

New Insights into the Pathophysiology of Chronic Myeloid Leukemia and Imatinib Resistance

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Chronic myeloid leukemia (CML) was the first human malignant disease to be linked to a single, acquired genetic abnormality. Identification of the BCR-ABL kinase fusion protein and its central role in the pathogenesis of CML provided new opportunities to develop rational molecular targeted therapies. This review provides an update on the underlying pathophysiologies of disease progression and imatinib mesylate resistance, leading to the development of new targeted tyrosine kinase inhibitors for managing CML. Imatinib, a selective inhibitor of BCR-ABL, represents a major success in the era of target-directed cancer chemotherapy. However, patients with advanced CML have been less sensitive to therapy and responses have been short. In addition, treatment resistance is an emerging problem at all disease stages. Insight into factors involved

in imatinib resistance and disease progression has highlighted a role for such BCR-ABL–dependent factors as amplification and overexpression of the *BCR-ABL* gene and the emergence of mutant isoforms of BCR-ABL. However, BCR-ABL–independent factors, including leukemogenic pathways involving kinases other than BCR-ABL, also play a part. In light of the limitations of imatinib against these factors, newer tyrosine kinase inhibitors, including dasatinib (a multitargeted kinase inhibitor of BCR-ABL and Src family kinases) and nilotinib (AMN107, a selective BCR-ABL inhibitor), may provide promising treatment options for patients with CML.

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The past few decades have witnessed considerable advances in our understanding of the pathophysiology underlying many diseases. This knowledge has provided a platform for the development of targeted molecular therapies. Defining the molecular basis of many types of cancer has shifted the focus of research toward identifying compounds that specifically inhibit proteins involved in signal transduction within malignant cells. Perhaps one of the best examples is the development of treatment strategies for chronic myeloid leukemia (CML); the first human malignant disease to be linked to an acquired genetic abnormality (1). This knowledge, combined with increased understanding of the signal transduction pathways activated in CML, has provided an exciting opportunity to develop rational molecular targeted therapies for this leukemia. This review provides an update on the pathophysiology of CML and how this insight has influenced the development of therapeutic strategies, specifically the small-molecule kinase inhibitors, for management of the disease.

PATHOPHYSIOLOGY OF CHRONIC MYELOID LEUKEMIA

Chronic myeloid leukemia accounts for approximately 20% of leukemia diagnosed in adults (2, 3). The disease characteristically develops in 3 phases. Left untreated, the initial chronic phase lasts approximately 3 to 6 years (4); the disease then progresses, often through an accelerated phase, to a terminal blastic phase. Most patients are diagnosed in chronic phase, which is characterized by an increased number of leukocytes and/or platelets and a bone marrow blast count less than 10%. The accelerated phase may be marked by 1 or more of the following: increasing splenomegaly and leukocytosis, an increase of blasts to 10% to 30% and an increase of basophils to 20% or greater, thrombocytopenia, and clonal evolution. In the blastic phase, for which the median survival is 2 to 4

months, 30% or more of blood and bone marrow cells are blasts, and myeloid precursors may also form tumors in the lymph nodes, skin, and bone. The goals of treatment for CML, therefore, are complete hematologic response (normalization of blood cell counts) and complete cytogenetic response (eradication of Philadelphia chromosome–positive marrow cells). Rapid achievement of a complete hematologic response is associated with a better outcome, whereas complete cytogenetic response is a statistically significant independent marker for improved survival (5–7). Patients with blastic-phase disease are the most refractory to treatment and can be divided into 1 of 2 categories: those with myeloid disease and those with lymphoid disease. The rate of response to standard induction chemotherapy for patients in the myeloid blastic phase is approximately 20%, and the rate of complete remission is less than 10%. In patients in the lymphoid blastic phase, the rate of response is approximately 50%, but remissions are transient (8, 9) and the median survival is 9 to 12 months.

The characteristic genetic abnormality of CML, the Philadelphia chromosome, is present in the marrow cells of more than 90% of all patients with CML and in 15% to 30% of adult patients with acute lymphoblastic leukemia (ALL); it results from a reciprocal chromosomal translocation between the long arms of chromosomes 9 and 22 (10). This process fuses the Abelson tyrosine kinase (*ABL*) gene on chromosome 9 with the breakpoint cluster region

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Imatinib resistance among patients with chronic myeloid leukemia (CML) is now a clinically significant problem and may limit the long-term benefits of the drug, particularly in advanced disease.

Resistance is often mediated by BCR-ABL mutations that prevent imatinib binding.

Recent evidence suggests that Src family kinases, implicated in driving the progression of CML, may also be involved in the development of BCR-ABL-independent mechanisms of imatinib resistance and disease progression.

Dasatinib is a novel, potent, multitargeted kinase inhibitor of BCR-ABL and Src family kinases. Results from phase I and phase II studies in more than 900 patients suggest that it is safe and effective in chronic and advanced imatinib-resistant and -intolerant CML, and in Philadelphia chromosome-positive acute lymphoblastic leukemia.

Phase I and II studies have also shown that the selective potent BCR-ABL inhibitor nilotinib is safe and effective in similar groups of patients.

(*BCR*) gene on chromosome 22, generating an oncogene that encodes the BCR-ABL protein, a constitutively active, cytoplasmic form of the ABL kinase (11–13). Two studies have shown the central role of BCR-ABL kinase activity in the pathogenesis of CML (11, 14). The activity of this fusion protein is no longer under the regulatory control mechanisms for ABL and thus induces malignant disease by activating multiple cytoplasmic and nuclear signal transduction pathways that influence the growth and survival of hematopoietic cells. **Figure 1** shows that the targets for BCR-ABL include members of the Ras, phosphatidylinositol-3 kinase (PI3K)/Akt, and Jak/Stat signaling pathways, which regulate cell proliferation and apoptosis (15–18). BCR-ABL abrogates cell dependence on external growth factors by upregulating interleukin-3 production (19) and alters the cell adhesion properties by modulating expression and activation of focal adhesion kinase and associated proteins (20, 21). The kinase also has diverse effects on the DNA repair response (22, 23), which may promote additional chromosomal alterations and mutations involved in the progression of the disease and may play a role in the aggressive nature of late-stage CML.

