0031-6997/03/5503-401–423$7.00
PHARMACOLOGICAL REVIEWS
Vol. 55, No. 3
30302/1082292
Pharmacol Rev 55:401–423, 2003
Printed in U.S.A

Specific Targeted Therapy of Chronic Myelogenous Leukemia with Imatinib

MICHAEL W. N. DEININGER AND BRIAN J. DRUKER

Oregon Health and Science University Cancer Institute, Portland, Oregon

Address correspondence to: Dr. Michael Deininger, BMT/Leukemia Center, Oregon Health and Science University, Mailcode L592, 3181 S.W. Sam Jackson Park Road, Portland, OR 97239. E-mail: deininge@ohsu.edu
Article, publication date, and citation information can be found at http://pharmrev.aspetjournals.org.
DOI: 10.1124/pr.55.3.4.

***Abstract*——Chronic myeloid leukemia (CML) is characterized by the Philadelphia translocation that fuses BCR sequences from chromosome 22 upstream of the ABL gene on chromosome 9. The chimerical Bcr-Abl protein expressed by CML cells has constitutive tyrosine kinase activity, which is essential for the pathogenesis of the disease. Imatinib, an ATP-competitive selective inhibitor of Bcr-Abl, has unprecedented efficacy for the treatment of CML. Most patients with early stage disease achieve durable complete hematological and complete cytogenetic remissions, with minimal toxicity. In contrast, responses are less stable in patients with advanced CML. This review highlights the pathogenesis of CML, its clinical features, and the development of imatinib as a specific molecularly targeted therapy. Aspects of disease monitoring and side effects are covered as well as resistance to imatinib and strategies to overcome resistance, such as alternative signal transduction inhibitors and drug combinations. Perspectives for further development are also discussed.**

I. Introduction

A. Historical Remarks

Chronic myelogenous leukemia (CML[1]) was described in 1845 by Hughes Bennet, a physician from Edinburgh who thought that the disease was an infection (Bennett, 1845). Rudolf Virchow, who published a similar case only a few weeks later, postulated that the disease was noninfectious and later coined the term leukemia (from $\lambda\epsilon\upsilon\kappa o\nu\ \alpha\iota\mu\alpha$ = white blood) (Virchow, 1845). That the leukemic cells originated from the bone marrow was recognized by Neumann in 1870 (Neumann, 1870). In 1960, Nowell and Hungerford (1960), two Philadelphia researchers noted that an abnormally small chromosome was consistently present in the cells of CML patients, and this chromosome was subsequently called the Philadephia (Ph)[1] chromosome. This was the first time that a chromosomal abnormality had been associated with a malignant disease. In 1973, Janet Rowley recognized that the Ph[1] chromosome was indeed the product of a reciprocal translocation between the long arms of chromosomes 9 and 22, the t(9;22)(q34;q11). The early 1980s saw the identification of the two genes that flank the translocation breakpoint. The *ABL* gene from chromosome 9 had been known as the human homolog of a murine leukemia virus (Abelson and Rabstein, 1970; Bartram et al., 1983); the translocation partner from chromosome 22 was termed *BCR* for breakpoint cluster region, since DNA breaks occurred in a relatively small genomic region (Groffen et al., 1984). Of paramount importance was the discovery that the protein derived from the chimeric *BCR-ABL* gene had protein-tyrosine kinase (PTK) activity that was deregulated compared with normal Abl and correlated with its ability to transform cells to a malignant phenotype (Lugo et al., 1990). In 1990, several groups reported that a CML-like disease could be induced in mice transplanted with bone marrow infected with a *BCR-ABL* retrovirus (Daley et al., 1990; Heisterkamp et al., 1990). This proved the point that *BCR-ABL* is the causative agent and not just a marker of the disease. In 1996, Druker and colleagues described CGP57148, a highly specific pharmacologic inhibitor of the Abl-tyrosine kinase that selectively sup-

[1]Abbreviations: CML, chronic myelogenous leukemia; A-loop, activation loop; ALL, acute lymphoblastic leukemia; ARG, ABL-related gene; CCR, complete cytogenetic response; CHR, complete hematological remission; FISH, fluorescence-in situ-hybridization; FTI, farnesyl transferase inhibitor; GIST, gastrointestinal stromal tumor; GM-CSF, granulocyte macrophage-colony-stimulating factor; HSP, heat shock protein; LTC-IC, long-term culture-initiating cells; MAP kinase, mitogen-activating protein kinase; MCR, major cytogenetic response; N-lobe, NH_2-terminal lobe; P-loop, phosphate binding loop; PDGF-R, platelet-derived growth factor receptor; Ph, Philadelphia (chromosome); PTK, protein-tyrosine kinase; RT-PCR, reverse transcription-polymerase chain reaction; SH, Src homology; STAT5, signal transducer and activator of transcription 5.

pressed the growth of *BCR-ABL*-positive cells. This compound, first renamed STI571 and then imatinib, has revolutionized the treatment of CML and set a precedent for the development of targeted therapies for malignant diseases.

B. Epidemiology

The incidence of CML is approximately 1 to $1.5/10^5$ (Sawyers, 1999). Thus, between 3500 and 5000 new cases per year are expected in the United States. The incidence rises slowly with age until the middle forties when it starts to rise more rapidly, resulting in a median age at diagnosis of about 60 years. There is no geographical or ethnic background that predisposes to CML. The only well characterized risk factor is exposure to ionizing radiation. An increased incidence of CML was observed approximately 8 years after the atomic bombings of Hiroshima and Nagasaki (Heyssel et al., 1960). Patients exposed to Thorotrast, an α-emitter that was used as a contrast medium in radiology in the 1930s, also have an increased risk of developing CML (Van Kaick et al., 1990), as do patients treated with radiation therapy (Corso et al., 1995). *BCR-ABL* fusion transcripts can be induced in vitro by high-dose ionizing radiation (Deininger et al., 1998). In contrast to acute myelogenous leukemia, there is no convincing evidence to support a link between exposure to organic solvents and CML.

C. Clinical Features

The clinical hallmarks of CML are leukocytosis, a left shift in the differential count, and splenomegaly. Importantly, in contrast to acute myelogenous leukemia, the disease is not restricted to the myeloid compartment, since the Philadelphia chromosome is regularly demonstrable in megakaryocytes and erythroid precursor cells. Thus, high platelet counts are frequent, but for unknown reasons, erythrocytosis is rarely seen. CML runs a three-phased course. During the initial *chronic phase*, there is gross expansion of the myeloid cell compartment, but the cells still retain the capacity to differentiate and function normally. Symptoms in the chronic phase are generally mild and many patients are asymptomatic, being diagnosed by routine blood sampling (Cervantes et al., 1999). After an average of 4 to 5 years, the disease typically progresses to *accelerated* phase, characterized by the appearance of more immature cells in the blood, frequent constitutional symptoms, and a less favorable response to therapy. The diagnostic criteria for accelerated phase are not universal, reflecting that disease progression from chronic to accelerated phase is a continuous process rather than a single step. Although the duration of accelerated phase varies from weeks to years, the disease inexorably progresses to the final stage of blast crisis, where immature cells dominate and survival is measured in weeks to months.

Given the highly diverse treatment options available for CML and its variable clinical course, methods have been developed to predict the biological behavior of individual cases based on information available at diagnosis. The most commonly used prognostic scores are the Sokal (Sokal et al., 1984) and European (Hasford et al., 1998) risk scores. They are based on parameters with a known adverse effect on outcome such as spleen size, platelet count, blast-, basophil-, and eosinophil counts as well as age. Although these scores are capable of separating patients into low, intermediate, and high risk groups, they are relatively crude indicators that have limited value for outcome prediction in individual patients. Moreover, it is not clear if they retain their predictive value in patients treated with imatinib. An important focus of CML research is the development of risk scores based on molecular rather than clinical features of the disease.

II. Pathogenesis of Chronic Myelogenous Leukemia

The leukemic cells of more than 90% of CML patients contain the Ph chromosome, and an additional 5% have a cytogenetically silent *BCR-ABL* translocation. The remaining 5% have truly *BCR-ABL*-negative CML, which constitutes a separate disease entity. In current usage, CML refers to *BCR-ABL*-positive CML only.

A. The Philadelphia Translocation and the BCR-ABL Fusion Gene

As a result of the exchange of genetic material, two fusion genes are produced: *BCR-ABL* on the derivative chromosome 22 and *ABL-BCR* on the derivative 9 (Fig. 1, A and B). Although *ABL-BCR* mRNA is expressed in approximately two-thirds of CML patients (Melo et al., 1993), expression of the protein has never been documented. Thus, the pathogenetically relevant principle is the *BCR-ABL* fusion gene and its cognate Bcr-Abl protein. All DNA breakpoints occur within introns, and regardless of their precise location, two types of fusion mRNA are generated that contain the first 13 or 14 exons of *BCR* fused to *ABL* exon 2 (e13a2 and e14a2 fusions, respectively) (Fig. 2) (Deininger et al., 2000a). Very rarely in CML, and much more frequently in acute lymphoblastic leukemia (ALL), the break in *BCR* occurs between the first and second exons, resulting in an e1a2 fusion mRNA (Melo et al., 1994). Yet other types of fusion have been described in isolated cases (Pane et al., 1996; Al Ali et al., 2002). The variation in the *BCR* part of the fusion mRNA contrasts with the constant *ABL* part. This is in itself an indication that *ABL* is likely to carry the relevant transforming principle.

Classical cytogenetics is still the mainstay of diagnosing and monitoring CML. However, fluorescence-in situ hybridization (FISH), used to detect the *BCR-ABL* translocation, has become an important complementary method (Fig. 1C). FISH is able to detect the 5% or so of CML cases with a masked translocation that escapes

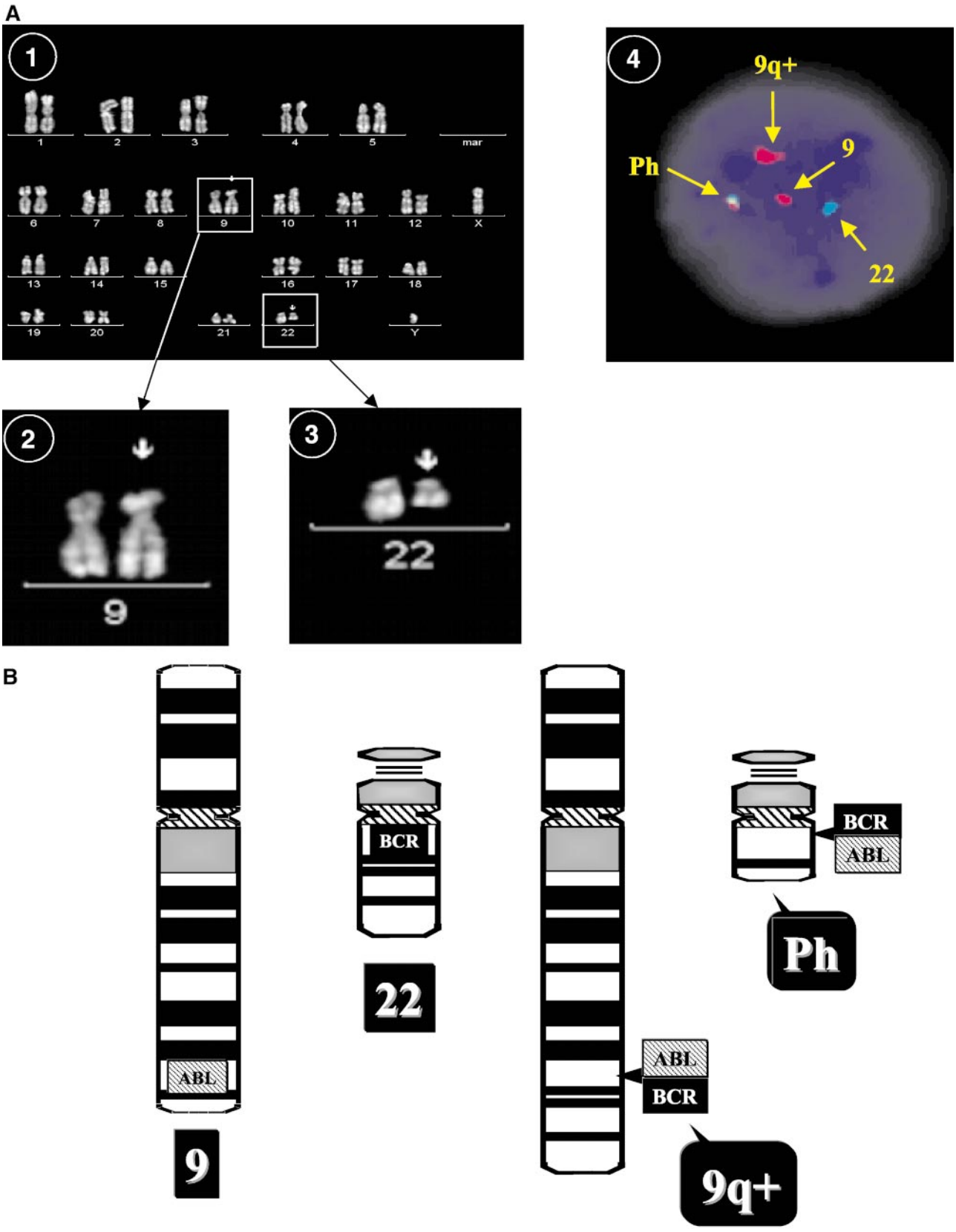

FIG. 1. Panel A, karyotype [46XY, t(9;22)(q34;q11)] of a patient with Philadelphia-positive CML (1); derivative chromosome 9 (arrow) (2); Philadelphia chromosome (arrow) (3). As a result of the translocation, the q (long) arm of chromosome 22 appears shortened and this chromosome was originally referred to as 22q-. FISH (4). As a result of the t(9;22), the *BCR* (green signal) and *ABL* genes (red signal) are juxtaposed and form a yellow fusion signal. Panel B, schematic illustration of the Philadelphia translocation. The *ABL* and *BCR* genes reside on the long arms of chromosomes 9 and 22, respectively. As a result of the translocation, an *ABL-BCR* chimeric gene is formed on the derivative chromosome 9 and a *BCR-ABL* gene on the derivative chromosome 22 (Philadelphia chromosome). Hatched areas, centromeric region.

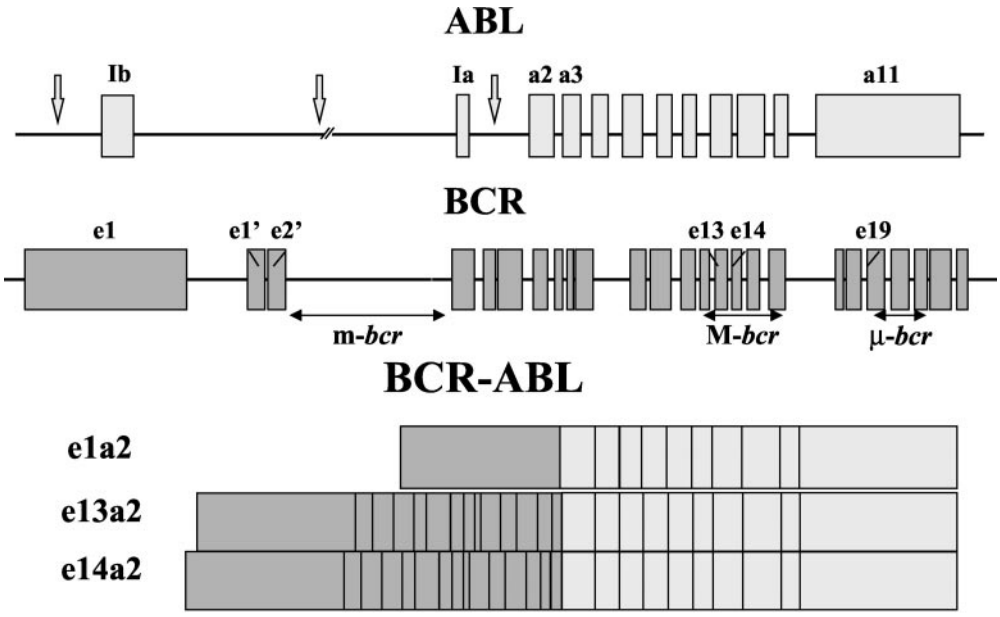

FIG. 2. Genomic organization of the *BCR* and *ABL* loci. The arrows indicate breakpoints within *ABL*, and the location of the minor (m-*BCR*), major (M-*BCR*) and micro breakpoint cluster region (*μ-BCR*) are shown. Regardless of the specific breakpoint in *ABL*, mRNAs are produced that fuse *BCR* sequences to the second *ABL* exon. Fusions between *BCR* exons e13 (previously b2) or e14 (previously b3) and *ABL* exon a2 produce $p210^{BCR\text{-}ABL}$ that is characteristic of CML, whereas fusions between *BCR* exon e1 and *ABL* exon a2 give rise to $p190^{BCR\text{-}ABL}$ (found in two of three of patients with Ph-positive acute lymphoblastic leukemia). Rare CML patients have a breakpoint in the so-called micro breakpoint cluster region (*μ-BCR*) and produce $p230^{BCR\text{-}ABL}$. Note that there are two alternative 1st exons in *ABL* (Ia and Ib) and two alternative 2nd exons in *BCR* (e1′ and e2′).

conventional cytogenetics. Moreover, it is useful for samples in which metaphases cannot be obtained. Its main limitation is the fact that it does not detect abnormalities other than the *BCR-ABL* translocation.

