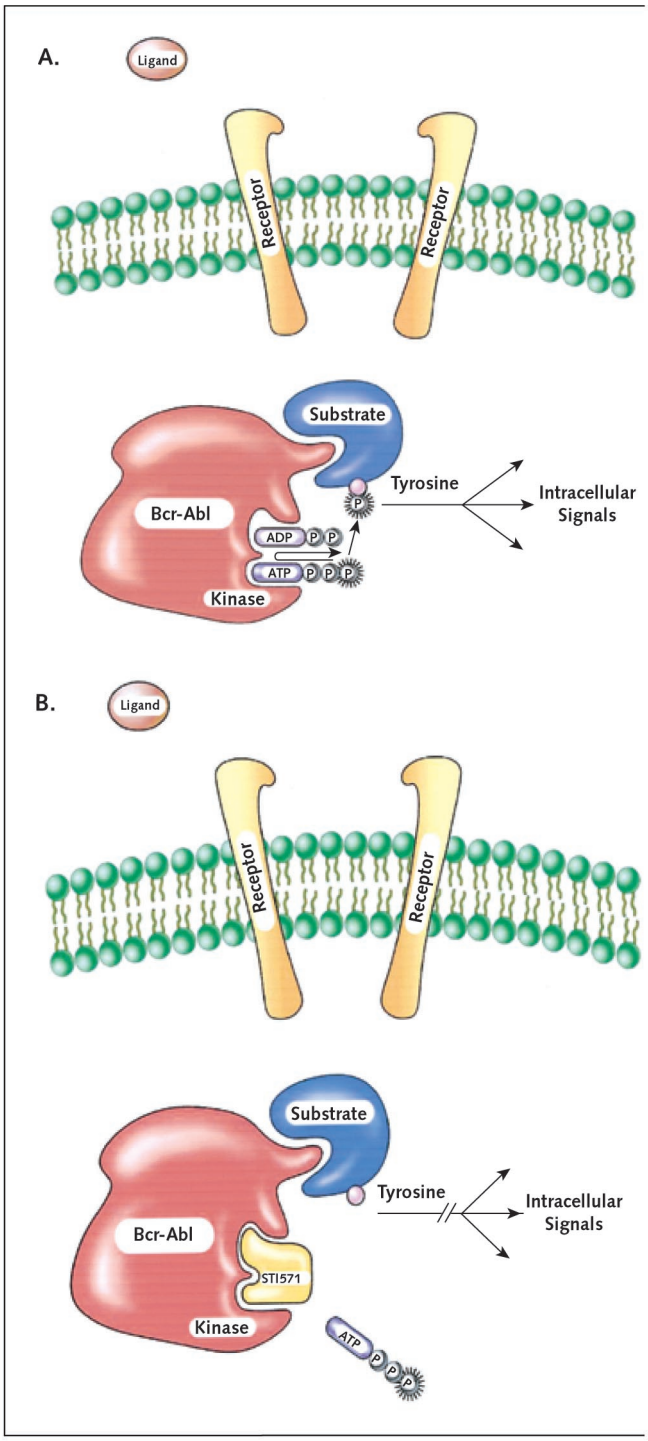
Growth Factor Independence

Bcr-Abl can abrogate growth factor dependence. Several mechanisms may be operative, including activation of intracellular signaling molecules (such as signal transducer and activator of transcription) (92), interaction with growth factor receptors (for example, receptors for interleukin-3 and stem-cell factor [81, 88]), and enhanced expression of growth factors themselves (interleukin-3 or granulocyte colony-stimulating factor) (89).

DNA Repair

Bcr-Abl affects the DNA damage response process in diverse ways (70, 84). It interacts with xeroderma pigmentosum B (XPB) gene product and increases radiosensitivity. It also enhances DNA double-strand break repair and, hence, resistance after drug therapy. Altered DNA repair may lead to subtle genetic errors, which manifest as clonal evolution and progression to blast crisis.

Figure 2. **The mechanism of action of imatinib mesylate.**



A. The Bcr-Abl tyrosine phosphokinase enzyme is constitutively active. Adenosine triphosphate (*ATP*) is an energy molecule used to drive Bcr-Abl enzymatic function. The enzyme's tyrosine kinase function is carried out at the kinase pocket. Bcr-Abl binds ATP and transfers phosphate from ATP to tyrosine residues on its substrates, thereby transmitting intracellular signals independently of ligand binding to growth factor receptors, such as that for interleukin-3. **B.** When imatinib mesylate (STI571) occupies the kinase pocket, it blocks the action of ATP, thereby suppressing phosphorylation of downstream effector molecules. ADP = adenosine diphosphate.