The BCR-ABL fusion protein can vary in size from 185 to 230 kD, depending on the breakpoint in the *BCR* gene. Nearly all patients with chronic-phase CML express a 210-kD protein; very few express the 230-kD protein associated with a more indolent CML course. Patients with Philadelphia chromosome-positive ALL express a 210-kD

(20% to 40%) or a 190-kD (60% to 80%) BCR-ABL protein. Studies indicate that the 190-kD BCR-ABL protein has higher tyrosine kinase activity than does the 210-kD protein, resulting in a greater potential to induce cancer (14, 24). This may explain the acute phenotype associated with Philadelphia chromosome-positive ALL.

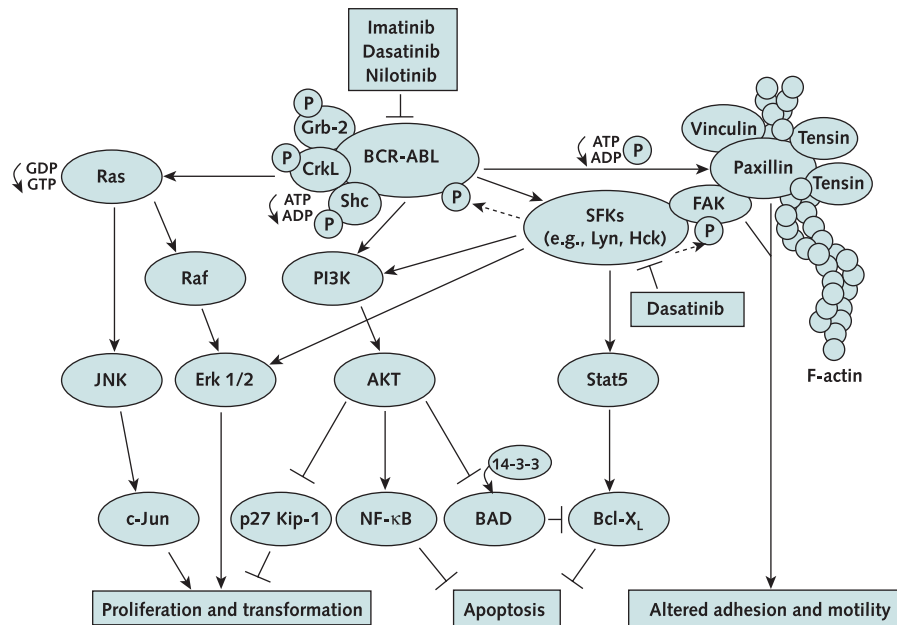
Although BCR-ABL triggers malignant transformation in CML, recent research has focused on the involvement of BCR-ABL-independent pathways in the development and progression of disease, in particular, the Src family kinases. Evidence suggests that Src family kinases are capable of promoting several aspects of tumor progression and metastasis (25). Lyn and other Src kinases support cell survival and are also critical in development of some BCR-ABL-dependent leukemias (26–29) that function downstream of BCR-ABL (30, 31) (**Figure 1**). The Src family kinases may play an important role in late-stage disease, functioning downstream and upstream of BCR-ABL and in pathways that are independent of BCR-ABL (28, 30, 31). The role for BCR-ABL-independent pathways in disease progression and treatment resistance is the subject of much ongoing research, particularly in the evolution of new treatment therapies for CML, as will be discussed.

IMATINIB MESYLATE

The development of imatinib mesylate (Gleevec, Novartis Pharmaceuticals, Basel, Switzerland) represented a major success for target-directed cancer chemotherapy and a breakthrough in the management of CML. Before this, treatment options for CML had been limited. Allogeneic stem-cell transplantation, although potentially curative, remains limited by suitable donor availability and by transplant-associated mortality and morbidities. Interferon- α induces complete cytogenetic responses at rates of 5% to 20% in early chronic-phase CML but is associated with serious toxicities and a reduction in efficacy with increasing duration of chronic-phase disease (7, 32). Imatinib selectively inhibits BCR-ABL by occupying the ABL domain adenosine triphosphate-binding site; it maintains the protein in an inactive conformation, thereby inhibiting its tyrosine kinase activity (33). Preclinical studies have shown that imatinib is effective at inhibiting autophosphorylation of ABL and the tyrosine kinases c-Kit and platelet-derived growth factor receptor- β (34–36).

In 2001, after many successful clinical trials, imatinib was approved for the treatment for Philadelphia chromosome-positive chronic-phase CML (400 mg/d) and accelerated- and myeloid-blastic phase CML (600 mg/d) after failure of interferon- α therapy (4, 37–41). Imatinib was particularly effective in newly diagnosed chronic-phase CML, in which the complete hematologic response rate was greater than 90%, and the complete cytogenetic response rate was between 70% and 80% (42). The drug was also generally well-tolerated. Adverse events were typically

Figure 1. A simplified illustration of BCR-ABL and Src family kinase involvement in oncogenic signaling pathways.



The inhibitory effect is indicated by the upside-down Ts. ABL = Abelson tyrosine kinase; BCR = breakpoint cluster region; FAK = focal adhesion kinase; Grb-2 = growth factor receptor-bound protein 2; Hck = hematopoietic cell kinase; JNK = Jun amino-terminal kinase; P = phosphate group; PI3K = phosphatidylinositol-3-kinase; SFK = Src family kinases; Stat5 = signal transducer and activator of transcription 5.

mild or moderate; common events included superficial edema, nausea, and muscle cramps (4). In a phase III trial (the international randomized interferon- α versus STI571 [IRIS] study), 1106 newly diagnosed patients with chronic-phase CML were treated with imatinib at 400 mg/d or interferon- α plus ara-C. The estimated complete cytogenetic response rate with imatinib was 82% over a median follow-up of 54 months (43). Table 1 shows that estimated rates of complete hematologic response, progression-free survival, and survival were also high with imatinib.

Patients with advanced CML are less sensitive to imatinib. Twenty-four percent and 66% of patients in accelerated and blastic phases, respectively, who were treated with imatinib at 600 mg/d did not achieve hematologic remission (44). Similarly, 76% and 91% of patients in accelerated and blastic phases, respectively, did not achieve complete cytogenetic response. Responses to imatinib in patients with advanced disease are often transient, generally lasting less than 6 months (37, 39–41, 44, 45). Furthermore, the emerging problem of resistance in chronic-phase CML, and particularly in advanced CML, may limit the long-term treatment benefits of imatinib. In patients with accelerated- and blastic-phase disease, 51% and 88% who initially responded to treatment had a relapse after 24 months of treatment with imatinib at 600 mg/d (44).