Reverse transcription-polymerase chain reaction (RT-PCR) is used for both diagnostic purposes and follow-up, particularly after allogeneic stem cell transplantation (Cross et al., 1993). It is the most sensitive method for monitoring residual disease. Improved methods of quantification enable reliable monitoring of leukemic burden and allow for therapeutic interventions before cytogenetic or hematological relapse occurs (Dazzi and Goldman, 1999).

B. Bcr and Abl Proteins

The function of the various structural motifs in the *Bcr* and Abl proteins have recently been reviewed (Deininger et al., 2000a). Both *Bcr* and Abl are multidomain proteins. The physiological function of *Bcr* is not well understood. The N terminus of the protein has serine/threonine kinase activity (Maru and Witte, 1991) and a dimerization domain (McWhirter et al., 1993). The central portion of the protein contains dbl-like and pleckstrin homology domains that stimulate GDP-GTP exchange on *rho* guanidine exchange factors (Ron et al., 1991) (Fig. 3A). The C terminus has GTPase activity for *Rac*, a *Ras*-family protein that activates an NADPH oxidase in neutrophils (Diekmann et al., 1991). Altogether, this suggests a function for *Bcr* in signal transduction. However, apart from an increased neutrophilic burst, the phenotype of mice with homozygous deletion of *BCR* is normal (Voncken et al., 1995).

The Abl protein contains several domains such as the Src homology domains 2 and 3 (SH2 and SH3), proline-rich regions in the center, and the actin-binding domain at the C terminus, which allow for interactions with other proteins (Fig. 3B). In addition, there is a DNA-binding domain as well as nuclear localization signals. The SH1 domain has the PTK activity that may be regulated by the NH_2-terminal SH3 domain. The data regarding the physiological function of Abl are complex (recently reviewed in Van Etten, 1999). The nuclear fraction appears to play an inhibitory role in cell cycle regulation, which led to the notion that *ABL* is a tumor suppressor gene (Sawyers et al., 1994). The cytoplasmic pool may function in the transmission of integrin-mediated signals from the cellular environment (Lewis and Schwartz, 1998). Importantly, Abl interacts with several proteins involved in DNA repair such as ataxia telangiectasia-mutated (*Atm*) (Baskaran et al., 1997; Shafman et al., 1997), *DNA-PK* (Kharbanda et al., 1997), and *Rad51* (Yuan et al., 1998; Chen et al., 1999). It appears that Abl kinase activity is important for the induction of apoptosis in response to genotoxic stress such as ionizing radiation (Yuan et al., 1997). *ABL* null mice have a high neonatal mortality, and the survivors exhibit a variety of defects such as disturbed immune function, bone defects, and a rather ill-defined wasting syndrome (Schwartzberg et al., 1991; Tybulewicz et al., 1991). There is, however, no increased incidence of tumors in these mice, which argues against the concept of *ABL* as a tumor suppressor. It is possible that *ARG* (*ABL*-related gene), a close homolog of *ABL*, is capable of compensating for the loss of some of the functions of *ABL*.

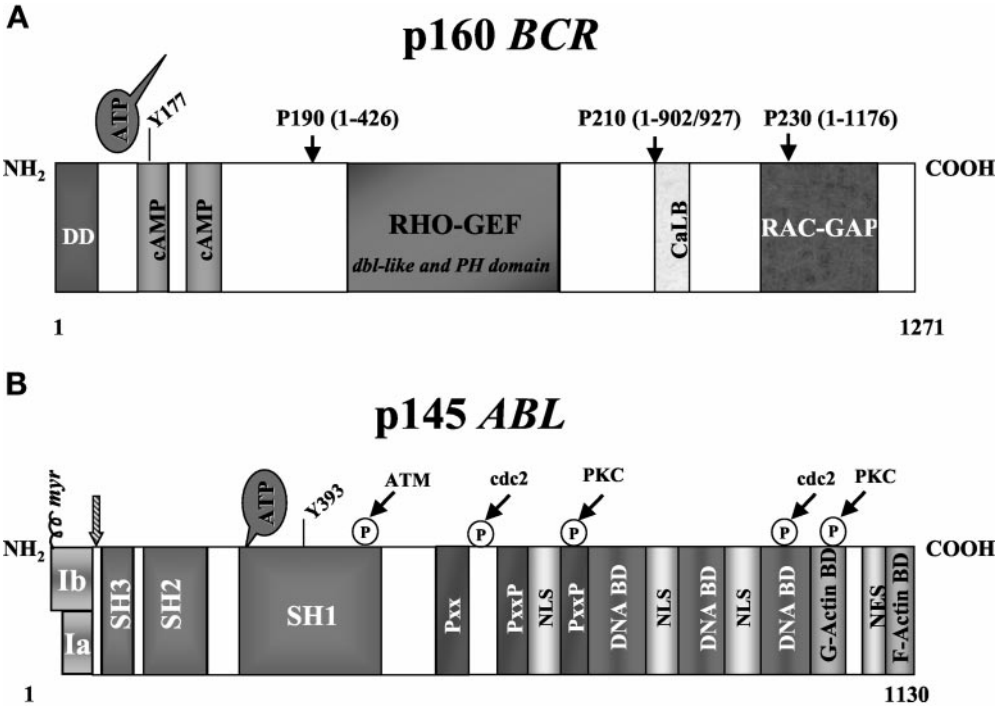

FIG. 3. Panel A, functional domains of the Bcr protein. Noted are the dimerization domain (DD) at the NH_2-terminal end and tyrosine 177, an important autophosphorylation site. The center of the protein has a Rho guanidine exchange factor domain. CalB is a lipid-binding domain, and RAC-GAP mediates GTP/GDP exchange on Rac proteins. Arrows indicate the breakpoints in the various types of Bcr-Abl fusion proteins. Panel B, functional domains of Abl. Noted are the alternative first exons and the Src homology domains (SH1–3) that form most of the NH_2 terminus. The SH1 domain carries the tyrosine kinase function, whereas the SH2 domain interacts with phosphotyrosines on other proteins. The center of Abl consists of proline-rich regions (PxxP) that allow for interaction with SH3 domains of other proteins, although toward the COOH terminus, nuclear localization signals (NLS), DNA-binding function (DNA-BD), a nuclear export signal (NES), and actin-binding motifs are found. Shown are also major phosphorylation sites for ATM, cdc2, and protein kinase C (PKC) as well as tyrosine 393, a major autophosphorylation site which regulates kinase activity.

Notably, homozygous deletion of both the *ABL* and the *ARG* locus results in embryonic lethality due to a block of neuronal development (Koleske et al., 1998).

C. Essential Features of Bcr-Abl

Several motifs within the chimeric protein are essential for malignant transformation. In Abl, they include the SH1 (kinase) domain (Lugo et al., 1990) and actin-binding domain (McWhirter and Wang, 1993). In *Bcr*, the dimerization domain is crucial (McWhirter et al., 1993); it can be replaced by other sequences that also allow for dimerization, such as Tel in the Tel-Abl fusion protein that is seen in rare patients with acute lymphoblastic leukemia (Papadopoulos et al., 1995). In addition, a tyrosine at position 177 of *Bcr* is essential for transformation of myeloid cells (Million and Van Etten, 2000). The requirement of specific features for transformation of cells by Bcr-Abl is dependent on the cellular background. For example, the SH2 domain is crucial for fibroblast transformation (Afar et al., 1995) but not for transformation of cytokine-dependent hematopoietic cells to factor independence (Ilaria and Van Etten, 1995). However, malignant transformation is absolutely dependent upon the kinase domain, although more subtle effects of the Bcr-Abl protein, such as the adhesion defect, may be independent of its tyrosine kinase activity (Wertheim et al., 2002). There is no consensus as to how Abl kinase is regulated under physiological circumstances. Both *trans*- and *cis*-acting mechanisms have been implicated. The fact that purified Abl protein is kinase active (Sawyers et al., 1992) is indirect evidence for a *trans*-acting inhibitory factor. Several proteins have been shown to bind to Abl in vivo (Dai and Pendergast, 1995; Shi et al., 1995; Wen and Van Etten, 1997), but their true relevance is presently not completely understood. Other data suggest an inhibitory role for the SH3 domain and more 5′ regions of the molecule (Mayer and Baltimore, 1994; Barila and Superti Furga, 1998). The SH3 domain may either bind Abl, leading to a kinase-inactive conformation, or it may bind to an inhibitory factor. In v-*abl*, the SH3 domain is deleted and replaced with viral Gag sequences, which leads to kinase activation and again argues for an inhibitory role of the SH3 domain.

D. Consequences of Deregulated Tyrosine Kinase Activity

The phosphorylation of cellular proteins on tyrosine residues is an important mechanism of intracellular signal transduction, used by many growth factor receptors. Normally, less than 1% of cellular tyrosine residues are phosphorylated, and the activity of tyrosine kinases is

counterbalanced by the activity of tyrosine phosphatases. In cells that express a constitutively active tyrosine kinase, this tight regulation is undermined, leading to a situation that resembles chronic growth factor stimulation. In the case of Bcr-Abl, a multitude of signal transduction pathways is activated. They appear to target three major cellular functions.

1. Increased Proliferation. Many cell lines derived from CML patients in blast crisis proliferate in the absence of growth factors. The defect in the chronic phase appears to be more subtle. These cells are not completely factor-independent, but compared with normal cells, they proliferate at lower cytokine concentrations (Jonuleit et al., 1998) or they may have interleukin-3-driven autocrine loops (Jiang et al., 1999). This may enable them to outpace normal hematopoiesis over time.

2. Reduced Apoptosis. Bcr-Abl stimulates pathways that transduce survival signals, leading to a decreased rate of apoptosis (Bedi et al., 1994). Again, this phenomenon is more easily demonstrable in cell lines (Cortez et al., 1996) than in primary chronic phase cells, which still require cytokines for survival. This may explain, why some studies found CML progenitor cells no more resistant to apoptosis induced by growth-factor withdrawal and ionizing radiation than normal cells (Amos et al., 1995). Bcr-Abl effects may also include a prolonged arrest in the G_2 phase of the cell cycle that allows for extensive DNA repair after DNA damage, whereas a normal cell would undergo apoptosis under the same circumstances (Bedi et al., 1995).

3. Disturbed Interaction with the Extracellular Matrix. Among the proteins that are tyrosine-phosphorylated in BCR-ABL-positve cells are several that are involved in the organization of the cytoskeleton, such as paxillin (Salgia et al., 1995) and focal adhesion kinase (Gotoh et al., 1995). Importantly, the COOH-terminal portion of Bcr-Abl binds actin (McWhirter and Wang, 1993). Thus, it is not surprising that BCR-ABL-positive cells show abnormalities in motility as well as adhesion to integrins and other components of the extracellular matrix (Wertheim et al., 2002). The extramedullary hematopoiesis that characterizes CML may be a consequence of a defective interaction with the bone marrow stroma (Gordon et al., 1987).

It is evident that some clinical features of CML are explicable by its molecular pathogenesis. However, a number of questions remain unsolved. For example, it is not clear why a lesion that occurs in a hematopoietic stem cell predominantly targets the myeloid compartment. Even more important, the genetic events that underlie progression to blast crisis are poorly understood. Cytogenetic abnormalities in addition to the Philadelphia chromosome are seen in at least 50% of patients (Johansson et al., 2002). The causative significance of such abnormalities for disease progression is frequently not clear. Deletion and inactivation of tumor suppresser genes such as p53 (Feinstein et al., 1991) and p16 (Sill et al., 1995) is seen in some patients but no universal genetic lesion has been associated with progression. Even less is known about the biological basis of the "genetic instability" that is thought to render chronic phase CML cells prone to the acquisition of further genetic damage. There is evidence that DNA repair may be less efficient in CML cells (Canitrot et al., 1999; Takedam et al., 1999), and the threshold for the induction of apoptosis in response to DNA damage may be unduly high. Finally, the high cell turnover in itself might predispose to a higher frequency of mutations.

III. Conventional Treatment Options for Chronic Myelogenous Leukemia

A. Assessment of Response to Therapy

As with any other disease, the ultimate measure of the efficacy of a therapy is survival. However, for a disease with a relatively long course such as CML, surrogate markers are often used to allow for an earlier assessment of efficacy. From historical data, it has been estimated that the median survival of CML patients without any treatment is between 2 and 3 years. Three levels of disease control can be defined in CML.

Complete hematological response (CHR) is defined as the normalization of the blood counts and the white cell differential as well as the disappearance of all symptoms and signs of disease.

Complete cytogenetic response (CCR) means that no Ph-positive metaphases are detectable using classical cytogenetics, with at least 20 metaphases available for analysis. Major cytogenetic response (MCR) is the presence of less than 35% Ph-positive metaphases.

Molecular remission implies that no BCR-ABL transcripts are detectable by RT-PCR. This assay is far less standardized than the other tests, and its sensitivity varies greatly between laboratories. However, there is a general consensus that PCR negativity requires a level of sensitivity that allows for detection of one BCR-ABL-positive cell in 10^5 to 10^6 normal cells (Bose et al., 1998).

There is good evidence that achievement of a major cytogenetic response on interferon-α therapy predicts improved survival, unless the patient belongs to the high risk group of patients (Hehlmann et al., 1994; Italian Cooperative Study Group on CML, 1994). Thus, cytogenetic response instead of survival is frequently used as a surrogate marker to assess efficacy. It must be stressed that such endpoints have been validated only in interferon-treated patients.

B. Conventional Cytotoxic Drugs

The first effective treatment for CML was Fowler's solution, which was widely used in the 19th century and contains arsenic as the active component. Recent in vitro studies have confirmed activity of arsenicals against CML cells, and the agent may see a comeback in the future (La Rosee et al., 2002a). With the advent of ra-

diotherapy, splenic irradiation became popular in the 1920s and 1930s. It offered symptomatic relief but probably did not prolong life. The first synthetic compound with activity in CML was busulfan, an alkylating agent. Busulfan is extremely toxic to stem cells, which may explain why it is particularly effective in the stem cell disease CML; it was the first therapeutic modality that offered a definitive survival benefit, although no randomized study was carried out. Interestingly, there are anecdotal cases of long-term remissions after high-dose busulfan (Djaldetti et al., 1966). Although superseded by more effective and less toxic alternatives, busulfan is still used in preparative regimens for allogeneic stem cell transplantation. The next effective drug to be introduced for CML was hydrea. Compared with busulfan, hydrea does not cause prolonged cytopenias, since it primarily targets the more mature myeloid cells. It also has a far more benign nonhematological toxicity profile than busulfan. A survival advantage for hydrea over busulfan was shown in a controlled randomized trial (Hehlmann et al., 1994). Another drug with significant single agent activity in CML is cytarabine, although it never became widely used. Neither busulfan, hydrea, nor cytarabine produced cytogenetic remissions in a significant number of cases. The 1970s saw a number of trials using acute leukemia-type multiagent chemotherapy. In contrast to conventional chemotherapy, a proportion of patients achieved some degree of Ph-negative hematopoiesis (Kantarjian et al., 1985). However, as a rule, these were transient responses. Given the very considerable toxicity of polychemotherapy in CML, this approach was abandoned for patients in chronic phase.

C. Interferon-α

At the beginning of the 1980s, interferon-α was introduced as a therapy for CML. In contrast to other drug treatments, interferon-α produced sustained cytogenetic responses in up to one-third of patients (Talpaz et al., 1991). The initial single center results were subsequently confirmed in randomized trials that demonstrated a survival advantage for interferon-α over hydrea and busulfan (Hehlmann et al., 1994; Italian Cooperative Study Group on CML, 1994). A large randomized trial suggested that the combination of interferon-α and cytarabine is superior to interferon alone (Guilhot et al., 1997), a finding that was not confirmed in a subsequent study (Baccarani et al., 2002). The cytogenetic remissions induced by interferon are durable in a proportion of patients, sometimes even after discontinuation of the agent (Bonifazi et al., 2001). Although, with RT-PCR, BCR-ABL mRNA is still detectable, these long-lasting remissions amount to a biological although not molecular cure of the disease.

D. Allogeneic Stem Cell Transplantation

A comprehensive evaluation of allografting for CML is beyond the scope of this review. As of now, allografting is the only treatment capable of disease eradication, with the majority of patients achieving RT-PCR negativity (Savage and Goldman, 1997). Long-term disease-free survival is in the range of 50 to 80% in most studies (Savage and Goldman, 1997; Hansen et al., 1998). However, allografting is limited to patients who have a suitable donor and are medically fit to undergo the procedure, which involves high-dose chemotherapy and total body irradiation. Less toxic (nonmyeloablative) transplant regimens make allografting an option for patients who do not qualify for a conventional transplant (McSweeney et al., 2001; Or et al., 2003). It is not yet clear if the durability of remissions with nonmyeloablative regimens equals that of conventional transplants, and transplant-related complications remain a problem (Bornhauser et al., 2001). Although no randomized trials have been conducted, there is no doubt that decisively more patients are long-term survivors after an allograft than with nontransplant therapies.