Animal Models

Animal studies provide cogent support for the oncogenic potential of *BCR-ABL* (100, 101). Several fundamental observations can be summarized. First, *BCR-ABL* alone seems to be sufficient to initiate leukemogenesis. Second, the cell type targeted may be a critical factor in determining the type of leukemia formed. Finally, as might be expected, the 210-kDa Bcr-Abl variant found in human CML induces leukemia less efficiently than the 190-kDa Bcr-Abl protein associated with the more aggressive human acute leukemias.

Therapeutic Implications

Initially, the thrust of therapy for CML was to control the high leukocyte count of the chronic phase, which was mostly cosmetic therapy. It did not eliminate the karyotypically abnormal clone or prevent the genomic instability that inevitably led to blast crisis. Therefore, the goal of newer therapies has been to eradicate the cells carrying the Philadelphia chromosome.

Most recently, approaches that target treatment of malignancy at the molecular level have been identified. The relatively specific *BCR-ABL* tyrosine protein kinase inhibitor imatinib mesylate (Gleevec, Novartis Pharmaceuticals, Basel, Switzerland) (originally known as STI571 and CGP57148B) targets the enzymatic activity of the Bcr-Abl protein (**Figure 2**). Early results suggest striking efficacy with little toxicity.

While the presence of an aberrant activated tyrosine protein kinase (Bcr-Abl) in CML made an appealing target for therapy, research floundered initially because of the ubiquitous effects of tyrosine protein kinases on cellular signal transduction pathways. The ATP-binding cleft, found in all members of this enzyme class, was believed to be a poor target for therapy until the advent of designer protein kinase inhibitors clearly demonstrated that selectivity was attainable (102).

Imatinib mesylate occupies the nucleotide-binding pocket of the Bcr-Abl protein and blocks access to ATP, thereby preventing phosphorylation of any substrate (102). It is not totally selective. Imatinib mesylate also blocks the kinase activity of stem-cell factor receptor (Kit), the platelet-derived growth factor (PDGF) receptor, Abl, and Arg (see Glossary) but has little effect on other kinases. Preclinical studies in which imatinib mesylate demonstrated potent growth inhibitory effects against Philadelphia chromosome–positive leukemias, both in vitro and in vivo (103), led to clinical trials (1, 104–111).