The success of imatinib in chronic-phase CML led to the approval of the drug as frontline therapy in 2002. Patients diagnosed in chronic phase are initiated on a regimen of oral imatinib at 400 mg/d. This regimen is main-

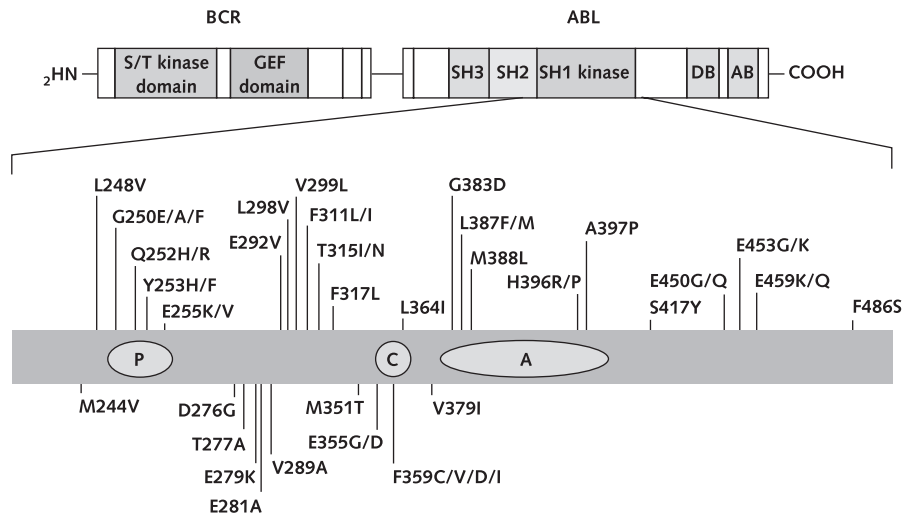
tained if patients achieve hematologic response after 3 months of therapy, cytogenetic response after 6 months, major cytogenetic response (Philadelphia chromosome-positive cells reduced to <35%) after 12 months, or complete cytogenetic response after 18 months. Failure to do so constitutes an indication for modifying imatinib therapy (46), which may include higher-dose imatinib, imatinib combinations, new tyrosine kinase inhibitors (dasatinib or nilotinib), allogeneic stem-cell transplantation, or investigational therapies. For patients who achieve complete cytogenetic response, hematologic assessment should continue every 4 to 6 weeks and cytogenetic assessment should be done every 12 to 18 months, because of the risk for

Table 1. Outcome with Frontline Imatinib Therapy: 54-Month Follow-up of the International Randomized Interferon versus STI571 Trial*

Variable	Estimated Rates for Patients Receiving Frontline Imatinib, %
Complete hematologic response	97
Major cytogenetic response	88
Complete cytogenetic response	82
Estimated 5-year progression-free survival	84
Estimated 5-year survival without progression to accelerated or blastic phase	93

* Reference 43.

Figure 2. BCR-ABL characterized mutants associated with clinical resistance to imatinib.



A = activation loop; AB = actin-binding domain; ABL = Abelson tyrosine kinase; BCR = breakpoint cluster region; C = helix α C; DB = DNA-binding domain; GEF = guanine exchange factor; SH = Src homology; P = phosphate loop; S/T = serine/threonine kinase. Information in this figure is summarized from references 51, 52, 56-62. Figure reprinted with permission from reference 57: Branford S, Hughes T. Detection of BCR-ABL mutations and resistance to imatinib mesylate. In: Iland H, Hertzberg M, Marlton P, eds. Myeloid Leukemia: Methods and Protocols. Totowa, NJ: Humana Pr; 2006:93-106.

acquired resistance (loss of response) to imatinib (47). In addition, molecular monitoring of *BCR-ABL* versus *ABL* transcript levels should be conducted every 3 to 6 months, because most patients have evidence of minimal residual disease, even after 2 to 3 years of imatinib therapy (48, 49). Increasing levels of BCR-ABL transcripts are associated with the emergence of BCR-ABL mutants (50). Patients identified with BCR-ABL mutants associated with imatinib resistance may be considered for alternative therapies. The prognosis for patients who do not respond to imatinib therapy is thought to be poor, and treatment options are limited. The imatinib dosage can be increased to 600 to 800 mg/d with the risk for increased adverse events. However, preferred alternatives include stem-cell transplantation, new tyrosine kinase inhibitors (dasatinib or nilotinib), and enrollment in clinical trials for novel kinase inhibitors or other agents with the potential to overcome mechanisms of resistance to imatinib.

DISEASE PROGRESSION AND RESISTANCE TO TREATMENT

Several mechanisms have been proposed to underlie the development of imatinib resistance in CML, including *BCR-ABL* gene mutations (51, 52); overexpression and amplification of the *BCR-ABL* gene locus (51, 52); activation of BCR-ABL-independent pathways, such as members of the Src kinase family (26); binding of imatinib to serum α -1 acid glycoprotein (53); and increased drug efflux through the multidrug resistance gene (54, 55).

Of the proposed mechanisms, a common cause of imatinib resistance seems to be point mutations in the ABL

kinase domain (51), which preclude the binding of imatinib (Figure 2). These mutations have been characterized into 2 groups. The first group includes mutations that impede contact between BCR-ABL and imatinib (63). Substitution of any 1 of approximately 20 ABL kinase domain residues involved in imatinib binding could result in a reduced affinity for imatinib binding or in steric inhibition of binding. The second group includes mutations that alter the spatial conformation of the protein (63). The BCR-ABL structure contains 2 flexible loop structures, the adenosine triphosphate-binding phosphate loop and the activation loop, which have specific arrangements in the inactive conformation of BCR-ABL that stabilize the structure (56). Mutations in these loops destabilize their arrangement such that the kinase domain cannot assume the inactive conformation required for imatinib binding (33, 56, 64).

In vitro screening has now identified a more comprehensive set of 50 BCR-ABL mutations associated with imatinib resistance (63). There are conflicting data regarding potential differences in the prognostic significance of these mutations in terms of time to progression and survival (52, 56, 65). Branford and colleagues (65) showed that in patients with late chronic-phase (that is, chronic-phase CML diagnosis ≥ 12 months) and accelerated-phase CML, a specific subgroup of mutations in the phosphate loop is associated with a poor prognosis in terms of survival. Increasing evidence suggests that Src family kinases may play a role in the development of treatment resistance and in disease progression. In vitro data with kinase-defective Src

mutants and Src kinase inhibitors show that Src family kinases mediate the oncogenic signaling of BCR-ABL (29, 66, 67) (Figure 1). Experiments in mouse models indicate that BCR-ABL and Src kinases are required to induce an ALL phenotype akin to lymphoid blast crisis CML and Philadelphia chromosome-positive ALL (28) and that blockade of Src kinase signaling prevents CML progression to lymphoid blast crisis (68). BCR-ABL-positive CML cells cultured in the continuous presence of imatinib or those obtained from patients who have disease progression while receiving imatinib therapy have a decrease in BCR-ABL protein or mRNA levels and a corresponding increase in the activity of Src family kinases (26). The role of Src kinases in drug-resistant BCR-ABL cells has been further supported by the decreased survival and proliferation of drug-resistant BCR-ABL cells after inhibition of Lyn expression by RNA interference (69).