IV. Imatinib

A. Development of Abl-Specific Tyrosine Kinase Inhibitors

Given that the Bcr-Abl protein has deregulated tyrosine kinase activity, it was logical to search for specific pharmacological inhibitors. In 1993, Anafi and colleagues (1993) reported a tyrphostin, related to erbstatin, that inhibited the PTK activity of Bcr-Abl and suggested that it might be possible to design specific compounds for the treatment of Abl-associated human leukemias (Anafi et al., 1993). In a more extensive analysis of number of tyrphostins, the compounds AG568, AG957, and AG1112 proved to be the most specific agents. Growth inhibition of the CML cell line K562 occurred at micromolar concentrations and was associated with inhibition of Bcr-Abl tyrosine kinase activity (Kaur et al., 1994). Tyrphostins are competitive toward ATP or substrate, or both (Kovalenko et al., 1997). Although active in vitro, tyrphostins have not been developed for clinical use.

Another compound with activity toward Bcr-Abl is herbimycin A, an antibiotic derived from *Streptomyces hygroscopicus*. Its efficacy in inhibiting transforming tyrosine kinases was recognized as early as 1988 (Uehara et al., 1988). Herbimycin was originally thought to inhibit Bcr-Abl PTK (Okabe et al., 1992), but it was subsequently shown that its mode of action is the acceleration of Bcr-Abl protein degradation (Shiotsu et al., 2000). Selective inhibition of primary CML cells was also shown for genistein, a flavonoid (Carlo Stella et al., 1996).

In 1995 and 1996, Buchdunger and colleagues reported the synthesis of a series of compounds that exhibited specific inhibitory activity against the platelet-derived growth factor receptor (PDGF-R) (Buchdunger et al., 1995) and Abl (Buchdunger et al., 1996). These

compounds emerged from a high-throughput screen of chemical libraries with the goal of identifying kinase inhibitors. From this time-consuming approach, a lead compound of the 2-phenylaminopyrimidine class was identified. This lead compound had weak inhibitory activity against both serine/threonine and tyrosine kinases, but served as a starting point for the synthesis of other related compounds. A key finding was that substitutions at the 6-position of the anilino phenyl ring led to loss of serine/threonine kinase inhibition, while the introduction of a methyl group at this position retained or enhanced activity against tyrosine kinases. The activity against the platelet-derived growth factor receptor tyrosine kinase was further enhanced by the introduction of a benzamide group at the phenyl ring. These compounds were also found to possess inhibitory activity toward Abl, with CGP57148 (STI571, now imatinib mesylate, Gleevec, Glivec) emerging as the lead compound for clinical development (Fig. 4). Introduction of *N*-methylpiperazine as a polar side chain greatly improved water solubility and oral bioavailability. Studies in our laboratory demonstrated that imatinib had extremely potent and specific in vitro and in vivo activity against BCR-ABL-transformed cells (Druker et al., 1996), results that were independently confirmed by other groups (Deininger et al., 1997; Gambacorti-Passerini et al., 1997).

B. Preclinical Evaluation of Imatinib

1. In Vitro Studies

a. Kinase Assays. The effects of imatinib on a number of serine/threonine as well as tyrosine kinases was tested using in vitro kinase assays with immunoprecipitated or purified proteins (Table 1), which are independent of the specific cellular environment. Imatinib showed activity toward Abl and its activated derivatives v-Abl, Bcr-Abl (Buchdunger et al., 1996; Druker et al., 1996) and Tel-Abl (Carroll et al., 1997), with IC_{50} values in the range of 0.025 μM for protein autophosphorylation. Activity against PDGF-R and c-*Kit* was found to be in a similar range. By contrast, the IC_{50} values for a large number of other tyrosine and serine/threonine kinases were generally at least 100-fold higher, demonstrating that imatinib exhibits a high level of selectivity.

TABLE 1
Inhibition of protein kinases by imatinib[a]

Enzyme	Substrate Phosphorylation IC_{50}	Cellular Tyrosine Phosphorylation IC_{50}
	μM	
c-Abl	0.2;0.025[b]	
v-Abl	0.038	0.1–0.3
$p210^{BCR-ABL}$	0.025[b]	0.25
$p185^{BCR-ABL}$	0.025[b]	0.25
Tel-Abl		0.35
PDGF-R α and β		0.1
Tel-PDGF-R		0.15
c-Kit		0.1
Flt-3		>10
c-Fms and v-Fms		>10
c-Src	>100	
v-Src		>10
c-Lyn	>100	
c-Fgr	>100	
Lck	9.0	
Syk (TPK-IIB)	>100	
Jak-2	>100[b]	>100
EGF-R	>100	>100
Insulin receptor		>100
IGF-IR		>100
FGF-R1	31.2	
VEGF-R2 (Kdr)	10.7	
VEGF-R1 (Flt-1)	19.5	
Tie-2 (Tek)	>50	
c-Met	>100	
PKA	>500	
PPK	>500	
PKCα, β1, β2, γ, δ, ε, ζ, or η	>100	
Protein kinase CK-1, CK-2	>100	
Cdc2/cyclin B	>100	

TPK, tyrosine-protein kinase; EGF-R, epidermal growth factor receptor; IGF-IR, insulin-like growth factor receptor I; FGF-R1, fibroblast growth factor receptor 1; VEGF-R, vascular endothelial growth factor receptor; PKA, cAMP-dependent protein kinase; PPK, phosphorylase kinase; PKC, protein kinase C; CK, casein kinase.

[a] Imatinib concentrations causing a 50% reduction in kinase activity (IC_{50}) are given.

[b] IC_{50} was determined in immunocomplex assays.

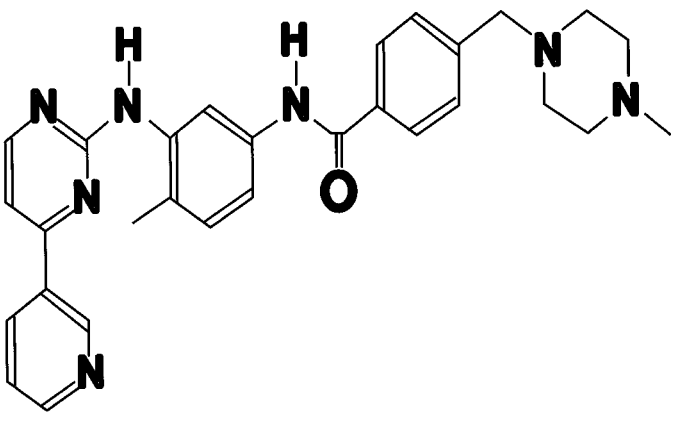

FIG. 4. Structure of imatinib.

b. Studies in Cell Lines. To determine whether imatinib was able to penetrate the cell membrane, studies were extended to intact cells. Most of these investigations used cell lines engineered to express Bcr-Abl or cell lines derived from CML patients in blast crisis. These experiments showed that the IC_{50} values for inhibition of Bcr-Abl tyrosine phosphorylation are in the range of 0.25 to 0.5 μM, approximately 10-fold higher than those measured in the in vitro kinase assays (Druker et al., 1996; Beran et al., 1998; Dan et al., 1998; Deininger et al., 2000b). The precise reason for this difference is not known, but it may be related to drug efflux or to intracellular regulatory mechanisms that affect the binding of imatinib to the Abl kinase.

Incubation of BCR-ABL-positive cell lines resulted in growth inhibition and induction of apoptosis in almost all lines studied (Druker et al., 1996; Deininger et al., 1997; Gambacorti-Passerini et al., 1997). The IC_{50} values for inhibition of cell proliferation mirrored those for inhibition of Bcr-Abl tyrosine phsophorylation in cellular assays. Importantly, the IC_{50} values seen in these

assays are well below the concentrations that can be achieved in patients. Of the many cell lines studied, only the KCL22 line derived from a CML patient in myeloid blast crisis, and SD1, an Epstein-Barr virus-transformed lymphoblastoid line derived from a patient with acute lymphoblastic leukemia, were primarily resistant to imatinib (Deininger et al., 1997) (Fig. 5). Given the fact that cell lines derived from CML blast crisis patients harbor multiple genetic abnormalities in addition to the Ph chromosome, these findings were impressive. Equally relevant, concentrations of up to 10 μM imatinib did not affect the growth of BCR-ABL-negative cell lines.

c. Studies in Primary Cells. To assess the effects of imatinib on committed hematopoietic progenitors, mononuclear cells from CML patients and normal individuals were studied in assays of colony formation. The formation of myeloid and erythroid colonies was reduced in CML samples using imatinib concentrations of up to 10 μM, whereas there was relatively little effect on normal cells (Fig. 6) (Druker et al., 1996; Deininger et al., 1997). A dose of approximately 1 μM offered the maximal differential effect. In re-plating experiments, it was also demonstrated that imatinib selectively suppressed the growth of secondary colonies from CML patients, similar to interferon-α (Marley et al., 2000). The differential sensitivity of CML versus normal cells was also confirmed using long-term culture-initiating cells (LTC-IC) that represent early hematopoietic progenitor cells (Kasper et al., 1999).

2. Animal Studies. The myeloid murine cell line 32D, engineered to express Bcr-Abl, forms tumors in syngeneic mice. Imatinib, at 10 to 50 mg/kg, given once daily intraperitoneally (i.p.), starting 1 week after the injection of $32D^{BCR\text{-}ABL}$, caused dose-dependent inhibition of tumor growth. In contrast, imatinib (50 mg/kg) was ineffective against tumor formation by v-Src-transformed 32D cells, again demonstrating the specificity of the compound (Druker et al., 1996). Another study tested imatinib against the human CML cell line KU812 injected into nude mice. These experiments demonstrated that continuous inhibition of the Bcr-Abl tyrosine kinase is required for maximal antitumor effect (le Coutre et al., 1999). In this model, a treatment schedule of three times daily administration of 50 mg/kg i.p. or daily administration of 160 mg/kg p.o. for 11 consecutive days, resulting in continuous inhibition of $p210^{BCR\text{-}ABL}$ tyrosine kinase activity and led to tumor-free survival after an injection of KU812 cells. Moreover, 160 mg/kg p.o. every 8 h for 11 days inhibited tumor growth even in the presence of advanced disease. Tumor nodules began to regress 48 h after the initiation of treatment; by day 8, no treated animal had measurable disease. However, 4 of 12 animals relapsed on days 48 through 60, whereas 8 animals remained tumor free after 200 days of follow-up. The specificity of the effect was demonstrated by the fact that imatinib did not inhibit tumor growth after injection of BCR-ABL-negative U937 cells.

Imatinib was also tested in a refined version of the transplantation model of CML (Pear et al., 1998). In these experiments, murine bone marrow is infected with a BCR-ABL retrovirus and subsequently transplanted into syngeneic recipients. Mice typically die within 3 weeks from CML. By contrast, animals treated with imatinib have prolonged survival. However, responses

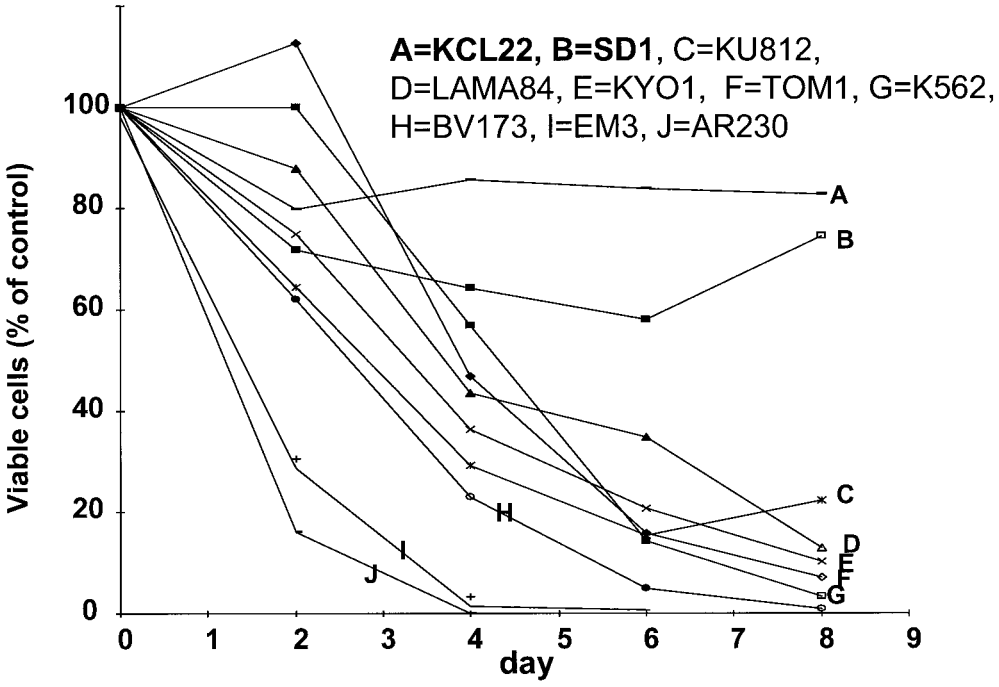

FIG. 5. BCR-ABL-positive cell lines were incubated with 1 μM imatinib, and viable cells were counted over an 8-day period. The KCL-22 and SD-1 cell lines were resistant, whereas all other lines were sensitive to imatinib.

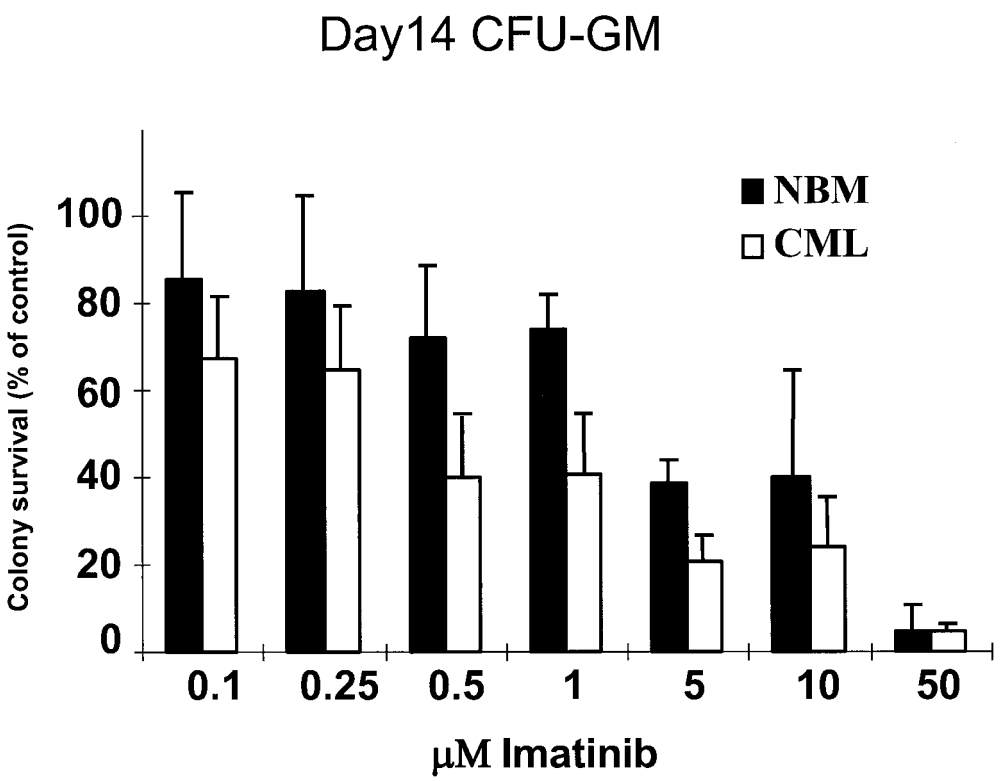

FIG. 6. Formation of myeloid colonies [colony-forming units-granulocyte macrophage (CFU-GM)] from normal individuals (NBM, ■) and CML patients (□) in the presence of imatinib.

were variable, and 25% of mice showed primary resistance to imatinib (Wolff and Ilaria, 2001). In no case was the compound able to prevent the disease, even if treatment was started as early as 48 h after injection of the BCR-ABL-infected bone marrow. No universal cause of resistance could be established in this study. Analysis of clonality showed, however, that there was "clonal depletion" in animals that responded to imatinib, indicating that the compound was able to successfully target some but not all leukemic clones.

C. Clinical Trials

1. Phase I. In June 1998, a phase I trial was initiated at three centers in the United States. The study population included CML patients in chronic phase who were resistant to or intolerant of interferon-α. The initial dose was 25 mg of imatinib daily. Hematological responses were seen with daily doses above 85 mg. Almost all patients (53/54, 98%) treated with at least 300 mg per day achieved complete hematological response. Thirty-one percent of patients obtained major cytogenetic responses, and 13% complete cytogenetic response. Importantly, responses were durable, with only 2/53 patients relapsing with a median follow-up of 265 days (Druker et al., 2001a).