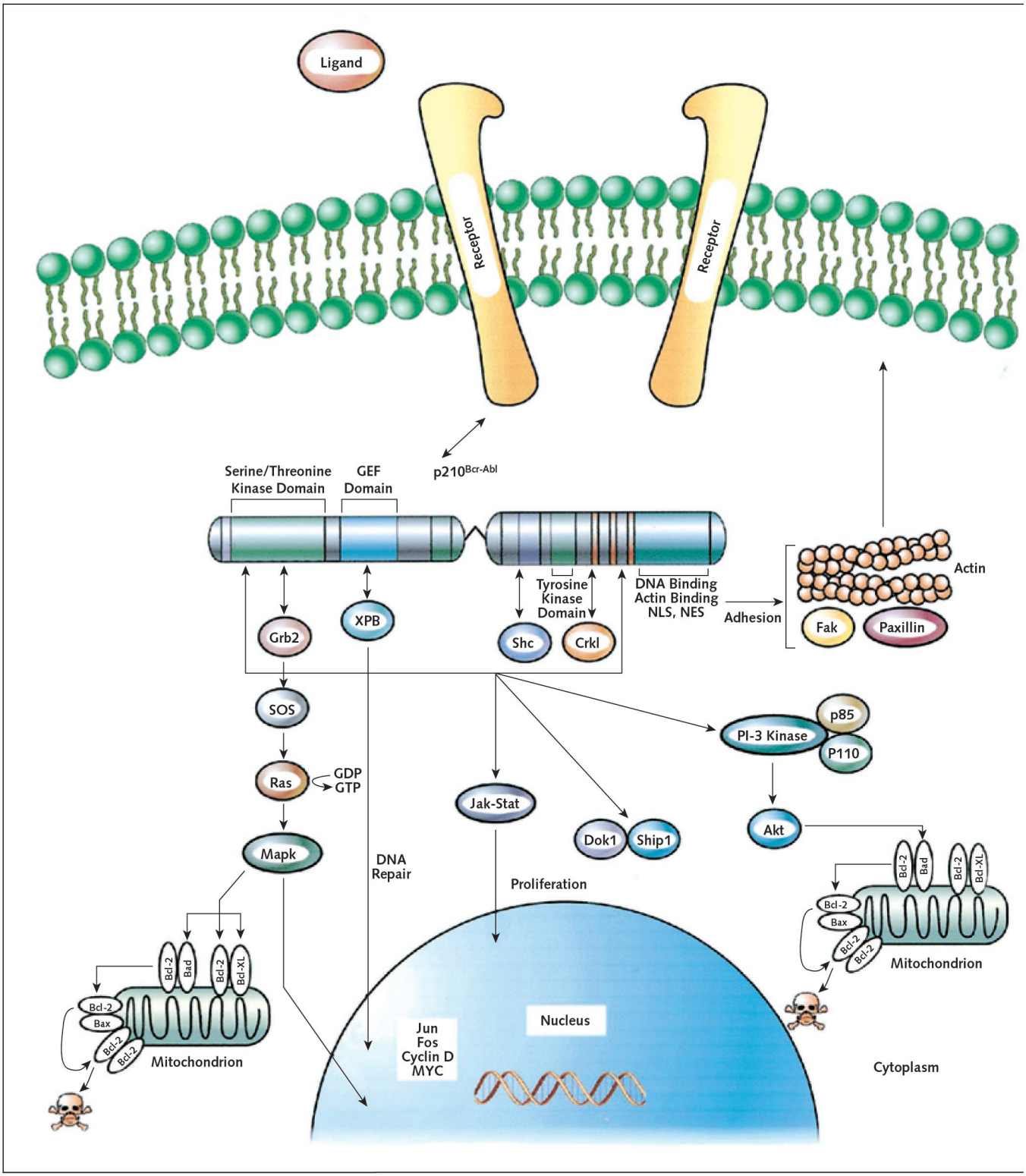
Clinical Trials in Human Disease

Bcr-Abl–Positive Leukemia

Chronic-Phase CML

A series of trials with imatinib mesylate have shown important therapeutic principles (**Table 4**). In the first study of patients with chronic-phase CML who had not responded to interferon-α, a daily oral dose of imatinib

Figure 3. **p210-encoding Bcr-Abl signaling pathways.**



Bcr-Abl interacts with the interleukin-3 receptor β(c) subunit and constitutively induces its phosphorylation. Downstream signaling occurs independently of ligand binding. Adaptor molecules connect Bcr-Abl to Ras and PI-3 kinase pathways; to focal adhesion complexes (affected molecules include focal adhesion kinase [*Fak*], paxillin, and actin cytoskeleton); and to messenger systems, such as Jak-Stat (Janus kinase signal transducer and activator of transcription) kinases. Downstream effectors involve mitogen-activated protein kinases (*MAPKs*) and survival proteins interacting with the Bcl-2 family. GDP = guanosine diphosphate; GEF = guanine exchange factor; GTP = guanosine triphosphate; NES = nuclear export signal; NLS = nuclear localization signal; PI-3 = phosphatidylinositol-3; SOS = son of sevenless; XPB = xeroderma pigmentosum B.

Table 4. **Response of Philadelphia Chromosome–Positive Disease to Imatinib Mesylate***

Stage and Status of Disease	Complete Hematologic Remission, *n/n* (%)†	Complete Cytogenetic Remission, *n/n* (%)	Comment	Reference
Chronic-phase CML; interferon-α failure	53/54 (98)	71/54 (13)	Dosages ≥300 mg/d, orally; results shown are early; full responses not yet evident	104
Chronic-phase CML; interferon-α failure	430/454 (95)	181/454 (44)	Dosage, 400 mg/d	105
Chronic-phase CML; no previous therapy	522/553 (94)	382/553 (69)	Randomized trial of imatinib mesylate vs. interferon-α plus cytosine arabinoside	109
Accelerated-phase CML	61/181 (34)	30/181 (17)	Dosage, 400 or 600 mg/d	106
Lymphoid blast crisis; Philadelphia chromosome–positive ALL	4/20 (20)	2/20 (10)		
			Median response duration, 3 mo for CML lymphoid blast crisis and ALL; 6 mo for CML myeloid blast crisis	1
Myeloid blast crisis	4/38 (11)	3/38 (7)		
Myeloid blast crisis	16/229 (7)	15/229 (7)	Median response duration, 8.3 mo; higher responses to imatinib mesylate at 600 mg/d than to 400 mg/d	107
Myeloid and lymphoid blast crisis	16/75 (21)	5/75 (7)	Seven patients (9%) reverted from blast phase to chronic phase	108