The inability of imatinib to inhibit Src kinases directly (70) further corroborates their potential role in the development of imatinib resistance. In vitro experiments with cells from imatinib-sensitive patients showed that imatinib inhibits Src family kinase activation through its effect on BCR-ABL (71). However, in multiple specimens from imatinib-resistant patients, imatinib inhibition of BCR-ABL had no effect on Src family kinase activation, which indicates that their activity in these patients had become uncoupled from BCR-ABL regulation (71). In animal models, loss of BCR-ABL-mediated inhibition of Lyn substantially impaired the antitumor activity of imatinib, which was recovered by the addition of an inhibitor of BCR-ABL and Src family kinase. These data support the hypothesis that increased Src kinase activity may reduce the dependence of leukemic cells on BCR-ABL kinase activity in some patients with imatinib-resistant CML. In these patients, inhibition of BCR-ABL and Src family kinase is required for apoptosis (26, 27). Studies investigating the pathways by which Src kinases may mediate disease progression and resistance are ongoing.

NEW TREATMENT OPTIONS FOR THE MANAGEMENT OF CHRONIC MYELOID LEUKEMIA

Several new targeted tyrosine kinase inhibitors are currently under development, the focus of which has been to target the underlying causes of imatinib resistance and disease progression (Table 2).

Multitargeted Inhibitors of BCR-ABL and Src Family Kinases

Insight into the mechanisms of disease progression and imatinib resistance suggests that compounds with inhibitory activity against BCR-ABL and Src kinases may provide more effective treatment for patients with CML and Philadelphia chromosome-positive ALL, including those with advanced disease or resistance to imatinib. Accordingly, many studies support the potential of such agents in patients with these diseases.

Table 2. Spectrum of Tyrosine Kinase Inhibition for Imatinib and Novel Compounds*

Drug	Kinase-Inhibited
Imatinib	BCR-ABL, c-Kit, and PDGFR
Dasatinib	BCR-ABL, Src family kinases, c-Kit, ephrin receptor kinases, and PDGFR
Nilotinib	BCR-ABL, c-Kit, and PDGFR
SKI-606	BCR-ABL and Src family kinases
VX-680	BCR-ABL, Aurora kinases, and Flt3 kinase
BIRB-796	BCR-ABL, p38 MAP kinase
ONO12380	BCR-ABL and Lyn kinase
Adaphostin	BCR-ABL and other tyrosine kinases

* PDGFR = platelet-derived growth factor β -receptor; MAP = mitogen-activated protein.

Originally described as Src kinase inhibitors, the pyrido[2, 3-d]pyrimidine class of Src/ABL inhibitors were subsequently found to also inhibit ABL. Compounds from this class of multitargeted kinase inhibitors, including PD180970, PD166326, and PD173955 (Pfizer Global Research and Development, Ann Arbor, Michigan), have shown promising in vitro activity against a subset of BCR-ABL mutants (72–74). PD166326 has also demonstrated greater antileukemic activity than imatinib in a mouse model of CML (75). This class of compounds potently suppresses the proliferation of imatinib-resistant cells in which the cause of the resistance is overexpression of BCR-ABL (76). The unfavorable safety profile of pyridopyrimidines has precluded their clinical development, but these studies have shown that resistance to imatinib can be overcome with kinase inhibitors of other structural classes.

Similarly, the multitargeted kinase inhibitors PP1 (AG Scientific, San Diego, California) and CGP76030 (Novartis Pharmaceuticals) have been shown to block cell growth and survival of 32D cells, a murine myeloid cell line expressing wild-type BCR-ABL or mutant, imatinib-resistant BCR-ABL, albeit at very high concentrations (66). These agents inhibited tyrosine phosphorylation of some, but not all, imatinib-resistant BCR-ABL mutants; they had little effect on phosphorylation of the adenosine triphosphate-binding site mutant BCR-ABL T315I, a residue that is directly involved in binding many adenosine triphosphate-competitive kinase inhibitors. However, PP1 and CGP76030 were able to inhibit the proliferation of 32D cells expressing BCR-ABL T315I. The observed inhibition was independent of BCR-ABL activity, but seemed to correlate, at least in part, with PP1 and CGP76030 action against Src (66). The multitargeted kinase inhibitor SKI-606 (Wyeth Pharmaceuticals, Madison, New Jersey) inhibits proliferation of human CML cell lines in a dose-dependent manner, with a potency that is 10-fold greater than that of imatinib. The compound is also effective in vivo: A regimen of 100 mg/kg SKI-606 once daily for 5 days was sufficient to eradicate large K562 xenograft tumors (800 to 900 mg) in mice (77). SKI-606 is currently being evaluated in clinical trials. Preclinical studies have also been reported

for many other multitargeted kinase inhibitors, including AZD0530 (AstraZeneca, London, United Kingdom) and AP23464 and AP23848 (Ariad Pharmaceuticals, Cambridge, Massachusetts).