The encouraging results in patients with CML in chronic phase led to an expansion of the protocol to 58 patients in blast crisis or with Ph-positive acute lymphoblastic leukemia. The minimum dose in this cohort was 300 mg of imatinib daily. Hematological responses were seen in 21/38 patients (55%) with myeloid phenotype and 14/20 of patients (70%) with lymphoid phenotype, including four complete responses in each group. Twelve percent of patients achieved a major and 8% a complete cytogenetic response. In contrast to patients treated in the chronic phase, approximately 50% of responders with myeloid disease and all but one responder with lymphoid disease relapsed between 42 and 193 days after initiating imatinib therapy (Druker et al., 2001b).

2. Phase II. Three international multicenter phase II protocols were initiated in the second half of 1999. They enrolled patients with CML in myeloid blast crisis, relapsed Ph-positive ALL, CML in accelerated phase, and patients with CML who had failed interferon-α.

In *myeloid blast crisis*, the results largely confirmed the results seen in the phase I study (Table 2) (Sawyers et al., 2002). Since no controlled trials are available for this patient population, it is difficult to conclude how the results of imatinib compare with conventional chemotherapeutic agents. However, the 1-year survival rate of 30% is better than in any reported study (Kantarjian et al., 1987, 1992, 1997). In addition, since imatinib is well tolerated (see below) and usually administered as an outpatient treatment, it can be assumed that there is a large gain in quality of life compared with conventional chemotherapy, which is associated with considerable toxicity. In contrast to myeloid blast crisis, where a small proportion of patients achieve durable remissions,

TABLE 2
Responses to imatinib in blast crisis and accelerated phase of CML

	Overall/Complete Hematological Responses	Sustained Responses (>4 weeks)	Major Cytogenetic Responses	Complete Cytogenetic Response	Median Survival	Overall Survival at 12 Months (Estimated)
	%	%	%	%	*months*	%
Myeloid blast crisis (n = 229)	52/15	31	16	7	6.8	30
Ph-positive acute lymphoblastic leukemia (n = 56)[a]	59/22	27	Not available	16	4.9	
Accelerated phase (n = 181)	82/53	69	24	17	Not reached	74

[a] And lymphoid blast crisis of CML.

there are practically no durable responses in blast crisis with a lymphoid phenotype and in Ph-positive acute lymphoblastic leukemia (Ottmann et al., 2002). The only exception may be patients treated for relapse after an allograft (Wassmann et al., 2001).

Patients in *accelerated phase* had not been studied in the phase I protocols. Accelerated phase was diagnosed when at least one of the following criteria was present: 1) bone marrow or peripheral blood blasts more than 15% but less than 30%; 2) blast combined with promyelocytes more than 30%; 3) basophils in the blood or marrow more than 20%; 4) platelets less than 100 $\times$ 10^9/liter (unrelated to therapy). Not surprisingly, the results for this group fall between those observed in myeloid blast crisis and chronic phase (Table 2) (Talpaz et al., 2002). As with myeloid blast crisis, there are very few controlled studies available for comparison. However, the 1-year survival rate of 74% with imatinib is twice as high as in the study with the best results previously published (Kantarjian et al., 1992). Moreover, cytogenetic responses in accelerated phase patients have rarely been observed, but 17% of patients treated with imatinib obtained a complete cytogenetic response. This might indicate that some of the responses may be maintained, a notion that is corroborated by the fact that achievement of a major cytogenetic response at 3 months was predictive of progression-free survival. The accelerated phase study had been initiated with an imatinib dose of 400 mg per day. When additional safety data became available, this dose was increased to 600 mg per day. Thus, a retrospective comparison between the two dose cohorts was possible and showed a significanty longer time to progression and overall survival for the 600 mg cohort.

The largest of the phase II trials enrolled patients in *chronic phase* who had previously failed interferon-α-based therapy. The patients were grouped as hematologically resistant or refractory, cytogenetically resistant or refractory and intolerant of interferon-α. Refractoriness implies that a response was never achieved, whereas resistance refers to loss of a response. The rate of complete hematological responses in all the patients was 95%, with an 89% progression-free survival at 18 months. Moreover, the rate of complete cytogenetic response was 41% with 60% major cytogenetic responses. The best results were seen in patients who had previously achieved a cytogenetic response to interferon-α (Table 3).

3. Phase III. A randomized study was initiated in June 2000 that compared imatinib to interferon-α plus cytarabine in newly diagnosed patients with CML in chronic phase. At that time, the combination of interferon and cytarabine was considered the best nontransplant therapy for CML (Guilhot et al., 1997). Results from the phase III study show that imatinib is vastly superior with respect to the rates of complete hematological remission, major cytogenetic remission, and complete cytogenetic remission. Most importantly, there was also a highly significant difference in the rate of progression to accelerated phase or blast crisis at 18 months (Table 4) (O'Brien et al., 2003). Based on these results, the FDA has approved imatinib as first-line treatment for newly diagnosed CML in December 2002.

D. Side Effects

Imatinib is generally very well tolerated. Although side effects are quite common, they are usually mild and only rarely lead to discontinuation of therapy. Side effects are more common in advanced phases of CML, reflecting the poorer performance status of many of these individuals. Toxicity can be broadly divided in hematological and nonhematological adverse events,

TABLE 3
Responses to imatinib in chronic phase of CML after failure of interferon-α

	Complete Hematological Response	Major Cytogenetic Response	Complete Cytogenetic Response
	%	%	%
Patients failing interferon (n = 454)	95	60	41
Hematologically refractory (n = 63)	89	41	25
Hematologically resistant (n = 70)	69	57	41
Cytogenetically refractory (n = 119)	97	55	31
Cytogenetically resistant (n = 41)	98	83	76
Intolerant (n = 161)	93	66	75

Median duration of follow-up = 18 months.

TABLE 4
Responses to imatinib vs. interferon + cytarabine in newly diagnosed CML patients in chronic phase

	Complete Hematological Response	Major Cytogenetic Response	Complete Cytogenetic Response	Progression-Free Survival (14 months)
		%		
Imatinib (n = 553)	95.3	85.2	73.8	92.1
Interferon-α + cytarabin (n = 553)	55.5	22.1	8.5	73.5
p =	0.001	0.001	0.001	0.001

Median duration of follow-up = 19 months.

and the following overview summarizes the observations from the controlled clinical trials as well as "expanded access" protocols. These less stringently controlled trials bridged the gap between the end of enrollment into the phase II trials and regulatory approval of imatinib.

1. Nonhematological Toxicity

a. Edema and Fluid Retention. Superficial edema, most frequently in the form of periorbital edema, is seen in approximately 50% of patients treated with imatinib. In some cases, more severe forms of fluid retention occurred, such as pleural and pericardial effusions, pulmonary edema, ascites, anasarca, and cerebral edema. The more serious adverse reactions necessitate interruption of therapy, while in the milder forms, diuretics may be used. The pathogenesis of the edema is not precisely understood, but current thinking links it to kinases inhibited by imatinib. For example, the PDGF-R has a role in the maintenance of the integrity of vessel walls, since mice with homozygous deletions of PDGF-R have defective blood vessels and, as a consequence, suffer from edema (Lindahl et al., 1997). In addition, mice with homozygous deletions of *ABL* and the *A*BL-related gene *ARG*, are also prone to edema (Koleske et al., 1998).

b. Gastrointestinal Side Effects. Mild nausea is common, particularly if imatinib is taken on an empty stomach. However, with studies indicating that there is no difference in absorption, when imatinib is taken together with food (Reckmann et al., 2002), the recommendation is now to take the drug with the largest meal of the day. It is thought that nausea and also the abdominal pain that some patients experience are due to the local irritative effects of imatinib. Mild diarrhea, also relatively common, may also be caused by local irritation. An alternative explanation, although unproven, would be that it results from the inhibition of c-*Kit* on the interstitial cells of Cajal, the pacemaker cells for gastrointestinal motility.

c. Skin Reactions. Skin rashes are seen in about one-third of patients. They vary greatly in appearance, and may be quite severe, with one patient developing Stevens-Johnson syndrome (Hsiao et al., 2002). Most of the rashes are mild and self-limited or respond to antihistamines or steroids. Rashes do not necessarily recur when therapy is resumed after being discontinued; however, skin reactions are the most frequent reason for permanent discontinuation of imatinib therapy. Apart from these drug-induced reactions, an urticarial rash has been seen at the start of treatment in patients with high basophil counts. It is likely that this reaction is the consequence of histamine release from the basophils, and it tends to subside when remission is induced. In a few cases, changes of skin pigmentation and darkening of the hair have been seen (Etienne et al., 2002). This may be due to imatinib effects on melanocytes that express c-*kit*.

d. Arthralgia, Myalgia, and Bone Pain. Bone, joint, and muscle pain are frequent side effects, although they are rarely severe enough to require discontinuation of treatment. Muscle cramps are very common, can be quite unpleasant, and frequently respond well to calcium supplements or quinine. Their pathogenesis is not known.

e. Liver Toxicity. Preclinical studies in rats and dogs suggested that liver toxicity would be a major problem in clinical trials of imatinib. Fortunately, liver toxicity has been relatively uncommon. One patient with advanced CML died of liver failure; besides imatinib, he was taking large quantities of acetaminophen, thus a causal relation to imatinib could not be established with certainty. Nonetheless, monitoring of liver function tests is mandatory and should be continued routinely for as long as patients are on imatinib, since toxicity may develop late. Histology has been consistent with a toxic drug reaction, without any peculiarities specific to imatinib.

2. Hematological Toxicity. Myelosuppression may reflect a therapeutic effect but could also be due to toxicity to normal hematopoietic cells. Generally, severe neutropenia and thrombocytopenia are more common in advanced disease, particularly blast crisis. This may be due to the smaller numbers of residual Ph-negative stem cells that are available for the reestablishment of normal hematopoiesis (Petzer et al., 1996). Another observation that supports this view is that in some cases the peripheral blood counts recover, when the patient achieves a cytogenetic response while remaining on imatinib therapy. The management of imatinib-induced myelosuppression requires experience. The guiding principle should be to match the aggressiveness of therapy with the aggressiveness of the disease. This implies that in patients with advanced disease it may be justified to continue imatinib in the face of myelosuppression although in early chronic phase interrupting therapy is advisable. Myeloid growth factors have been used suc-

cessfully to treat neutropenia and do not seem to adversely affect prognosis (Mauro et al., 2001).

V. Pharmakokinetics

Imatinib is well absorbed after oral administration, and no significant interaction with food intake is observed (Reckmann et al., 2002). The half-life of the drug is approximately 18 h. With once daily administration of 400 mg orally per day, median peak plasma concentrations at steady state are 5.4 μM, and median trough levels are 1.43 μM (Druker et al., 2001a). Thus, concentrations are achieved that are several times higher than the IC_{50} values in assays of intracellular tyrosine phosphorylation. Imatinib is metabolized by the CYP3A4/5 enzyme system. Thus inhibitors and inducers of this system are expected to alter plasma concentrations. One patient who was treated with phenytoin and imatinib failed to achieve a hematological response and was found to have drug levels that were 4-fold lower than expected. He obtained a complete hematological remission after discontinuation of phenytoin and a 1.5-fold dose increase (Druker et al., 2001a). Imatinib may also alter the plasma levels of other drugs that are metabolized by the CYP3A4/5 system, such as cyclosporine A or simvastatin. The interaction with cyclosporine A may be particularly relevant if imatinib is used in patients after allogeneic stem cell transplantation.

VI. Monitoring Patients on Imatinib

CML patients are usually monitored with blood counts and bone marrow cytogenetics, and these methods have been used to assess response to treatment in the clinical studies of imatinib. Complemented with quantitative RT-PCR, these tests will continue to be used to monitor therapy. However, given that suppression of Bcr-Abl tyrosine kinase activity is likely to be crucial for therapeutic success, monitoring of phosphotyrosine levels in the leukemic target cells would also be desirable. This would allow adjusting the dose in the case of insufficient suppression of Bcr-Abl kinase activity, assuming that such insufficient dosing would lead to relapse. Thus far, the phosphorylation of CrkL, the major tyrosine-phosphorylated protein in CML neutrophils (Oda et al., 1994), has been used for monitoring (Druker et al., 2001a). This is a cumbersome test that requires immunoblotting of protein from patient cells and is difficult to use in a routine setting. Other options that are being developed include fluorescence-activated cell sorting analysis and enzyme-linked immunosorbent assay tests that might be more suitable for routine detection of cellular phosphotyrosine. The second important area of development is monitoring for mutations of the BCR-ABL kinase domain. As discussed below, resistance is frequently caused or associated with kinase domain mutations that interfere with imatinib binding. Early detection of such mutations would be desirable, since it might influence therapeutic decisions.

VII. Imatinib in Drug Combinations

Imatinib has been tested in combination with a large number of conventional cytotoxic agents (for a recent review, see La Rosee et al., 2002b). The rationale is that the Bcr-Abl tyrosine kinase confers resistance to the induction of apoptosis, and this should be correctable by inhibition of Bcr-Abl. Most of the combinations show additive to synergistic anti-proliferative effects (Thiesing et al., 2000; Kano et al., 2001; Topaly et al., 2001). Importantly, interferon-α and cytarabine act synergistically with imatinib, which forms the basis for using them in combination with imatinib in clinical trials. In contrast, the results for hydrea, the third agent commonly used in CML, are conflicting, with ambiguous results in cell lines (Kano et al., 2001; Topaly et al., 2001) but with additive or synergistic effects in primary cells (Thiesing et al., 2000). By contrast, combinations of imatinib with methotrexate or topotecan were consistently antagonistic. It remains to be seen whether drug combinations are really superior to imatinib alone. At least in newly diagnosed patients, it will be difficult to improve upon the results of imatinib monotherapy in terms of cytogenetic response. Other endpoints such as molecular response may be suitable if they are demonstrated to be useful surrogates for survival.

VIII. Mechanism of Action

A. Binding of Imatinib to the Abl Kinase Domain

The catalytic domains of eukaryotic serine/threonine and tyrosine kinases have a highly conserved bilobed structure. The NH_2-terminal lobe (N-lobe) contains a β-sheet and a conserved α helix (helix C), whereas the C-lobe is helical. In the interface between the two lobes, a series of highly conserved residues form the ATP binding and catalytic sites (Nagar et al., 2002). The activation state of kinases is dependent on the position of the activation loop (A-loop), a portion of the C-lobe, which in Abl comprises amino acid residues 381–402 [numbering according to Shtivelman et al. (1986)]. In active kinases, the A-loop is in an "open" conformation, because it swings away from the catalytic center of the kinase (Fig. 7). The three NH_2-terminal residues of the A-loop (amino acids 381–383) contain a strictly conserved DFG (aspartate-phenylalanine-glycine) motif, which is crucial for catalytic activity. Aspartate 381 is able to bind Mg^{2+}, which in turn coordinates the phosphate groups of ATP. The COOH-terminal portion of the A-loop serves as a platform for substrate binding.