* Results of selected, representative studies are given. ALL = acute lymphoblastic leukemia; CML = chronic myelogenous leukemia.
† Refers to remissions sustained for at least 4 weeks.

mesylate at 300 mg or more led to a complete hematologic remission rate of 98% (104) (**Table 4**). Responses were evident within days, and hematologic remission, achieved within 4 weeks, was durable. In a large phase II study of 454 patients who had also not responded to interferon-α and who were then treated with 400 mg of imatinib mesylate daily, 44% achieved a complete cytogenetic response (105). Cytogenetic response was often evident as early as 3 months after initiation of therapy. Only 2% of patients discontinued treatment as a result of toxicity.

When imatinib mesylate was given as first-line therapy, the results were even more impressive (109). Ninety-four percent of patients ($n = 553$) achieved complete hematologic remission and 69% had a complete cytogenetic response. The effect of imatinib mesylate on survival has not yet been definitively ascertained, but previous studies of patients with CML treated with interferon have shown that survival is prolonged when cytogenetic remission is achieved. Furthermore, 12-month progression-free survival was 97% for patients treated with imatinib mesylate. In the front-line trial referred to earlier, patients had been randomly assigned to receive imatinib mesylate or a combination of interferon-α and cytosine arabinoside (previously considered first-line therapy for CML). In patients receiving the combination therapy, responses were poor; only 7% achieved a complete cytogenetic response. Finally, at 6 and 9 months of follow-up, 15% to 30% of patients with newly diagnosed CML who were treated with imatinib mesylate are showing molecular remissions (elimination of the Bcr-Abl anomaly, as determined by highly sensitive polymerase chain reaction techniques) (111).

These observations have led many physicians to use imatinib mesylate as front-line therapy for chronic-phase CML. Complete hematologic remission is expected by 3 months and major (>65% diploid metaphases) or complete cytogenetic response by 12 months. In patients who do not achieve these milestones, the imatinib mesylate dose can be increased or a different treatment strategy may be considered, such as therapy with interferon-α plus cytosine arabinoside, allogeneic stem-cell transplantation, or entry into a clinical trial with a new agent.

Advanced CML and Philadelphia Chromosome–Positive ALL

Surprisingly, even patients with more advanced disease respond to imatinib mesylate. This agent achieved complete hematologic responses lasting at least 4 weeks in 34% of patients with CML in accelerated phase ($n = 181$) (106) and led to a complete cytogenetic response in 17% of patients. Higher dosages of imatinib mesylate (600 mg/d vs. 400 mg/d) were associated with longer time to disease progression in multivariate analysis.

Blast crisis is believed to be driven by secondary genetic events, which supplant the role of Bcr-Abl. Therefore, the fact that imatinib mesylate alone was also effective in blast crisis as well as in Philadelphia chromosome–positive acute leukemia (although the duration of remission was short) is unexpected. A recent study showed a response rate of 55% in patients with myeloid blast crisis; the complete hematologic response rate was 11% (1). Patients with Philadelphia chromosome–positive ALL or lymphoid blast crisis had a response rate of 70% and a complete hematologic response rate of 20%. Complete cytogenetic responses were seen in 7% to 10% of patients. The median duration of response was approximately 3 months in the patients with lymphoid disease and 6 months in those with myeloid blast crisis. In larger phase II studies of blast crisis, these results were confirmed (107, 108). The median duration of response was more than 8 months. Both previously treated and untreated patients responded, although the latter had better responses. Few patients (9%) also demonstrated reversion to the chronic phase (108).

Relapsed CML after Allogeneic Stem-Cell Transplantation

Of patients with CML who had relapse after transplantation, 74% achieved a complete hematologic remission after therapy with imatinib mesylate and 35% attained a complete cytogenetic response (110). Responses were more common in the chronic phase than in the accelerated or blast phase. The 1-year estimated survival rate was 74%.