Of the new targeted therapies for CML in development, the clinical evaluation of dasatinib (Sprycel, Bristol-Myers Squibb, New York, New York) is most advanced, with more than 900 patients having received the drug to date. Phase II trials of dasatinib have been completed: In June 2006, the U.S. Food and Drug Administration approved dasatinib for the treatment for adults with all phases of CML and Philadelphia chromosome-positive ALL with resistance or intolerance to previous therapy, including imatinib. Dasatinib is a novel, oral multitargeted kinase inhibitor of BCR-ABL and Src family kinases and ephrin receptor kinases, platelet-derived growth factor receptor, and c-Kit (78). The high potency of dasatinib has been demonstrated in preclinical comparisons with imatinib and nilotinib (Novartis Pharmaceuticals) (79, 80). These studies showed that nilotinib was 20- to 50-fold more potent than imatinib against cells expressing wild-type BCR-ABL, in agreement with previous studies (81, 82), although dasatinib was 325-fold more potent (79). Similar relative improvements were maintained for various imatinib-resistant mutants (79). The increased potency of dasatinib compared with imatinib and nilotinib may be due to its ability to bind to the active (open) and inactive (closed) conformations of ABL, as revealed in x-ray crystallography studies (83). In vitro and in vivo preclinical studies have shown the inhibitory activity of dasatinib against 18 of 19 tested BCR-ABL mutations associated with resistance to imatinib (78, 79). To date, the limited spectrum of BCR-ABL point mutations found to confer resistance map almost exclusively to critical contact residues in the adenosine triphosphate-binding pocket directly involved in dasatinib binding (84). Of these, only BCR-ABL T315I is clinically relevant. Indeed, residue 315 seems to be critical for binding most adenosine triphosphate-competitive kinase inhibitors, because BCR-ABL T315I also confers resistance to imatinib, nilotinib, and many other drugs. However, 2 adenosine triphosphate-competitive compounds, BIRB-796 (Boehringer Ingelheim, Ridgefield, Connecticut) and VX-680 (Vertex Pharmaceuticals, Cambridge, Massachusetts), have shown in vitro activity against this mutant (85, 86).

Results have been reported from a phase I study of dasatinib in patients with chronic-, accelerated-, and blastic-phase imatinib-resistant or -intolerant CML and Philadelphia chromosome-positive ALL (87). In the chronic phase, the complete hematologic response rate was 93% with dasatinib, and the major cytogenetic response rate was 45%, including a complete cytogenetic response rate of 35% (87). Among 44 evaluable patients with advanced CML, the complete hematologic response rates were 45% in accelerated phase, 35% in myeloid blastic phase, and 70% in lymphoid blastic phase and Philadelphia chromo-

Table 3. Response to Dasatinib in Patients with Chronic Myeloid Leukemia after Imatinib Failure: Results from a Phase I Study*

Disease	Patients, n	Complete Hematologic Response, %	Complete Cytogenetic Response, %
Chronic-phase CML	40	93	35
Accelerated-phase CML	11	45	25
Myeloid blastic-phase CML	23	35	
Lymphoid blastic-phase CML/Philadelphia chromosome-positive ALL	10	70	

* Reference 87. ALL = acute lymphoblastic leukemia; CML = chronic myeloid leukemia.

some-positive ALL (Table 3). Cytogenetic responses were observed in patients with a wide range of BCR-ABL mutations and in patients who had had little or no previous response to imatinib.

Src kinases are involved in numerous cellular processes in normal cells. This involvement has led to concerns over potential safety issues with dasatinib; however, the drug was well tolerated in a phase I study (87). The rate of severe cytopenia (grade 3 to 4 reductions in leukocytes, neutrophils, platelets and hemoglobin, as defined by the National Cancer Institute Common Toxicity Criteria, version 3.0, Bethesda, Maryland) was 45% in chronic phase, 82% in accelerated phase, 96% in myeloid blastic phase, and 80% in lymphoid blastic phase and Philadelphia chromosome-positive ALL; cytopenia was reversible when managed with dose reductions and interruptions in dasatinib treatment. Nonhematologic adverse events were also reported; 15 patients developed unexplained pleural effusion (5 in chronic phase and 10 in blastic phase).

Dasatinib has been evaluated further in a phase II trial program entitled START (Src/ABL tyrosine kinase inhibition activity: research trials of dasatinib). This program comprised 5 trials; 4 single-group studies in patients with all stages of imatinib-resistant or -intolerant CML or Philadelphia chromosome-positive ALL, and 1 randomized trial that evaluated dasatinib versus high-dose imatinib in patients with chronic-phase disease, after failure of standard-dose imatinib. Data from the 4 single-group studies have been reported (88–91) (Table 4) and support the phase I observations. In chronic-phase CML, the complete hematologic response rate was 90%, and the major cytogenetic response rate was 45%. In accelerated-phase CML, the major hematologic response rate was 59% (33% complete hematologic response), and the major cytogenetic response rate was 32% (22% complete cytogenetic response). In myeloid blastic-phase CML, the major hematologic response rate was 32% (24% complete hematologic response), and the major cytogenetic response rate was 30%

Table 4. Initial Phase II Data for Second-line Therapy with Dasatinib after Imatinib Failure*

CML Disease Phase	Patients, <i>n</i>	Hematologic Response, %		Cytogenetic Response, %	
		Complete	Major	Complete	Major
Chronic	186	90	–	40	50
Accelerated	107	33	59	22	32
Myeloid blastic	74	24	32	27	30
Lymphoid blastic	42	26	31	–	50
Philadelphia chromosome–positive ALL	36	31	42	–	58

* References 88–91. ALL = acute lymphoblastic leukemia; CML = chronic myeloid leukemia.

(27% complete cytogenetic response). In lymphoid blastic-phase CML, the major hematologic response rate was 31% (26% complete hematologic response), and the major cytogenetic response rate was 50%. Finally, in Philadelphia chromosome–positive ALL, the major hematologic response rate was 42% (31% complete hematologic response), and the major cytogenetic response rate was 58%. Response rates to dasatinib were encouraging, given the fact that many patients had a long duration of CML and were heavily pretreated for their conditions.

In all 4 studies, dasatinib was well-tolerated (88–91). Reversible severe cytopenia was the most common adverse event, although a notable proportion of patients with advanced CML entered the studies with severe cytopenia, probably associated with their CML phase or previous treatment. Nonhematologic side effects were infrequent and usually were mild to moderate; diarrhea, nausea, peripheral edema, rash, gastrointestinal bleeding, and pleural effusion were most commonly observed. Pleural effusions were noted in 5% to 20% of patients. These initial reports suggest that dasatinib may provide safe and effective treatment for patients with CML following failure of imatinib therapy. Additional follow-up of the phase II studies is ongoing.

Selective Inhibitors of BCR-ABL

Nilotinib has been developed from its parent compound, imatinib, as a more potent inhibitor of BCR-ABL (80–82, 92, 93). Like imatinib, nilotinib also inhibits platelet-derived growth factor receptor and c-Kit but does not inhibit Src kinases and binds only to the inactive conformation of BCR-ABL (92). The compound shows 20-

50-fold greater potency than imatinib, as determined by its ability to block proliferation of BCR-ABL–dependent cells derived from patients with CML (K562 and Ku-812F cells) and cell lines 32D and BaF3 (79, 80). The potency of nilotinib against imatinib-resistant BCR-ABL mutants seems variable, depending on the mutation that is examined (80). Nilotinib shows 26-fold greater potency compared with imatinib in cells resistant to imatinib as a result of *BCR-ABL* gene amplification but is not able to overcome imatinib-resistance associated with the critical contact residue point mutation T315I (81). In vivo assessment has shown prolonged survival of mice injected with BCR-ABL–transformed hematopoietic cell lines or primary marrow cells, and in imatinib-resistant CML mouse models (80, 81). Nilotinib is also 30- to 40-fold more potent than imatinib in inhibiting the proliferation of p190 BCR-ABL–expressing Philadelphia chromosome–positive ALL cell lines (82).