Although the conformation of the A-loop is highly conserved in kinases when they are in the active, open conformation, there are considerable differences between their inactive (closed) conformations. Kinases are

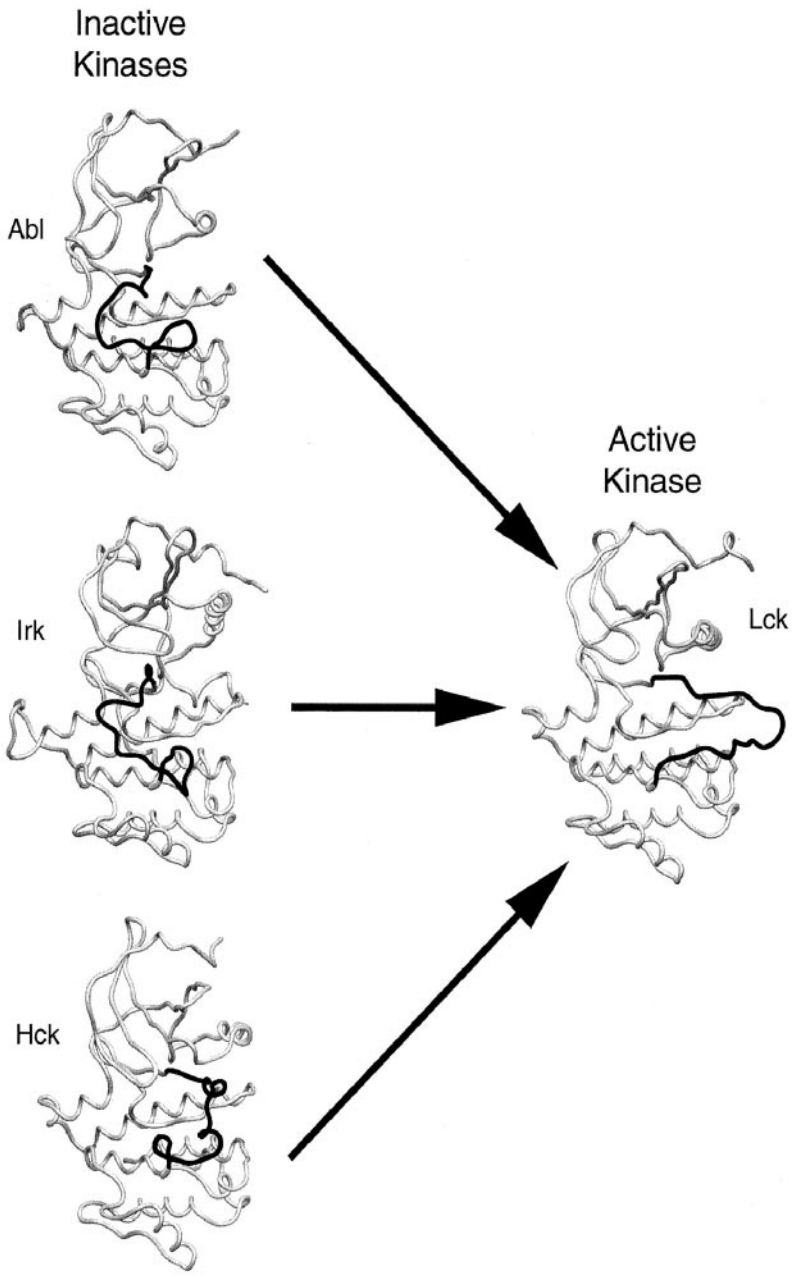

FIG. 7. Structure of the activation loop (in black) in the kinase domains of Abl, Hck, and Irk in their inactive state. Note that in Abl, the activation loop is folded back over the catalytic center of the molecule and occludes the mouth of the kinase. In the inactive state, the activation loops have distinct configurations, which form the basis for specific inhibition. In contrast, tyrosine kinase domains are very similar in their active states, when the activation loop is folded away from the catalytic center as in active Lck (adapted from Nagar et al., 2002 with permission from the American Association for Cancer Research).

activated by phosphorylation of key serine/threonine or tyrosine residues within the A-loop. In the case of Abl, tyrosine 393 is phosphorylated and points away from the center of the kinase, allowing substrates to bind. In the inactive state of Abl, tyrosine 393 is unphosphorylated and points toward the center of the kinase, mimicking a substrate by forming a hydrogen bond with asparagine 363. In this conformation, the mouth of the kinase is occluded, preventing substrate binding (Schindler et al., 2000; Nagar et al., 2002).

Imatinib functions as a competitive inhibitor of ATP binding, with a K_i value of 85 nM (Buchdunger et al., 1995). The crystal structure of the catalytic domain of the Abl kinase in complex with an imatinib analog (Schindler et al., 2000) and with imatinib (Nagar et al., 2002) has been solved. The most important finding of these studies is that the compound binds to the inactive conformation of Abl, contacting 21 amino acid residues (Nagar et al., 2002). By exploiting the distinct inactive conformation of the A-loop of Abl, imatinib is able to achieve its high specificity. No major structural rearrangements are required for imatinib to bind to the A-loop. In contrast, there is an induced-fit mechanism for binding to occur in the N-lobe, which normally ac-

commodates the phosphate groups of ATP and is therefore referred to as the P-loop. The P-loop is a glycine-rich and highly flexible structure, which folds down upon binding of imatinib, resulting in increased surface complementarity. This change in position is stabilized by a newly formed hydrogen bond between Tyr-253 and Asn-322 (Schindler et al., 2000). A consequence of the induced fit is the formation of a hydrophobic cage that surrounds imatinib, engaging van der Waals interactions with residues Tyr-253, Leu-370, and Phe-382 (Fig. 8A) (Nagar et al., 2002). Moreover, imatinib forms a number of hydrogen bonds with the kinase domain. Methionine 318 [which normally binds N1 of ATP (Schindler et al., 2000)], threonine 315, methionine 290, glutamine 286, lysine 270, and asparagine 381, together with water molecules, form a network of hydrogen bonds around the imatinib molecule (Fig. 8B). Given this extremely tight fit, it is not surprising that changes of single amino acids can affect the binding of imatinib. For example, threonine 315 is replaced by methionine in the insulin receptor kinase (Schindler et al., 2000), which is completely insensitive to imatinib (Table 1) (Nagar et al., 2002).

In agreement with these predictions, Abl phosphorylated on tyrosine 393 is much less sensitive to imatinib, since phosphorylation of tyrosine 393 stabilizes the active, open conformation of the A-loop (Schindler et al., 2000; Roumiantsev et al., 2002), to which imatinib does not bind. Bcr-Abl is constitutively tyrosine-phosphorylated and will thus be in a conformation that is unable to bind imatinib. From experiments with BCR-ABL-positive cell lines treated with imatinib, it is known that exposure to the compound results in inhibition of kinase activity within minutes. This indicates that there is rapid turnover between the phosphorylated form (constituting the bulk of the protein) and the unphosphorylated form, which is capable of binding imatinib. This points to an important role of tyrosine phosphatases.

IX. Resistance to Imatinib

A. In Vitro Models

Several groups have generated cell lines that are resistant to imatinib (le Coutre et al., 2000; Mahon et al., 2000; Weisberg and Griffin, 2000). In most cases, these lines were generated by continuous growth of cells in increasing concentrations of the inhibitor. Both human CML lines and murine lines engineered to express a BCR-ABL construct were used. The most common mechanism of resistance was increased expression of BCR-ABL mRNA and protein, caused by gene amplification or increased transcription. In one line, overexpression of the *MDR-1* gene was demonstrated in addition to overexpression of Bcr-Abl (Mahon et al., 2000), suggesting that drug efflux may contribute to resistance. In several cases, it was not possible to define a specific mechanism of resistance. In contrast to clinical resistance (see be-

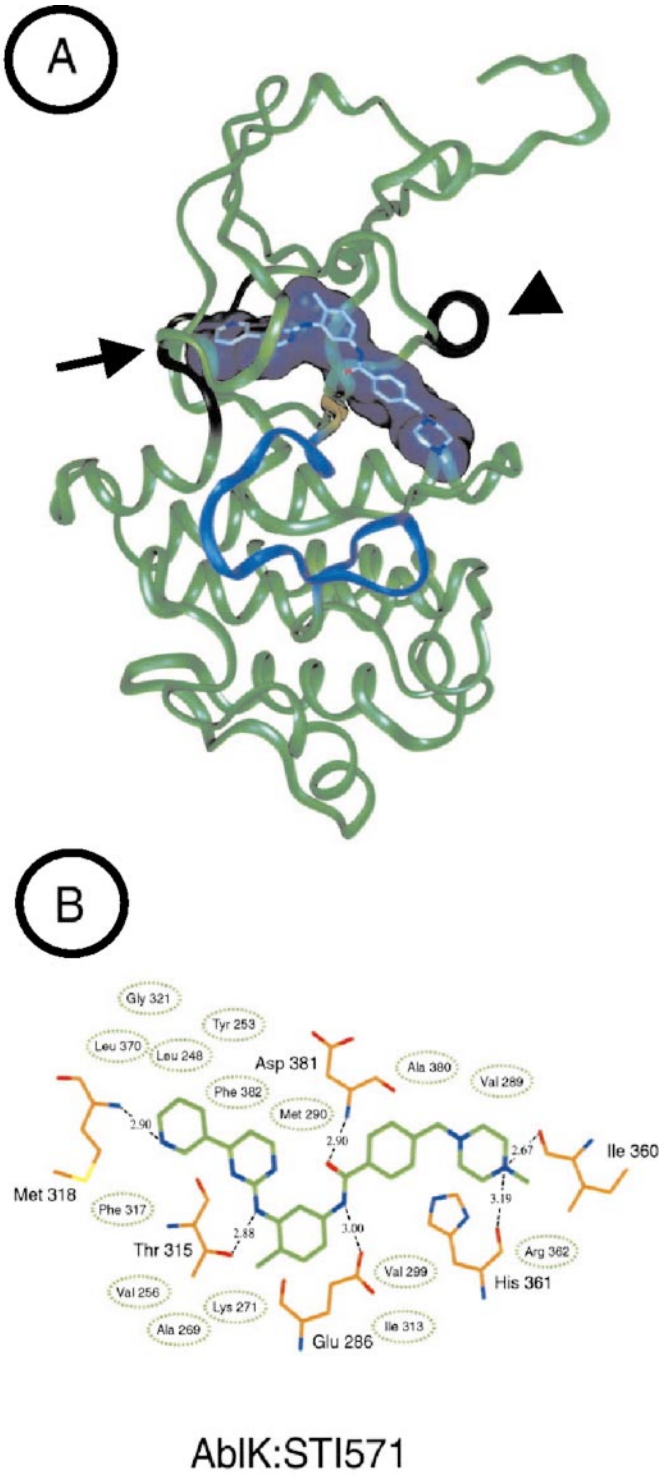

FIG. 8. A, structure of imatinib bound to the kinase domain of Abl. Arrow, P-loop; arrowhead, helix αC. The activation loop is shown in blue, with the conserved DFG motif in gold. Imatinib penetrates the center of the kinase, stabilizing the inactive conformation of the activation loop. Binding to the activation loop occurs without major steric clashes, whereas binding to the P-loop involves an induced fit mechanism. B, amino acids contacting imatinib. Imatinib: carbon is shown in green, nitrogen in blue, and oxygen in red. Carbons of the protein backbone are shown in orange. Hydrogen bonds are indicated as dashed lines. Numbers represent distances in Angström units. Residues that form hydrophobic interactions are circled. Imatinib contacts 21 amino acids within the Abl kinase domain (adapted from Nagar et al., 2002 with permission from the American Association for Cancer Research).

low), mutations of the kinase domain of Abl were not detected in any of these studies, although recently a cell line was described that acquired a mutation in vitro (Ricci et al., 2002).

B. Resistance in Patients

Most patients who acquire resistance to imatinib after an initial response have reactivated Bcr-Abl kinase activity (Gorre et al., 2001; Hochhaus et al., 2002; Shah et al., 2002; von Bubnoff et al., 2002). In these individuals, mutations of the Abl kinase domain are very common. Many of these mutations affect residues that form hydrogen bonds or hydrophobic interactions with imatinib (Fig. 8B). Mutations cluster in four regions of the kinase, the P-loop (the region that normally accomodates the phosphate residues of ATP), threonine 315, methionine 351, and the A-loop (activation loop). In vitro studies using purified proteins demonstrated that some mutants (e.g., T315I, E255K) have a one to two log increase of their IC_{50} values for imatinib compared with wild-type Bcr-Abl (Corbin et al., 2002; Roumiantsev et al., 2002). These findings were confirmed in cellular assays of inhibition of kinase activity and proliferation (Shah et al., 2002; von Bubnoff et al., 2002; Corbin et al., 2003). However, other mutants have less dramatic shifts of their IC_{50} values. Thus, determining the precise mutation may be of major clinical relevance. For example, in a patient with a mutation that confers a moderate shift in the IC_{50}, dose escalation of imatinib may be effective, whereas this would probably be useless for one of the highly resistant mutations such as T315I (Gorre et al., 2001). This biochemical characterization is also important because it allows one to infer that mutants that are highly resistant to imatinib are causal events. In contrast, other mutants are merely as sensitive as wild-type Bcr-Abl to imatinib and may be epiphenomena as opposed to causal events (Corbin et al., 2003).

One intriguing question is when the mutations arise. Although originally described only at the time of resistance to imatinib, several groups reported the presence of mutations even before therapy with the inhibitor (Roche-Lestienne et al., 2002; Shah et al., 2002). Thus, the selective pressure exerted by imatinib appears to favor the outgrowth of pre-existing resistant clones, in striking analogy to a bacterial culture treated with an antibiotic. It is likely that if more sensitive technology were available, mutations would be detectable in even more patients before imatinib therapy. Importantly, there is no evidence of a pronounced general increase in the rate of point mutations in resistant patients: analysis of the BCR and KIT genes in resistant patients revealed only wild-type sequences (Shah et al., 2002). Whether some of the mutations confer a growth advantage to the malignant clone even in the absence of imatinib is also of interest. This possibility has been suggested by the finding of increased kinase activity of mutations involving tyrosine 253 (Roumiantsev et al., 2002) and threonine 315 (Warmuth et al., 2003).

The second well defined mechanism of resistance in patients is overexpression of Bcr-Abl. In some cases, gene amplification has been demonstrated by FISH (Gorre et al., 2001; Hochhaus et al., 2002), whereas others have an increase in BCR-ABL transcripts (measured by quantitative PCR) without amplification at the DNA level (Hochhaus et al., 2002). Regardless of the precise mechanism, the end result is expected to be an increase of Bcr-Abl protein that out-competes imatinib, although increased expression of Bcr-Abl has not been demonstrated in patients, most likely because it is notoriously difficult to analyze Bcr-Abl protein in clinical specimens. Dose escalation of imatinib might restore responsiveness to imatinib in patients with increased expression of Bcr-Abl as the mechanism of resistance.

There are also cases where no mechanism of resistance can be identified. Not infrequently, there is clonal cytogenetic evolution in such patients, i.e., the acquisition of cytogenetic abnormalities in addition to the Ph chromosome (Hochhaus et al., 2002). If and how these cytogenetic abnormalities are causally related to resistance is unknown, although clonal evolution is associated with a higher risk of disease progression (Marktel et al., 2001). Some patients with myeloid blast crisis exhibit primary resistance to imatinib, without BCR-ABL mutations, gene amplification or additional chromosomal abnormalities. In these patients, drug efflux may play a role, because they tend to express high levels of *MRP1* mRNA (Lange et al., 2003).

A question that arises from the clinical studies with imatinib is whether the mechanisms of resistance will be similar in other malignancies treated with a specific targeted inhibitor or whether they will vary with the type of malignancy. As yet, there are not enough additional examples to draw firm conclusions. However, mutations of c-*KIT* that confer resistance to imatinib have been observed in patients with GISTS treated with the inhibitor (M. C. Heinrich, personal communication). It will be of interest to see if the same phenomena will be observed in acute myeloid leukemia patients treated with specific inhibitors of the Flt3 tyrosine kinase (Kelly et al., 2002).

X. Other Novel Agents for the Treatment of Chronic Myelogenous Leukemia

The observation of resistance to imatinib has led to a renewed interest in other drugs that may be useful in this situation. The agents that will be discussed belong to one of three categories: 1) agents that target pathways that are activated by Bcr-Abl; 2) agents that affect the stability of the Bcr-Abl protein; 3) alternative inhibitors of the Abl kinase.

A. Agents That Target Pathways Downstream of Bcr-Abl

A multitude of signaling pathways are activated by Bcr-Abl. These pathways are potential targets for intervention where inhibition of Bcr-Abl itself is not complete. It is also conceivable that oncogenic stimuli other than Bcr-Abl activate such pathways.

1. Farnesyl Transferase Inhibitors. Farnesylation, i.e., the transfer of an isoprenoid moiety to the C terminus of the protein, is required for *Ras* to localize to the cell membrane. This subcellular localization is necessary for *Ras* to activate *Raf1* and the MAP kinase pathway (Marais et al., 1995). Numerous cellular proteins are farnesylated and the precise mechanism of growth inhibition by FTIs is not known. Since *Ras* is a major pathway activated by Bcr-Abl, this pathway is presumed to be the target of FTIs in these cells. FTIs inhibit the proliferation of Bcr-Abl-positive cells (Peters et al., 2001), including those that are resistant to imatinib (Hoover et al., 2002) but do not induce apoptosis. Simultaneous treatment of Bcr-Abl expressing cells with imatinib and FTIs results in apoptosis only if some inhibition of Bcr-Abl is achieved, as in cell lines with increased expression of Bcr-Abl. In contrast, apoptosis is not induced in cell lines that express Bcr-Abl with the T315I mutation, which has an extremely high IC_{50} (Hoover et al., 2002). This suggests that for the induction of apoptosis, Bcr-Abl tyrosine kinase activity must be reduced below a certain threshold.

2. Mitogen-Activated Protein Kinase Inhibitors. As mentioned, activation of *Ras* by Bcr-Abl is thought to activate the MAP kinase pathway. Synergism of MAP kinase inhibitors with imatinib was demonstrated in BCR-ABL-positive cell lines, whereas this drug combination did not affect the proliferation of normal mononuclear cells (Yu et al., 2002).

3. Phosphatidylinositol-3 Kinase Inhibitors. Phosphatidylinositol-3 kinase activity is another major downstream target of Bcr-Abl that has been shown to be required for Bcr-Abl to induce leukemia in mice (Skorski et al., 1995). In cell lines, phosphatidylinositol-3 kinase inhibitors are synergistic with imatinib. The situation is less clear in primary cells, where there is great variation between individual patients (Klejman et al., 2002a).