Toxicity

Imatinib mesylate differs from traditional therapies because of its more favorable toxicity profile. The most common side effect is mild nausea. Edema, myalgias, arthralgias, diarrhea, and skin rash occur in about 10% of patients. Rarely, a fluid retention syndrome occurs, as does an unusual phenomenon of periorbital edema. Myelosuppression may be seen, but it is more common in the blast phase than in the chronic phase. It is also seen more often in patients with relapses after allogeneic stem-cell transplantation (110). Recurrence of graft-versus-host disease also occurs after transplantation in patients treated with imatinib mesylate. In most patients, side effects are mild.

Other Targets: Tumors with Activation of Kit or PDGF Receptor

Since imatinib mesylate targets other kinases (Kit and PDGF receptor) in addition to Bcr-Abl, its activity was explored for gastrointestinal stromal tumors with mutations that activate Kit kinase, myeloproliferative disorders harboring an ETV6-PDGF receptor-β fusion gene, dermatofibrosarcoma protuberans with a COL1A1-PDGF fusion anomaly, and hypereosinophilic syndrome with a FIP1-PDGFRα fusion gene (112–116). In all of these examples, imatinib mesylate was efficacious. Indeed, 54% to 81% of patients with gastrointestinal stromal tumors, a notoriously chemotherapy-resistant mesenchymal tumor of the intestines, respond to imatinib mesylate; responses are sustained after 6 to 12 months of follow-up (112, 113). Strikingly, positron emission tomography could discern responses within 8 days or less. Remissions in chronic myeloproliferative disorders with a chimeric ETV6-PDGF receptor-β molecular anomaly secondary to a translocation between chromosomes 5 and 12 also show rapid and durable responses (114). A recent report also describes a response to imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans, a fibrohistiocytic tumor characterized by a translocation involving PDGFβ (rather than PDGF receptor itself), which, nevertheless, exerts its pathogenic effects through interaction with PDGF receptor (115). Finally, hypereosinophilic syndrome, caused by a novel fusion kinase involving PDGFRα, also responds well to imatinib mesylate (116).

FUTURE DIRECTIONS

Despite the dramatic success with imatinib mesylate, the issue of how to maximize response and defy resistance remains. In chronic-phase CML, not all patients will attain cytogenetic remission; in blast transformation phase and Philadelphia chromosome–positive acute leukemia, most patients who respond will relapse quickly. The role of imatinib mesylate compared with matched sibling allogeneic stem-cell transplantation remains to be elucidated. Since imatinib mesylate is commonly used as front-line therapy in CML, its impact on survival warrants full study.

The mechanisms mediating failure are being studied. These include upregulation of multidrug-resistance proteins, functional inactivation of imatinib mesylate, and *BCR-ABL* gene amplification or mutations (117–119). Alternative innovative approaches that directly interfere with Bcr-Abl function or enhance imatinib mesylate efficacy have therefore been suggested: 1) targeting *BCR-ABL* RNA with antisense oligonucleotides or with ribozymes (120); 2) using Bcr fragments as therapy (based on the observation that high levels of Bcr attenuate Bcr-Abl kinase activity) (121); 3) exploiting molecules, such as tyrphostins, which alter the binding of peptide substrates (rather than ATP) to Bcr-Abl; 4) combining imatinib mesylate with inhibitors of other signaling molecules (Jak_2 or Ras) (122, 123) or with interferon-α, which has known activity in CML; and 5) using suppressors of nuclear export to entrap Bcr-Abl in the nucleus, where it induces apoptosis (124). The efficacy of these strategies may depend on the mechanism of resistance, which could vary among patients. For instance, approaches that target Bcr-Abl function or levels may be moot in persons in whom molecular pathways other than Bcr-Abl mediate resistance to imatinib mesylate.

The development of imatinib mesylate serves as a model for harnessing the remarkable salutary potential of targeted therapies. Indeed, each of the known kinase targets of imatinib mesylate—Bcr-Abl, Kit, and PDGF receptor—can be suppressed in vivo, and suppression is associated with clinical response. Furthermore, a synthesis of the clinical trial data indicates that the unifying feature for disorders responsive to imatinib mesylate is not whether they belong to a certain disease category or anatomic locale but rather their underlying molecular abnormality. Hence, the paradigm for gene-directed therapeutics may need to shift to defining cancer by its molecular fingerprint. The wealth of data now available on the fundamental aberrations that characterize other malignant conditions can be similarly exploited for targeted intervention.

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