Initial results have been reported for a phase I study of nilotinib therapy in patients with CML and Philadelphia chromosome–positive ALL who are resistant or intolerant to imatinib (94). Nilotinib was administered orally to 119 patients in a continuous modified reassessment design. The compound was well-tolerated. Adverse events were generally mild to moderate; the most common events were elevated bilirubin levels and skin symptoms (pruritis, dry skin, and exanthem). Dose-limiting toxicities, including neutropenia and hyperbilirubinemia, were observed at 600 mg twice daily. Table 5 summarizes the response rates with nilotinib in the phase I study. Hematologic responses were noted in 89% of imatinib-resistant patients with chronic-

Table 5. Response to Nilotinib in Patients with Imatinib Resistance: Initial Results from a Phase I Study*

Disease Phase	Patients, <i>n</i>	Hematologic Response, %	Cytogenetic Response, %
Chronic-phase CML	16	89	50
Clonal evolution CML	9	100	100
Accelerated-phase CML	49	69	29
Myeloid blastic–phase CML	23	57	22
Lymphoid blastic–phase CML	9	44	22
Philadelphia chromosome–positive ALL	10	10	NA
Philadelphia chromosome–positive ALL (minimal residual disease)	3	33	NA

* Reference 94. ALL = acute lymphoblastic leukemia; CML = chronic myeloid leukemia; NA = not available.

Table 6. Response to Nilotinib in Preliminary Phase II Studies*

Disease	Evaluable Patients, <i>n</i>	Hematologic Response, %	Cytogenetic Response, %	
			Any	Major
Chronic-phase CML	81	69 (complete response)	55	46
Accelerated-phase CML	22	64	25	–
Blastic-phase CML/Philadelphia chromosome–positive ALL	16/6	38/33	17	–

* References 95–97. ALL = acute lymphoblastic leukemia; CML = chronic myeloid leukemia.

phase disease, and cytogenetic responses were noted in 50%. The results were also encouraging in patients with accelerated and myeloid and lymphoid blastic phases of CML. Phase II evaluation of nilotinib in these patients is currently ongoing. Table 6 summarizes the preliminary results (95–97).

Nonadenosine Triphosphate–Competitive BCR-ABL Inhibitors

Most BCR-ABL inhibitors, including imatinib, dasatinib, and nilotinib, act by occupying the kinase's adenosine triphosphate–binding site. Alternative strategies have also been sought in an effort to develop compounds that may complement these drugs. One such strategy is to disrupt BCR-ABL–substrate interactions by occupying the kinase substrate-binding site. ON012380 (Onconova, Princeton, New Jersey) is a specific inhibitor of BCR-ABL substrate binding that shows approximately 10-fold greater potency against wild-type BCR-ABL than does imatinib. It has a synergistic effect in combination with imatinib and is effective against a range of BCR-ABL mutants that confer imatinib resistance, including the adenosine triphosphate–binding site mutation T315I (98). The compound also inhibits platelet-derived growth factor receptor kinases and Src family members, Lyn and Fyn, but has a limited inhibitory effect against c-kit.

Other nonadenosine triphosphate–competitive BCR-ABL inhibitors include ON01910 (Novonex, Onconova) and adaphostin. Like ON012380, ON01910 is a multitargeted inhibitor of kinase substrate binding that potently inhibits polo-like kinase-1 but also has lower levels of activity against BCR-ABL, Src, and Fyn (99). ON01910 has in vitro tumor-killing activity against various malignant cell lines, including leukemic cells; the drug is currently in phase I clinical development for advanced solid tumors. Adaphostin inhibits BCR-ABL and stimulates apoptosis in imatinib-sensitive and -resistant CML cells and also has a synergistic effect with imatinib against imatinib-sensitive CML cells (100). Adaphostin also increases levels of intracellular reactive oxygen species and can stimulate apoptosis in Philadelphia chromosome–negative malignant cells (101).

CONCLUSIONS

Increased understanding of the underlying pathogenesis of CML has marked the development of targeted therapies for the treatment for this disease. The successful introduction of the BCR-ABL inhibitor imatinib was a paradigm shift in the treatment for CML, resulting in its use as frontline treatment for newly diagnosed CML. Consequently, treatment guidelines for patients with CML are undergoing substantial changes (46, 102, 103). The success of this agent has been hampered by issues of clinical resistance at all stages of disease and transient responses to therapy for patients with advanced disease. Further understanding of the molecular basis of CML progression and the reasons behind transient responses and resistance to imatinib treatment have provided an opportunity for the development of novel kinase inhibitors with superior potency that are able to overcome mechanisms of resistance, both BCR-ABL–dependent and –independent. Multitargeted inhibitors of BCR-ABL and Src family kinases and more potent, selective BCR-ABL inhibitors have the attraction of reducing disease progression and potentially preventing acquired resistance to imatinib. After the encouraging results of the phase I and phase II trials with dasatinib and nilotinib, full reports of these trials are now eagerly awaited.