One general caveat with all agents that target pathways downstream of Bcr-Abl may be the fact that inhibition of individual pathways may not be sufficient to shut down the entire system. This notion is derived from studies of mice with homozygous deletions of the genes for STAT5 (Sexl et al., 2000), IL-3, or GM-CSF (Li et al., 2001), respectively. In the transplantation model of CML, these mice still develop BCR-ABL-positive leukemias, which implies that STAT5, GM-CSF, and IL-3 are dispensable for leukemogenesis, although previous studies had indicated otherwise (Sillaber et al., 2000).

B. Agents That Affect the Bcr-Abl Protein

Geldanamycin and derivatives inhibit heat shock protein 90 (HSP90), a molecular chaperone that stabilizes the Bcr-Abl protein, among others. Treatment of Bcr-Abl expressing cell lines with these agents suppresses and induces apoptosis (Shiotsu et al., 2000). In their presence, Bcr-Abl is degraded via the proteasome pathway. Interestingly, Bcr-Abl proteins with mutations of the kinase domain may be even more sensitive to geldanamycin than wild-type protein (Gorre et al., 2002), which makes these agents particularly attractive for therapy in this situation. Arsenic trioxide also leads to the degradation of Bcr-Abl protein, although the mechanism is not understood (Perkins et al., 2000).

C. Alternative Abl Inhibitors

Several compounds, originally described as Src kinase inhibitors were subsequently shown to inhibit Abl at nanomolar concentrations (Dorsey et al., 2000). One eminent alternative Abl inhibitor is PD180970, a pyrido-[2,3-*d*]pyrimidine. The crystal structure of PD173955, a closely related member of this group, in complex with imatinib has been solved. These studies indicate that the most important difference between the binding of imatinib and PD173955 is the fact that the latter binds both active and inactive conformations of Abl (Nagar et al., 2002). Although PD173955 contacts far fewer amino acid residues than does imatinib, it inhibits the tyrosine kinase at approximately 100-fold lower concentrations. A recent study in our laboratory showed that PD180970 is active against Abl mutations affecting the P-loop and A-loop of Abl (La Rosee et al., 2002c). In contrast, there is no activity toward the T315I mutant. Although the pharmacological properties of this compound make it unsuitable for clinical use, these data suggest that it will be possible to target imatinib-resistant Abl mutants. Given the fact that reactivation of Bcr-Abl is observed in most patients who develop imatinib resistance (Gorre et al., 2001), Bcr-Abl tyrosine kinase activity remains a good target for specific interaction.

D. Partially Selective Inhibitors

Several compounds have less biochemical specificity for inhibition of Abl, but may still have biological selectivity. Examples are the tyrphostin AG957 and its derivative Adaphostin (NSC860410), which has improved pharmacokinetic properties. Both agents exhibit selective anti-proliferative effects against BCR-ABL-positive cells. Adaphostin is synergistic with imatinib and has activity in imatinib-resistant cell lines with Bcr-Abl overexpression (Mow et al., 2002). The activity against Bcr-Abl mutants with kinase domain mutations has not been evaluated.

Src kinases may be involved in Bcr-Abl-mediated malignant transformation. It has been shown that HCK, a Src kinase, phosphorylates Bcr-Abl independent of Abl

kinase activity (Warmuth et al., 1997). HCK may also be responsible for the activation of STAT5 in BCR-ABL-positive cells (Klejman et al., 2002b). As noted above, many Src inhibitors are also active against Abl. Two such Abl-Src inhibitors, PP1 and CGP76030, have been tested in cell lines expressing several types of imatinib-resistant Bcr-Abl mutants (Warmuth et al., 2003). Several of these Bcr-Abl mutants (A380C, A380T, D246S/E249S) were sensitive to these compounds by in vitro kinase assays, whereas mutations involving Thr315 were resistant. Nonetheless, the compounds inhibited the growth of cell lines expressing any of these mutants, presumably due to inhibition of an Src kinase. Not surprisingly, both compounds also affected the growth of IL-3-dependent cells that do not express Bcr-Abl. Thus, broadening the range of targets may lead to a loss of specificity.

XI. Imatinib for the Treatment of Malignancies Other Than Philadelphia-Positive Leukemia

Imatinib is also a potent inhibitor of c-*Kit* and PDGF-R. Activating mutations of c-*Kit* are found in the majority of patients with GISTs, a neoplasm that is practically unresponsive to conventional cytotoxic drugs (Blanke et al., 2001). GIST patients with c-*Kit* mutations respond dramatically to imatinib (Joensuu et al., 2001; van Oosterom et al., 2001). The PDGF-R tyrosine kinase is activated as a result of the t(5;12) translocation in some patients with chronic myelomonocytic leukemia, and rapid and sustained responses have also been observed in these patients (Apperley et al., 2002). Another disease that responds to imatinib is the hypereosinophilic syndrome, although the target of imatinib is not yet known (Gleich et al., 2002).

Targeted therapies for conditions with other defined molecular lesions are also being developed. For example, approximately 30% of patients with acute myeloid leukemia have activating mutations of the Flt3-tyrosine kinase (Schnittger et al., 2002). Various Flt3 inhibitors are currently being evaluated in clinical trials (Kelly et al., 2002). Another well defined target is the Alk-tyrosine kinase that is overexpressed as a consequence of the NPM-ALK fusion in some patients with anaplastic large cell lymphoma (Elmberger et al., 1995). This approach will not be restricted to tyrosine kinases: activating mutations of *B-Raf*, a serine/threonine kinase involved in the *Ras* signaling cascade, have recently been detected in 66% of melanomas and also in a number of other solid tumors (Davies et al., 2002). This suggests a role for *B-Raf* activation in these malignancies and that targeting this kinase may have therapeutic utility.

XII. Future Perspectives

The unprecedented response rates seen with imatinib have profoundly changed the management of CML. However, only isolated patients have become negative by RT-PCR, whereas most patients treated with an allogeneic stem cell transplant will achieve this landmark. As long as there is residual disease detectable, there is still a risk of relapse, and longer follow-up will be required to see how durable the responses to imatinib will be. Two recent reports showed that the primary effect of imatinib on primitive CML progenitor cells is inhibition of proliferation rather than induction of apoptosis (Graham et al., 2002; Holtz et al., 2002). One of these reports demonstrated the existence of a population of quiescent BCR-ABL-positive stem cells that are resistant to the induction of apoptosis by imatinib (Graham et al., 2002). It is not known why these cells escape the effects of imatinib. One possibility is that they express high levels of a drug efflux protein, similar to an imatinib-resistant cell line generated in vitro (Mahon et al., 2000). Another explanation could be that the survival of Ph-positive stem cells might rely on external growth factors rather than Bcr-Abl, making these cells immune to the effects of a Bcr-Abl inhibitor. If there is a high rate of relapse in newly diagnosed patients who achieve a complete cytogenetic remission, efforts will have to be directed at eliminating these residual stem cells. This will in part depend on the durability of cytogenetic remissions and the long-term toxicity profile of imatinib.

Imatinib is generally very well tolerated, particularly in patients with early CML, and grade 3 or 4 side effects are infrequent. Nonetheless, continuous monitoring of patients is necessary not only for response to therapy but also for late side effects. For example, some patients developed liver toxicity after more than 2 years of treatment. In addition, some patients in complete cytogenetic remission have developed cytogenetic abnormalities in their Ph-negative cells (Andersen et al., 2002; Bumm et al., 2003; O'Dwyer et al., 2003). The frequency of this phenomenon is currently unknown as are its prognostic implications. However, some of these individuals developed myelodysplasia, indicating that this may not be a benign condition (Bumm et al., 2003).

Another area of interest is the prediction of cytogenetic response to imatinib, and long-term disease-free survival. Although several prognostic factors have been identified in the phase II and III studies, it is currently not possible to predict responses in individual patients with certainty. Microarrays have been used to establish patterns of gene expression that distinguish between responders and nonresponders before therapy (Kaneta et al., 2002). If it were possible to prospectively identify nonresponders up-front, this would allow such patients to receive more aggressive treatments such as allogeneic stem cell transplantation.

The role of allografting for CML has changed already, with the numbers of patients undergoing this procedure dropping sharply after the introduction of imatinib (Gratwohl et al., 2002). However, there are unequivocal transplantation candidates with aggressive CML, in whom the role of imatinib would be the induction of

remission prior to allografting and possibly maintenance of remission thereafter. The available data regarding the use of imatinib in the transplant setting are limited, but a retrospective analysis found no evidence for increased transplant-related complications in patients who had previously been treated with imatinib (Deininger et al., 2002). Murine data indicate that administration of imatinib does not adversely affect engraftment of transplanted marrow (Hoepfl et al., 2002).

Imatinib has set a precedent for the approach of molecularly targeted therapy. It is hoped that this will lead to specific therapies for many other malignant conditions whose prognosis with conventional cytotoxic treatment is still dismal. The most important lesson to learn is that the identification of the right target and, thus, the choice of the right group of patients for an agent is crucial for success.

Note Added in Proof. While this manuscript was under review, important contributions elucidating the regulation of the Abl kinase were made that point to a crucial role of the N-terminal region and its myristoylation (Hantschel et al., 2003; Nagar et al., 2003). The molecular abnormality responsible for most cases of hypereosinophilic syndrome was identified as a fusion between the FIP1L1 and PDGFR-α genes (Cools et al., 2003).

Acknowledgments. We thank Chris Koontz for editorial assistance.

References

Abelson HT and Rabstein LS (1970) Lymphosarcoma: virus-induced thymic-independent disease in mice. *Cancer Res* **30:**2213–2222.

Afar DE, McLaughlin J, Sherr CJ, Witte ON, and Roussel MF (1995) Signaling by ABL oncogenes through cyclin D1. *Proc Natl Acad Sci USA* **92:**9540–9544.

Al Ali HK, Leiblein S, Kovacs I, Hennig E, Niederwieser D, and Deininger MW (2002) CML with an E1a3 BCR-ABL fusion: rare, benign and a potential diagnostic pitfall. *Blood* **100:**1092–1093.

Amos TA, Lewis JL, Grand FH, Gooding RP, Goldman JM, and Gordon MY (1995) Apoptosis in chronic myeloid leukaemia: normal responses by progenitor cells to growth factor deprivation, X-irradiation and glucocorticoids. *Br J Haematol* **91:** 387–393.

Anafi M, Gazit A, Zehavi A, Ben Neriah Y, and Levitzki A (1993) Tyrphostin-induced inhibition of p210bcr-abl tyrosine kinase activity induces K562 to differentiate. *Blood* **82:**3524–3529.

Andersen MK, Pedersen-Bjergaard J, Kjeldsen L, Dufva IH, and Brondum-Nielsen K (2002) Clonal Ph-negative hematopoiesis in CML after therapy with imatinib mesylate is frequently characterized by trisomy 8. *Leukemia* **16:**1390–1393.

Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter EJ, Chase A, Chessells JM, Colombat M, Dearden CE, et al. (2002) Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N Engl J Med* **347:**481–487.

Baccarani M, Rosti G, de Vivo A, Bonifazi F, Russo D, Martinelli G, Testoni N, Amabile M, Fiacchini M, Montefusco E, et al. (2002) A randomized study of interferon-alpha versus interferon-alpha and low-dose arabinosyl cytosine in chronic myeloid leukemia. *Blood* **99:**1527–1535.

Barila D and Superti Furga G (1998) An intramolecular SH3-domain interaction regulates c-Abl activity. *Nat Genet* **18:**280–282.

Bartram CR, de Klein A, Hagemeijer A, van Agthoven T, Geurts van Kessel A, Bootsma D, Grosveld G, Ferguson Smith MA, Davies T, and Stone M (1983) Translocation of c-abl oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. *Nature (Lond)* **306:**277–280.

Baskaran R, Wood LD, Whitaker LL, Canman CE, Morgan SE, Xu Y, Barlow C, Baltimore D, Wynshaw Boris A, Kastan MB, et al. (1997) Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. *Nature (Lond)* **387:**516–519.

Bedi A, Barber JP, Bedi GC, El-Deiry WS, Sidransky D, Vala MS, Akhtar AJ, Hilton J, and Jones RJ (1995) BCR-ABL-mediated inhibition of apoptosis with delay of G_2/M transition after DNA damage: a mechanism of resistance to multiple anti-cancer agents. *Blood* **86:**1148–1158.

Bedi A, Zehnbauer BA, Barber JP, Sharkis SJ, and Jones RJ (1994) Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. *Blood* **83:**2038–2044.

Bennett JH (1845) Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood. *Edinb Med Surg J* **64:**413–423.

Beran M, Cao X, Estrov Z, Jeha S, Jin G, O'Brien S, Talpaz M, Arlinghaus RB, Lydon NB and Kantarjian H (1998) Selective inhibition of cell proliferation and BCR-ABL phosphorylation in acute lymphoblastic leukemia cells expressing Mr 190,000 BCR-ABL protein by a tyrosine kinase inhibitor (CGP-57148). *Clin Cancer Res* **4:**1661–1672.

Blanke CD, Eisenberg BL, and Heinrich MC (2001) Gastrointestinal stromal tumors. *Curr Treat Options Oncol* **2:**485–491.

Bonifazi F, de Vivo A, Rosti G, Guilhot F, Guilhot J, Trabacchi E, Hehlmann R, Hochhaus A, Shepherd PC, Steegmann JL, et al. (2001) Chronic myeloid leukemia and interferon-alpha: a study of complete cytogenetic responders. *Blood* **98:**3074–3081.

Bornhauser M, Kiehl M, Siegert W, Schetelig J, Hertenstein B, Martin H, Schwerdtfeger R, Sayer HG, Runde V, Kroger N, et al. (2001) Dose-reduced conditioning for allografting in 44 patients with chronic myeloid leukaemia: a retrospective analysis. *Br J Haematol* **115:**119–124.

Bose S, Deininger M, Gora-Tybor J, Goldman JM, and Melo JV (1998) The presence of BCR-ABL fusion genes in leukocytes of normal individuals: implications for the assessment of minimal residual disease. *Blood* **92:**3362–3367.

Buchdunger E, Zimmermann J, Mett H, Meyer T, Muller M, Druker BJ, and Lydon NB (1996) Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. *Cancer Res* **56:**100–104.

Buchdunger E, Zimmermann J, Mett H, Meyer T, Muller M, Regenass U, and Lydon NB (1995) Selective inhibition of the platelet-derived growth factor signal transduction pathway by a protein-tyrosine kinase inhibitor of the 2-phenylaminopyrimidine class. *Proc Natl Acad Sci USA* **92:**2558–2562.

Bumm T, Muller C, Al Ali HK, Krohn K, Shepherd P, Schmidt E, Leiblein S, Franke C, Hennig E, Friedrich T, et al. (2003) Emergence of clonal cytogenetic abnormalities in Ph- cells in some CML patients in cytogenetic remission to imatinib but restoration of polyclonal hematopoiesis in the majority. *Blood* **101:**1941–1949.

Canitrot Y, Lautier D, Laurent G, Frechet M, Ahmed A, Turhan AG, Salles B, Cazaux C, and Hoffmann JS (1999) Mutator phenotype of BCR–ABL transfected Ba/F3 cell lines and its association with enhanced expression of DNA polymerase beta. *Oncogene* **18:**2676–2680.

Carlo Stella C, Dotti G, Mangoni L, Regazzi E, Garau D, Bonati A, Almici C, Sammarelli G, Savoldo B, Rizzo MT, et al. (1996) Selection of myeloid progenitors lacking BCR/ABL mRNA in chronic myelogenous leukemia patients after in vitro treatment with the tyrosine kinase inhibitor genistein. *Blood* **88:**3091–3100.

Carroll M, Ohno Jones S, Tamura S, Buchdunger E, Zimmermann J, Lydon NB, Gilliland DG, and Druker B (1997) CGP 57148, a tyrosine kinase inhibitor, inhibits the growth of cells expressing BCR-ABL, TEL-ABL and TEL-PDGFR fusion proteins. *Blood* **90:**4947–4952.

Cervantes F, Hernandez-Boluda JC, Ferrer A, Cid J, and Montserrat E (1999) The changing profile of Ph-positive chronic myeloid leukemia at presentation: possible impact of earlier diagnosis on survival. *Haematologica* **84:**324–327.

Chen G, Yuan SS, Liu W, Xu Y, Trujillo K, Song B, Cong F, Goff SP, Wu Y, Arlinghaus R, et al. (1999) Radiation-induced assembly of Rad51 and Rad52 recombination complex requires ATM and c-Abl. *J Biol Chem* **274:**12748–12752.

Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, Kutok J, Clark J, Galinsky I, Griffin JD, et al. (2003) A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N Engl J Med* **348:**1201–1214.

Corbin AS, Buchdunger E, Pascal F, and Druker BJ (2002) Analysis of the structural basis of specificity of inhibition of the Abl kinase by STI571. *J Biol Chem* **277:** 32214–32219.

Corbin AS, La Rosee P, Stoffregen EP, Druker BJ, and Deininger MW (2003) Several bcr-abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. *Blood* **101:**4611–4614.

Corso A, Lazzarino M, Morra E, Merante S, Astori C, Bernasconi P, Boni M, and Bernasconi C (1995) Chronic myelogenous leukemia and exposure to ionizing radiation—a retrospective study of 443 patients. *Ann Hematol* **70:**79–82.

Cortez D, Stoica G, Pierce JH, and Pendergast AM (1996) The BCR-ABL tyrosine kinase inhibits apoptosis by activating a Ras-dependent signaling pathway. *Oncogene* **13:**2589–2594.

Cross NC, Feng L, Chase A, Bungey J, Hughes TP, and Goldman JM (1993) Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. *Blood* **82:**1929–1936.

Dai Z and Pendergast AM (1995) Abi-2, a novel SH3-containing protein interacts with the c-Abl tyrosine kinase and modulates c-Abl transforming activity. *Genes Dev* **9:**2569–2582.

Daley GQ, Van Etten RA, and Baltimore D (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science (Wash DC)* **247:**824–830.

Dan S, Naito M, and Tsuruo T (1998) Selective induction of apoptosis in Philadelphia chromosome-positive chronic myelogenous leukemia cells by an inhibitor of BCR-ABL tyrosine kinase, CGP 57148. *Cell Death Differ* **5:**710–715.

Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, et al. (2002) Mutations of the BRAF gene in human cancer. *Nature (Lond)* **417:**949–954.

Dazzi F and Goldman J (1999) Donor lymphocyte infusions. *Curr Opin Hematol* **6:**394–399.

Deininger M, Goldman JM, Lydon NB, and Melo JV (1997) The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL positive cells. *Blood* **90:**3691–3698.

Deininger MW, Bose S, Gora-Tybor J, Yan XH, Goldman JM, and Melo JV (1998) Selective induction of leukemia-associated fusion genes by high-dose ionizing radiation. *Cancer Res* **58:**421–425.

Deininger MW, Goldman JM, and Melo JV (2000a) The molecular biology of chronic myeloid leukemia. *Blood* **96:**3343–3356.

Deininger MW, Vieira S, Mendiola R, Schultheis B, Goldman JM, and Melo JV (2000b) BCR-ABL tyrosine kinase activity regulates the expression of multiple

genes implicated in the pathogenesis of chronic myeloid leukemia. *Cancer Res* **60:**2049–2055.

Deininger MWN, Schleuning M, Sayer HG, Greinix H, Fischer T, Olavarria E, Maziarz RT, Martinez J, and Niederwieser D (2002) Allografting after imatinib therapy: no evidence for increased transplant-related mortality and favorable results in patients transplanted in remission. A retrospective study by the EBMT. *Blood* **100:**783a.

Diekmann D, Brill S, Garrett MD, Totty N, Hsuan J, Monfries C, Hall C, Lim L, and Hall A (1991) Bcr encodes a GTPase-activating protein for p21rac. *Nature (Lond)* **351:**400–402.

Djaldetti M, Padeh B, Pinkhas J, and De Vries A (1966) Prolonged remission in chronic myeloid leukemia after one course of busulfan. *Blood* **27:**103–109.

Dorsey JF, Jove R, Kraker AJ, and Wu J (2000) The pyrido[2,3-d]pyrimidine derivative PD180970 inhibits p210Bcr-Abl tyrosine kinase and induces apoptosis of K562 leukemic cells. *Cancer Res* **60:**3127–3131.

Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, and Talpaz M (2001b) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* **344:**1038–1042.

Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, et al. (2001a) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* **344:**1031–1037.

Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, and Lydon NB (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* **2:**561–566.

Elmberger PG, Lozano MD, Weisenburger DD, Sanger W, and Chan WC (1995) Transcripts of the Npm-Alk fusion gene in anaplastic large cell lymphoma, Hodgkin's disease and reactive lymphoid lesions. *Blood* **86:**3517–3521.

Etienne G, Cony-Makhoul P, and Mahon FX (2002) Imatinib mesylate and gray hair. *N Engl J Med* **347:**446.

Feinstein E, Cimino G, Gale RP, Alimena G, Berthier R, Kishi K, Goldman J, Zaccaria A, Berrebi A, and Canaani E (1991) p53 in chronic myelogenous leukemia in acute phase. *Proc Natl Acad Sci USA* **88:**6293–6297.

Gambacorti-Passerini C, le Coutre P, Mologni L, Fanelli M, Bertazzoli C, Marchesi E, di Nicola M, Biondi A, Corneo GM, Belotti D, et al. (1997) Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis. *Blood Cells Mol Dis* **23:**380–394.

Gleich GJ, Leiferman KM, Pardanani A, Tefferi A, and Butterfield JH (2002) Treatment of hypereosinophilic syndrome with imatinib mesilate. *Lancet* **359:**1577–1578.

Gordon MY, Dowding CR, Riley GP, Goldman JM, and Greaves MF (1987) Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. *Nature (Lond)* **328:**342–344.

Gorre ME, Ellwood-Yen K, Chiosis G, Rosen N, and Sawyers CL (2002) BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. *Blood* **100:**3041–3044.

Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, and Sawyers CL (2001) Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science (Wash DC)* **293:**876–880.

Gotoh A, Miyazawa K, Ohyashiki K, Tauchi T, Boswell HS, Broxmeyer HE, and Toyama K (1995) Tyrosine phosphorylation and activation of focal adhesion kinase (P125FAK) by BCR-ABL oncoprotein. *Exp Hematol (Copenh)* **23:**1153–1159.

Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, and Holyoake TL (2002) Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood* **99:**319–325.

Gratwohl A, Baldomero H, Horisberger B, Schmid C, Passweg J, and Urbano-Ispizua A (2002) Current trends in hematopoietic stem cell transplantation in Europe. *Blood* **100:**2374–2386.

Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, and Grosveld G (1984) Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell* **36:**93–99.

Guilhot F, Chastang C, Michallet M, Guerci A, Harousseau JL, Maloisel F, Bouabdallah R, Guyotat D, Cheron N, Nicolini F, et al. (1997) Interferon alfa-2b combined with cytarabine versus interferon alone in chronic myelogenous leukemia. French Chronic Myeloid Leukemia Study Group. *N Engl J Med* **337:**223–229.

Hansen JA, Gooley TA, Martin PJ, Appelbaum F, Chauncey TR, Clift RA, Petersdorf EW, Radich J, Sanders JE, Storb RF, et al. (1998) Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. *N Engl J Med* **338:**962–968.

Hantschel O, Nagar B, Guettler S, Kretzschmar J, Dorey K, Kuriyan J, and Superti-Furga G (2003) A myristoyl/phosphotyrosine switch regulates c-Abl. *Cell* **112:**845–857.

Hasford J, Pfirrmann M, Hehlmann R, Allan NC, Baccarani M, Kluin-Nelemans JC, Alimena G, Steegmann JL, and Ansari H (1998) A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. *J Natl Cancer Inst* **90:**850–858.

Hehlmann R, Heimpel H, Hasford J, Kolb HJ, Pralle H, Hossfeld DK, Queisser W, Loffler H, Hochhaus A, Heinze B, et al. (1994) Randomized comparison of interferon-alpha with busulfan and hydroxyurea in chronic myelogenous leukemia. The German CML Study Group. *Blood* **84:**4064–4077.

Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, and Groffen J (1990) Acute leukaemia in bcr/abl transgenic mice. *Nature (Lond)* **344:**251–253.

Heyssel R, Brill B, and Woodbury L (1960) Leukemia in Hiroshima bomb survivors. *Blood* **15:**313.

Hochhaus A, Kreil S, Corbin AS, La Rosee P, Muller MC, Lahaye T, Hanfstein B, Schoch C, Cross NC, Berger U, et al. (2002) Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia* **16:**2190–2196.

Hoepfl J, Miething C, Grundler R, Gotze KS, Peschel C, and Duyster J (2002) Effects of imatinib on bone marrow engraftment in syngeneic mice. *Leukemia* **16:**1584–1588.

Holtz MS, Slovak ML, Zhang F, Sawyers CL, Forman SJ, and Bhatia R (2002) Imatinib mesylate (STI571) inhibits growth of primitive malignant progenitors in chronic myelogenous leukemia through reversal of abnormally increased proliferation. *Blood* **99:**3792–3800.

Hoover RR, Mahon FX, Melo JV, and Daley GQ (2002) Overcoming STI571 resistance with the farnesyl transferase inhibitor SCH66336. *Blood* **100:**1068–1071.

Hsiao LT, Chung HM, Lin JT, Chiou TJ, Liu JH, Fan FS, Wang WS, Yen CC, and Chen PM (2002) Stevens-Johnson syndrome after treatment with STI571: a case report. *Br J Haematol* **117:**620–622.

Ilaria RL Jr and Van Etten RA (1995) The SH2 domain of P210BCR/ABL is not required for the transformation of hematopoietic factor-dependent cells. *Blood* **86:**3897–3904.

Italian Cooperative Study Group on CML (1994) Interferon alfa-2a as compared with conventional chemotherapy for the treatment of chronic myeloid leukemia. The Italian Cooperative Study Group on Chronic Myeloid Leukemia. *N Engl J Med* **330:**820–825.

Jiang X, Lopez A, Holyoake T, Eaves A, and Eaves C (1999) Autocrine production and action of IL-3 and granulocyte colony-stimulating factor in chronic myeloid leukemia. *Proc Natl Acad Sci USA* **96:**12804–12809.

Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B, et al. (2001) Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* **344:**1052–1056.

Johansson B, Fioretos T, and Mitelman F (2002) Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. *Acta Haematol* **107:**76–94.

Jonuleit T, Peschel C, Schwab R, van der Kuip H, Buchdunger E, Fischer T, Huber C, and Aulitzky WE (1998) Bcr-Abl kinase promotes cell cycle entry of primary myeloid CML cells in the absence of growth factors. *Br J Haematol* **100:**295–303.

Kaneta Y, Kagami Y, Katagiri T, Tsunoda T, Jin-nai I, Taguchi H, Hirai H, Ohnishi K, Ueda T, Emi N, et al. (2002) Prediction of sensitivity to STI571 among chronic myeloid leukemia patients by genome-wide CDNA microarray analysis. *Jpn J Cancer Res* **93:**849–856.

Kano Y, Akutsu M, Tsunoda S, Mano H, Sato Y, Honma Y, and Furukawa Y (2001) In vitro cytotoxic effects of a tyrosine kinase inhibitor STI571 in combination with commonly used antileukemic agents. *Blood* **97:**1999–2007.

Kantarjian HM, Keating MJ, Estey EH, O'Brien S, Pierce S, Beran M, Koller C, Feldman E, and Talpaz M (1992) Treatment of advanced stages of Philadelphia chromosome-positive chronic myelogenous leukemia with interferon-alpha and low-dose cytarabine. *J Clin Oncol* **10:**772–778.

Kantarjian HM, Keating MJ, Talpaz M, Walters RS, Smith TL, Cork A, McCredie KB, and Freireich EJ (1987) Chronic myelogenous leukemia in blast crisis. Analysis of 242 patients. *Am J Med* **83:**445–454.

Kantarjian HM, O'Brien SM, Keating M, Beran M, Estey E, Giralt S, Kornblau S, Rios MB, de Vos D, and Talpaz M (1997) Results of decitabine therapy in the accelerated and blastic phases of chronic myelogenous leukemia. *Leukemia* **11:**1617–1620.

Kantarjian HM, Vellekoop L, McCredie KB, Keating MJ, Hester J, Smith T, Barlogie B, Trujillo J, and Freireich EJ (1985) Intensive combination chemotherapy (ROAP 10) and splenectomy in the management of chronic myelogenous leukemia. *J Clin Oncol* **3:**192–200.

Kasper B, Fruehauf S, Schiedlmeier B, Buchdunger E, Ho AD, and Zeller WJ (1999) Favorable therapeutic index of a p210(BCR-ABL)-specific tyrosine kinase inhibitor; activity on lineage-committed and primitive chronic myelogenous leukemia progenitors. *Cancer Chemother Pharmacol* **44:**433–438.

Kaur G, Gazit A, Levitzki A, Stowe E, Cooney DA, and Sausville EA (1994) Tyrphostin induced growth inhibition: correlation with effect on p210bcr-abl autokinase activity in K562 chronic myelogenous leukemia. *Anticancer Drugs* **5:**213–222.

Kelly LM, Yu JC, Boulton CL, Apatira M, Li J, Sullivan CM, Williams I, Amaral SM, Curley DP, Duclos N, et al. (2002) CT53518, a novel selective FLT3 antagonist for the treatment of acute myelogenous leukemia (AML). *Cancer Cell* **1:**421–432.

Kharbanda S, Pandey P, Jin S, Inoue S, Bharti A, Yuan ZM, Weichselbaum R, Weaver D, and Kufe D (1997) Functional interaction between DNA-PK and c-Abl in response to DNA damage. *Nature (Lond)* **386:**732–735.

Klejman A, Rushen L, Morrione A, Slupianek A, and Skorski T (2002a) Phosphatidylinositol-3 kinase inhibitors enhance the anti-leukemia effect of STI571. *Oncogene* **21:**5868–5876.

Klejman A, Schreiner SJ, Nieborowska-Skorska M, Slupianek A, Wilson M, Smithgall TE, and Skorski T (2002b) The Src family kinase Hck couples BCR/ABL to STAT5 activation in myeloid leukemia cells. *EMBO (Eur Mol Biol Organ) J* **21:**5766–5774.

Koleske AJ, Gifford AM, Scott ML, Nee M, Bronson RT, Miczek KA, and Baltimore D (1998) Essential roles for the Abl and Arg tyrosine kinases in neurulation. *Neuron* **21:**1259–1272.

Kovalenko M, Ronnstrand L, Heldin CH, Loubtchenkov M, Gazit A, Levitzki A, and Bohmer FD (1997) Phosphorylation site-specific inhibition of platelet-derived growth factor beta-receptor autophosphorylation by the receptor blocking tyrphostin AG1296. *Biochemistry* **36:**6260–6269.

Lange T, Gunther C, Kohler T, Krahl R, Musiol S, Leiblein S, Al Ali HK, van Hoomissen I, Niederwieser D, and Deininger MW (2003) High levels of BAX, low levels of MRP-1 and high platelets are independent predictors of response to imatinib in myeloid blast crisis of CML. *Blood* **101:**2152–2155.

La Rosee P, Corbin AS, Stoffregen EP, Deininger MW, and Druker BJ (2002c) Activity of the Bcr-Abl kinase inhibitor PD180970 against clinically relevant Bcr-Abl isoforms that cause resistance to imatinib mesylate (Gleevec, STI571). *Cancer Res* **62:**7149–7153.

La Rosee P, Johnson K, O'Dwyer ME, and Druker BJ (2002a) In vitro studies of the combination of imatinib mesylate (Gleevec) and arsenic trioxide (Trisenox) in chronic myelogenous leukemia. *Exp Hematol (Copenh)* **30:**729–737.

La Rosee P, O'Dwyer ME, and Druker BJ (2002b) Insights from pre-clinical studies for new combination treatment regimens with the Bcr-Abl kinase inhibitor imatinib mesylate (Gleevec/Glivec) in chronic myelogenous leukemia: a translational perspective. *Leukemia* **16:**1213–1219.

le Coutre, Mologni L, Cleris L, Marchesi E, Buchdunger E, Giardini R, Formelli F, and Gambacorti-Passerini C (1999) In vivo eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. *J Natl Cancer Inst* **91:**163–168.

le Coutre P, Tassi E, Varella-Garcia M, Barni R, Mologni L, Cabrita G, Marchesi E, Supino R, and Gambacorti-Passerini C (2000) Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. *Blood* **95:**1758–1766.

Lewis JM and Schwartz MA (1998) Integrins regulate the association and phosphorylation of paxillin by c-Abl. *J Biol Chem* **273:**14225–14230.

Li S, Gillessen S, Tomasson MH, Dranoff G, Gilliland DG, and Van Etten RA (2001) Interleukin 3 and granulocyte-macrophage colony-stimulating factor are not required for induction of chronic myeloid leukemia-like myeloproliferative disease in mice by BCR/ABL. *Blood* **97:**1442–1450.

Lindahl P, Johansson BR, Leveen P, and Betsholtz C (1997) Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science (Wash DC)* **277:**242–245.

Lugo TG, Pendergast AM, Muller AJ, and Witte ON (1990) Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science (Wash DC)* **247:**1079–1082.

Mahon FX, Deininger MW, Schultheis B, Chabrol J, Reiffers J, Goldman JM, and Melo JV (2000) Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. *Blood* **96:**1070–1079.

Marais R, Light Y, Paterson HF, and Marshall CJ (1995) Ras recruits Raf-1 to the plasma membrane for activation by tyrosine phosphorylation. *EMBO (Eur Mol Biol Organ) J* **14:**3136–3145.

Marktel S, Bua M, Marin D, Chase A, Udom C, Armstrong L, Apperley JF, Olavarria E, and Goldman JM (2001) Emergence of additional chromosomal abnormalities following treatment with STI571 (imatinib mesylate) for Philadelphia positive chronic myeloid leukemia in chronic phase (Abstract). *Blood* **98:**217a.