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References

- Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. *J Natl Cancer Inst.* 1960;25:85-109. [PMID: 14427847]
- Faderl S, Talpaz M, Estrov Z, Kantarjian HM. Chronic myelogenous leukemia: biology and therapy. *Ann Intern Med.* 1999;131:207-19. [PMID: 10428738]
- Sawyers CL. Chronic myeloid leukemia. *N Engl J Med.* 1999;340:1330-40. [PMID: 10219069]
- Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med.* 2002;346:645-52. [PMID: 11870241]
- Guilhot F, Chastang C, Michallet M, Guerci A, Harousseau JL, Maloisel F, et al. Interferon alfa-2b combined with cytarabine versus interferon alone in chronic myelogenous leukemia. French Chronic Myeloid Leukemia Study Group. *N Engl J Med.* 1997;337:223-9. [PMID: 9227927]
- Mahon FX, Montastruc M, Faberes C, Reiffers J. Predicting complete cytogenetic response in chronic myelogenous leukemia patients treated with recombinant interferon alpha [Letter]. *Blood.* 1994;84:3592-4. [PMID: 7832859]
- Kantarjian HM, Smith TL, O'Brien S, Beran M, Pierce S, Talpaz M. Prolonged survival in chronic myelogenous leukemia after cytogenetic response to interferon-alpha therapy. The Leukemia Service. *Ann Intern Med.* 1995;122:254-61. [PMID: 7825760]
- Kantarjian HM, Talpaz M, Keating MJ, Estey EH, O'Brien S, Beran M, et al. Intensive chemotherapy induction followed by interferon-alpha maintenance in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. *Cancer.* 1991;68:1201-7. [PMID: 1873771]
- Sacchi S, Kantarjian HM, O'Brien S, Cortes J, Rios MB, Giles FJ, et al. Chronic myelogenous leukemia in nonlymphoid blastic phase: analysis of the results of first salvage therapy with three different treatment approaches for 162 patients. *Cancer.* 1999;86:2632-41. [PMID: 10594858]
- Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. [Letter]. *Nature.* 1973;243:290-3. [PMID: 4126434]
- Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science.* 1990;247:824-30. [PMID: 2406902]
- Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffon J. Acute leukaemia in bcr/abl transgenic mice. *Nature.* 1990;344:251-3. [PMID: 2179728]
- Faderl S, Kantarjian HM, Thomas DA, Cortes J, Giles F, Pierce S, et al. Outcome of Philadelphia chromosome-positive adult acute lymphoblastic leukemia. *Leuk Lymphoma.* 2000;36:263-73. [PMID: 10674898]
- Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science.* 1990;247:1079-82. [PMID: 2408149]
- Tauchi T, Okabe S, Miyazawa K, Ohyashiki K. The tetramerization domain-independent Ras activation by BCR-ABL oncoprotein in hematopoietic cells. *Int J Oncol.* 1998;12:1269-76. [PMID: 9592185]
- Skorski T, Kanakaraj P, Nieborowska-Skorska M, Ratajczak MZ, Wen SC, Zon G, et al. Phosphatidylinositol-3 kinase activity is regulated by BCR/ABL and is required for the growth of Philadelphia chromosome-positive cells. *Blood.* 1995;86:726-36. [PMID: 7606002]
- Chai SK, Nichols GL, Rothman P. Constitutive activation of JAKs and STATs in BCR-Abl-expressing cell lines and peripheral blood cells derived from leukemic patients. *J Immunol.* 1997;159:4720-8. [PMID: 9366395]
- Iliara RL Jr, Van Etten RA. P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. *J Biol Chem.* 1996;271:31704-10. [PMID: 8940193]
- Jiang X, Lopez A, Holyoake T, Eaves A, Eaves C. Autocrine production and action of IL-3 and granulocyte colony-stimulating factor in chronic myeloid leukemia. *Proc Natl Acad Sci U S A.* 1999;96:12804-9. [PMID: 10536003]
- Cheng K, Kurzrock R, Qiu X, Estrov Z, Ku S, Dulski KM, et al. Reduced focal adhesion kinase and paxillin phosphorylation in BCR-ABL-transfected cells. *Cancer.* 2002;95:440-50. [PMID: 12124845]
- Gotoh A, Miyazawa K, Ohyashiki K, Tauchi T, Boswell HS, Broxmeyer HE, et al. Tyrosine phosphorylation and activation of focal adhesion kinase (p125FAK) by BCR-ABL oncoprotein. *Exp Hematol.* 1995;23:1153-9. [PMID: 7556524]
- Deutsch E, Dugray A, Abdulkarim B, Marangoni E, Maggiorella L, Vaganay S, et al. BCR-ABL down-regulates the DNA repair protein DNA-PKcs. *Blood.* 2001;97:2084-90. [PMID: 11264175]
- Slupianek A, Schmutte C, Tomblin G, Nieborowska-Skorska M, Hoser G, Nowicki MO, et al. BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. *Mol Cell.* 2001;8:795-806. [PMID: 11684015]
- Voncken JW, Kaartinen V, Pattengale PK, Germeraad WT, Groffon J, Heisterkamp N. BCR/ABL P210 and P190 cause distinct leukemia in transgenic mice. *Blood.* 1995;86:4603-11. [PMID: 8541551]
- Warmuth M, Damoiseaux R, Liu Y, Fabbro D, Gray N. SRC family kinases: potential targets for the treatment of human cancer and leukemia. *Curr Pharm Des.* 2003;9:2043-59. [PMID: 14529415]
- Donato NJ, Wu JY, Stapley J, Gallick G, Lin H, Arlinghaus R, et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. *Blood.* 2003;101:690-8. [PMID: 12509383]
- Dai Y, Rahmani M, Corey SJ, Dent P, Grant S. A Bcr/Abl-independent, Lyn-dependent form of imatinib mesylate (STI-571) resistance is associated with altered expression of Bcl-2. *J Biol Chem.* 2004;279:34227-39. [PMID: 15175350]
- Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, et al. Requirement of Src kinases Lyn, Hck and Fgr for BCR-ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. *Nat Genet.* 2004;36:453-61. [PMID: 15098032]
- Lionberger JM, Wilson MB, Smithgall TE. Transformation of myeloid leukemia cells to cytokine independence by Bcr-Abl is suppressed by kinase-defective Hck. *J Biol Chem.* 2000;275:18581-5. [PMID: 10849448]
- Roginskaya V, Zuo S, Caudell E, Nambudiri G, Kraker AJ, Corey SJ. Therapeutic targeting of Src-kinase Lyn in myeloid leukemic cell growth. *Leukemia.* 1999;13:855-61. [PMID: 10360372]
- Danhauser-Riedl S, Warmuth M, Druker BJ, Emmerich B, Hallek M. Activation of Src kinases p53/56lyn and p59hck by p210bcr/abl in myeloid cells. *Cancer Res.* 1996;56:3589-96. [PMID: 8758931]
- Silver RT, Woolf SH, Hehlmann R, Appelbaum FR, Anderson J, Bennett C, et al. An evidence-based analysis of the effect of busulfan, hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia: developed for the American Society of Hematology. *Blood.* 1999;94:1517-36. [PMID: 10477676]
- Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science.* 2000;289:1938-42. [PMID: 10988075]
- Heinrich MC, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol.* 2002;20:1692-703. [PMID: 11896121]
- Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med.* 2001;344:1052-6. [PMID: 11287975]
- Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter EJ, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N Engl J Med.* 2002;347:481-7. [PMID: 12181402]
- Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. 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.* 2001;344:1038-42. [PMID: 11287973]
- Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med.* 2001;344:1031-7. [PMID: 11287972]
- Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, Silver RT, et al. A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. *Blood.* 2002;100:1965-71. [PMID: 12200353]
- Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, Ottmann OG, et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood.* 2002;99:3530-9. [PMID: 11986204]
- Talpaz M, Silver RT, Druker BJ, Goldman JM, Gambacorti-Passerini C, Guilhot F, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2