Marley SB, Deininger MW, Davidson RJ, Goldman JM, and Gordon MY (2000) The tyrosine kinase inhibitor STI571, like interferon-alpha, preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors from patients with chronic myeloid leukemia. *Exp Hematol (Copenh)* **28:**551–557.

Maru Y and Witte ON (1991) The BCR gene encodes a novel serine/threonine kinase activity within a single exon. *Cell* **67:**459–468.

Mauro MJ, Kurilik G, Balleisen S, O'Dwyer ME, Reese SF, and Druker BJ (2001) Myeloid growth factors for neutropenia during imatinib mesylate (STI571) therapy for CML: preliminary evidence of safety and efficacy. *Blood* **98:**139a.

Mayer BJ and Baltimore D (1994) Mutagenic analysis of the roles of SH2 and SH3 domains in regulation of the Abl tyrosine kinase. *Mol Cell Biol* **14:**2883–2894.

McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, Chauncey TR, Gooley TA, Hegenbart U, Nash RA, et al. (2001) Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* **97:**3390–3400.

McWhirter JR, Galasso DL, and Wang JY (1993) A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. *Mol Cell Biol* **13:**7587–7595.

McWhirter JR and Wang JY (1993) An actin-binding function contributes to transformation by the Bcr-Abl oncoprotein of Philadelphia chromosome-positive human leukemias. *EMBO (Eur Mol Biol Organ) J* **12:**1533–1546.

Melo JV, Gordon DE, Cross NC, and Goldman JM (1993) The ABL-BCR fusion gene is expressed in chronic myeloid leukemia. *Blood* **81:**158–165.

Melo JV, Myint H, Galton DA, and Goldman JM (1994) P190BCR-ABL chronic myeloid leukaemia: the missing link with chronic myelomonocytic leukaemia? *Leukemia* **8:**208–211.

Million RP and Van Etten RA (2000) The Grb2 binding site is required for the induction of chronic myeloid leukemia-like disease in mice by the Bcr/Abl tyrosine kinase. *Blood* **96:**664–670.

Mow BM, Chandra J, Svingen PA, Hallgren CG, Weisberg E, Kottke TJ, Narayanan VL, Litzow MR, Griffin JD, Sausville EA, et al. (2002) Effects of the Bcr/Abl kinase inhibitors STI571 and adaphostin (NSC 680410) on chronic myelogenous leukemia cells in vitro. *Blood* **99:**664–671.

Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, Miller WT, Clarkson B, and Kuriyan J (2002) Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res* **62:**4236–4243.

Nagar B, Hantschel O, Young MA, Scheffzek K, Veach D, Bornmann W, Clarkson B, Superti-Furga G, and Kuriyan J (2003) Structural basis for the autoinhibition of c-Abl tyrosine kinase. *Cell* **112:**859–871.

Neumann E (1870) Ein Fall Von Leukämie Mit Erkrankung Des Knochenmarks. *Archive der Heilkunde.*

Nowell P and Hungerford D (1960) A minute chromosome in human chronic granulocytic leukemia. *Science (Wash DC)* **132:**1497.

O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, et al. (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* **348:**994–1004.

Oda T, Heaney C, Hagopian JR, Okuda K, Griffin JD, and Druker BJ (1994) Crkl is the major tyrosine-phosphorylated protein in neutrophils from patients with chronic myelogenous leukemia. *J Biol Chem* **269:**22925–22928.

O'Dwyer ME, Gatter K, Loriaux M, Druker B, Olsen JH, Lawce H, Mauro MJ, Maziarz RT, and Braziel RM (2003) The demonstration of clonal abnormalities in Philadelphia chromosome negative cells following imatinib mesylate induced major cytogenetic responses in patients with chronic myeloid leukemia. *Leukemia* **17:**481–487.

Okabe M, Uehara Y, Miyagishima T, Itaya T, Tanaka M, Kuni Eda Y, Kurosawa M, and Miyazaki T (1992) Effect of herbimycin A, an antagonist of tyrosine kinase, on bcr/abl oncoprotein-associated cell proliferations: abrogative effect on the transformation of murine hematopoietic cells by transfection of a retroviral vector expressing oncoprotein P210bcr/abl and preferential inhibition on Ph1-positive leukemia cell growth. *Blood* **80:**1330–1338.

Or R, Shapira MY, Resnick I, Amar A, Ackerstein A, Samuel S, Aker M, Naparstek E, Nagler A, and Slavin S (2003) Nonmyeloablative allogeneic stem cell transplantation for the treatment of chronic myeloid leukemia in first chronic phase. *Blood* **101:**441–445.

Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, Silver RT, Tura S, Fischer T, Deininger MW, Schiffer CA, et al. (2002) A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. *Blood* **100:**1965–1971.

Pane F, Frigeri F, Sindona M, Luciano L, Ferrara F, Cimino R, Meloni G, Saglio G, Salvatore F, and Rotoli B (1996) Neutrophilic chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). *Blood* **88:**2410–2414.

Papadopoulos P, Ridge SA, Boucher CA, Stocking C, and Wiedemann LM (1995) The novel activation of ABL by fusion to an ets-related gene, TEL. *Cancer Res* **55:**34–38.

Pear WS, Miller JP, Xu L, Pui JC, Soffer B, Quackenbush RC, Pendergast AM, Bronson R, Aster JC, Scott ML, et al. (1998) Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. *Blood* **92:**3780–3792.

Perkins C, Kim CN, Fang G, and Bhalla KN (2000) Arsenic induces apoptosis of multidrug-resistant human myeloid leukemia cells that express Bcr-Abl or overexpress MDR, MRP, Bcl-2, or Bcl-x(L). *Blood* **95:**1014–1022.

Peters DG, Hoover RR, Gerlach MJ, Koh EY, Zhang H, Choe K, Kirschmeier P, Bishop WR, and Daley GQ (2001) Activity of the farnesyl protein transferase inhibitor SCH66336 against BCR/ABL-induced murine leukemia and primary cells from patients with chronic myeloid leukemia. *Blood* **97:**1404–1412.

Petzer AL, Eaves CJ, Lansdorp PM, Ponchio L, Barnett MJ, and Eaves AC (1996) Characterization of primitive subpopulation of normal and leukemic cells present in the blood of patients with newly diagnosed as well as established chronic myeloid leukemia. *Blood* **88:**2162–2171.

Reckmann AH, Fischer T, and Peng B (2002) Effect of food on STI571 (Glivec) pharmacokinetics and bioavailability. *Proc Am Soc Clin Oncol* **20:**1223a.

Ricci C, Scappini B, Divoky V, Gatto S, Onida F, Verstovsek S, Kantarjian HM, and Beran M (2002) Mutation in the ATP-binding pocket of the ABL kinase domain in an STI571-resistant BCR/ABL-positive cell line. *Cancer Res* **62:**5995–5998.

Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, Lai JL, Philippe N, Facon T, Fenaux P, and Preudhomme C (2002) Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571 and they can pre-exist to the onset of treatment. *Blood* **100:**1014–1018.

Ron D, Zannini M, Lewis M, Wickner RB, Hunt LT, Graziani G, Tronick SR, Aaronson SA, and Eva A (1991) A region of proto-dbl essential for its transforming activity shows sequence similarity to a yeast cell cycle gene, CDC24 and the human breakpoint cluster gene, bcr. *New Biol* **3:**372–379.

Roumiantsev S, Shah NP, Gorre ME, Nicoll J, Brasher BB, Sawyers CL, and Van Etten RA (2002) Clinical resistance to the kinase inhibitor STI-571 in chronic myeloid leukemia by mutation of Tyr-253 in the Abl kinase domain P-loop. *Proc Natl Acad Sci USA* **99:**10700–10705.

Rowley JD (1973) A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature (Lond)* **243:**290–293.

Salgia R, Li JL, Lo SH, Brunkhorst B, Kansas GS, Sobhany ES, Sun Y, Pisick E, Hallek M, Ernst T, et al. (1995) Molecular cloning of human paxillin, a focal adhesion protein phosphorylated by P210BCR/ABL. *J Biol Chem* **270:**5039–5047.

Savage DG and Goldman JM (1997) Allografting for chronic myeloid leukemia. *Curr Opin Hematol* **4:**369–376.

Sawyers CL (1999) Chronic myeloid leukemia. *N Engl J Med* **340:**1330–1340.

Sawyers CL, Callahan W, and Witte ON (1992) Dominant negative MYC blocks transformation by ABL oncogenes. *Cell* **70:**901–910.

Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, Ottmann OG, Schiffer CA, Talpaz M, Guilhot F, Deininger MW, et al. (2002) Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood* **99:**3530–3539.

Sawyers CL, McLaughlin J, Goga A, Havlik M, and Witte O (1994) The nuclear tyrosine kinase c-Abl negatively regulates cell growth. *Cell* **77:**121–131.

Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, and Kuriyan J (2000) Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science (Wash DC)* **289:**1938–1942.

Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, Loffler H, Sauerland CM, Serve H, Buchner T, et al. (2002) Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood* **100:**59–66.

Schwartzberg PL, Stall AM, Hardin JD, Bowdish KS, Humaran T, Boast S, Harbison ML, Robertson EJ, and Goff SP (1991) Mice homozygous for the ablm1 mutation show poor viability and depletion of selected B and T cell populations. *Cell* **65:**1165–1175.

Sexl V, Piekorz R, Moriggl R, Rohrer J, Brown MP, Bunting KD, Rothammer K, Roussel MF, and Ihle JN (2000) Stat5a/b contribute to interleukin 7-induced B-cell precursor expansion, but abl- and bcr/abl-induced transformation are independent of stat5. *Blood* **96:**2277–2283.

Shafman T, Khanna KK, Kedar P, Spring K, Kozlov S, Yen T, Hobson K, Gatei M, Zhang N, Watters D, et al. (1997) Interaction between ATM protein and c-Abl in response to DNA damage. *Nature (Lond)* **387:**520–523.

Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, and Sawyers CL (2002) Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to

the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* **2:**117–125.

Shi Y, Alin K, and Goff SP (1995) Abl-interactor-1, a novel SH3 protein binding to the carboxy-terminal portion of the Abl protein, duppresses v-abl transforming activity. *Genes Dev* **9:**2583–2597.

Shiotsu Y, Neckers LM, Wortman I, An WG, Schulte TW, Soga S, Murakata C, Tamaoki T, and Akinaga S (2000) Novel oxime derivatives of radicicol induce erythroid differentiation associated with preferential G(1) phase accumulation against chronic myelogenous leukemia cells through destabilization of Bcr-Abl with Hsp90 complex. *Blood* **96:**2284–2291.

Shtivelman E, Lifshitz B, Gale RP, Roe BA, and Canaani E (1986) Alternative splicing of RNAs transcribed from the human abl gene and from the bcr-abl fused gene. *Cell* **47:**277–284.

Sill H, Goldman JM, and Cross NC (1995) Homozygous deletions of the P16 tumor-suppressor gene are associated with lymphoid transformation of chronic myeloid leukemia. *Blood* **85:**2013–2016.

Sillaber C, Gesbert F, Frank DA, Sattler M, and Griffin JD (2000) STAT5 activation contributes to growth and viability in Bcr/Abl-transformed cells. *Blood* **95:**2118–2125.

Skorski T, Kanakaraj P, Nieborowska Skorska M, Ratajczak MZ, Wen SC, Zon G, Gewirtz AM, Perussia B, and Calabretta B (1995) Phosphatidylinositol-3 kinase activity is regulated by BCR/ABL and is required for the growth of Philadelphia chromosome- positive cells. *Blood* **86:**726–736.

Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robertson JE, Tso CY, Braun TJ, Clarkson BD, Cervantes F, et al. (1984) Prognostic discrimination in "good-risk" chronic granulocytic leukemia. *Blood* **63:**789–799.

Takedam N, Shibuya M, and Maru Y (1999) The BCR-ABL oncoprotein potentially interacts with the xeroderma pigmentosum group B protein. *Proc Natl Acad Sci USA* **96:**203–207.

Talpaz M, Kantarjian H, Kurzrock R, Trujillo JM, and Gutterman JU (1991) Interferon-alpha produces sustained cytogenetic responses in chronic myelogenous leukemia. Philadelphia chromosome-positive patients. *Ann Intern Med* **114:**532–538.

Talpaz M, Silver RT, Druker BJ, Goldman JM, Gambacorti-Passerini C, Guilhot F, Schiffer CA, Fischer T, Deininger MW, Lennard AL, et al. (2002) Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood* **99:**1928–1937.

Thiesing JT, Ohno-Jones S, Kolibaba KS, and Druker BJ (2000) Efficacy of STI571, an abl tyrosine kinase inhibitor, in conjunction with other antileukemic agents against bcr-abl-positive cells. *Blood* **96:**3195–3199.

Topaly J, Zeller WJ, and Fruehauf S (2001) Synergistic activity of the new ABL-specific tyrosine kinase inhibitor STI571 and chemotherapeutic drugs on BCR-ABL-positive chronic myelogenous leukemia cells. *Leukemia* **15:**342–347.

Tybulewicz VL, Crawford CE, Jackson PK, Bronson RT, and Mulligan RC (1991) Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-abl proto-oncogene. *Cell* **65:**1153–1163.

Uehara Y, Murakami Y, Mizuno S, and Kawai S (1988) Inhibition of transforming activity of tyrosine kinase oncogenes by herbimycin A. *Virology* **164:**294–298.

Van Etten RA (1999) Cycling, stressed-out and nervous: cellular functions of c-abl. *Trends Cell Biol* **9:**179–186.

Van Kaick G, Wesch H, Luhrs H, Liebermann D, and Kaul A (1991) Neoplastic diseases induced by chronic alpha-irradiation—epidemiological, biophysical and clinical results of the German Thorotrast Study. *J Radiat Res Tokyo* **32 (Suppl 2):**20–33.

van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, Martens M, Webb A, Sciot R, Van Glabbeke M, et al. (2001) Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* **358:**1421–1423.

Virchow R (1845) Weisses blut. *Frorieps Notizen* **36:**151–156.

von Bubnoff N, Schneller F, Peschel C, and Duyster J (2002) BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. *Lancet* **359:**487–491.

Voncken JW, van Schaick H, Kaartinen V, Deemer K, Coates T, Landing B, Pattengale P, Dorseuil O, Bokoch GM, Groffen J, et al. (1995) Increased neutrophil respiratory burst in bcr-null mutants. *Cell* **80:**719–728.

Warmuth M, Bergmann M, Priess A, Hauslmann K, Emmerich B, and Hallek M (1997) The Src family kinase Hck interacts with Bcr-Abl by a kinase-independent mechanism and phosphorylates the Grb2-binding site of Bcr. *J Biol Chem* **272:**33260–33270.

Warmuth M, Simon N, Mitina O, Mathes R, Fabbro D, Manley PW, Buchdunger E, Forster K, Moarefi I, and Hallek M (2003) Dual-specific Src and Abl kinase inhibitors, PP1 and CGP76030, inhibit growth and survival of cells expressing imatinib mesylate-resistant Bcr-Abl kinases. *Blood* **101:**664–672.

Wassmann B, Klein SA, Scheuring U, Pfeifer H, Martin H, Gschaidmeier H, Hoelzer D, and Ottmann OG (2001) Hematologic and cytogenetic remission by STI571 (Glivec) in a patient relapsing with accelerated phase CML after second allogeneic stem cell transplantation. *Bone Marrow Transplant* **28:**721–724.

Weisberg E and Griffin JD (2000) Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. *Blood* **95:**3498–3505.

Wen ST and Van ER (1997) The PAG gene product, a stress-induced protein with antioxidant properties, is an Abl SH3-binding protein and a physiological inhibitor of c-Abl tyrosine kinase activity. *Genes Dev* **11:**2456–2467.

Wertheim JA, Forsythe K, Druker BJ, Hammer D, Boettiger D, and Pear WS (2002) BCR-ABL-induced adhesion defects are tyrosine kinase-independent. *Blood* **99:**4122–4130.

Wolff NC and Ilaria RL Jr (2001) Establishment of a murine model for therapy-treated chronic myelogenous leukemia using the tyrosine kinase inhibitor STI571. *Blood* **98:**2808–2816.

Yu C, Krystal G, Varticovksi L, McKinstry R, Rahmani M, Dent P, and Grant S (2002) Pharmacologic mitogen-activated protein/extracellular signal-regulated kinase kinase/mitogen-activated protein kinase inhibitors interact synergistically with STI571 to induce apoptosis in Bcr/Abl-expressing human leukemia cells. *Cancer Res* **62:**188–199.

Yuan ZM, Huang Y, Ishiko T, Kharbanda S, Weichselbaum R, and Kufe D (1997) Regulation of DNA damage-induced apoptosis by the c-Abl tyrosine kinase. *Proc Natl Acad Sci USA* **94:**1437–1440.

Yuan ZM, Huang Y, Ishiko T, Nakada S, Utsugisawa T, Kharbanda S, Wang R, Sung P, Shinohara A, Weichselbaum R, et al. (1998) Regulation of Rad51 function by c-Abl in response to DNA damage. *J Biol Chem* **273:**3799–3802.