- study. *Blood*. 2002;99:1928-37. [PMID: 11877262]
42. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2003;348:994-1004. [PMID: 12637609]
43. Druker B, Guilhot F, O'Brien S, Larson R. Long-term benefits of imatinib (IM) for patients newly diagnosed with chronic myelogenous leukemia in chronic phase (CML-CP): The 5-year update from the IRIS study [Abstract]. *J Clin Oncol*. 2006;24:18S. Abstract no. 6506.
44. Talpaz M, Goldman J, Sawyers C, Hochhaus A, Silver RT, Douglas Smith BD, et al. High dose imatinib (STI571, Gleevec®) provides durable long-term outcomes for patients (pts) with chronic myeloid leukemia (CML) in accelerated phase (AP) or myeloid blast crisis (BC): follow-up of the Phase II studies [Abstract]. *Blood*. 2003;102:905a-6a. Abstract no. 3369.
45. Silver RT, Talpaz M, Sawyers CL, Druker BJ, Hochhaus A, Schiffer CA, et al. Four years follow-up of 1027 patients with late chronic phase (L-CP), accelerated phase (AP), or blast crisis (BC) chronic myeloid leukemia (CML) treated with imatinib in three large Phase II trials [Abstract]. *Blood*. 2004;104:10a. Abstract no. 23.
46. Baccarani M, Saglio G, Goldman J, Hochhaus A, Simonsson B, Appelbaum F, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood*. 2006;108:1809-20. [PMID: 16709930]
47. Mauro MJ, Deininger MW. Chronic myeloid leukemia in 2006: a perspective [Editorial]. *Haematologica*. 2006;91:152. [PMID: 16461297]
48. Müller MC, Gattermann N, Lahaye T, Deininger MW, Berndt A, Fruehauf S, et al. Dynamics of BCR-ABL mRNA expression in first-line therapy of chronic myelogenous leukemia patients with imatinib or interferon alpha/ara-C. *Leukemia*. 2003;17:2392-400. [PMID: 14523462]
49. Branford S, Rudzki Z, Harper A, Grigg A, Taylor K, Durrant S, et al. Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. *Leukemia*. 2003;17:2401-9. [PMID: 14523461]
50. Branford S, Rudzki Z, Parkinson I, Grigg A, Taylor K, Seymour JF, et al. Real-time quantitative PCR analysis can be used as a primary screen to identify patients with CML treated with imatinib who have BCR-ABL kinase domain mutations. *Blood*. 2004;104:2926-32. [PMID: 15256429]
51. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science*. 2001;293:876-80. [PMID: 11423618]
52. Hochhaus A, Kreil S, Corbin AS, La Rosée P, Müller MC, Lahaye T, et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia*. 2002;16:2190-6. [PMID: 12399961]
53. Gambacorti-Passerini C, Barni R, le Coutre P, Zucchetti M, Cabrera G, Cleris L, et al. Role of alpha 1 acid glycoprotein in the in vivo resistance of human BCR-ABL(+) leukemic cells to the abl inhibitor STI571. *J Natl Cancer Inst*. 2000;92:1641-50. [PMID: 11036109]
54. Thomas J, Wang L, Clark RE, Pirmohamed M. Active transport of imatinib into and out of cells: implications for drug resistance. *Blood*. 2004;104:3739-45. [PMID: 15315971]
55. Illmer T, Schaich M, Platzbecker U, Freiberg-Richter J, Oelschlägel U, von Bonin M, et al. P-glycoprotein-mediated drug efflux is a resistance mechanism of chronic myelogenous leukemia cells to treatment with imatinib mesylate. *Leukemia*. 2004;18:401-8. [PMID: 14724652]
56. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, et al. 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*. 2002;2:117-25. [PMID: 12204532]
57. Branford S, Hughes T. Detection of BCR-ABL mutations and resistance to imatinib mesylate. In: Iland H, Hertzberg M, Marlton P, eds. *Myeloid Leukemia: Methods and Protocols*. Totowa, NJ: Humana Pr; 2006:93-106.
58. von Bubnoff N, Schneller F, Peschel C, Dwyer J. BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. *Lancet*. 2002;359:487-91. [PMID: 11853795]
59. Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, Taylor K, et al. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood*. 2002;99:3472-5. [PMID: 11964322]
60. Hofmann WK, Jones LC, Lemp NA, de Vos S, Gschaidmeier H, Hoelzer D, et al. Ph(+) acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique BCR-ABL gene mutation. *Blood*. 2002;99:1860-2. [PMID: 11861307]
61. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, Lai JL, Philippe N, Facon T, et al. 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*. 2002;100:1014-8. [PMID: 12130516]
62. Al-Ali HK, Heinrich MC, Lange T, Krahl R, Mueller M, Müller C, et al. High incidence of BCR-ABL kinase domain mutations and absence of mutations of the PDGFR and KIT activation loops in CML patients with secondary resistance to imatinib. *Hematol J*. 2004;5:55-60. [PMID: 14745431]
63. Azam M, Latek RR, Daley GQ. Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR-ABL. *Cell*. 2003;112:831-43. [PMID: 12654249]
64. Corbin AS, La Rosée P, Stoffregen EP, Druker BJ, Deininger MW. Several Bcr-Abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. *Blood*. 2003;101:4611-4. [PMID: 12576318]
65. Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood*. 2003;102:276-83. [PMID: 12623848]
66. Warmuth M, Simon N, Mitina O, Mathes R, Fabbro D, Manley PW, et al. Dual-specific Src and Abl kinase inhibitors, PP1 and CGP76030, inhibit growth and survival of cells expressing imatinib mesylate-resistant Bcr-Abl kinases. *Blood*. 2003;101:664-72. [PMID: 12393636]
67. Wilson MB, Schreiner SJ, Choi HJ, Kamens J, Smithgall TE. Selective pyrrolo-pyrimidine inhibitors reveal a necessary role for Src family kinases in Bcr-Abl signal transduction and oncogenesis. *Oncogene*. 2002;21:8075-88. [PMID: 12444544]
68. Li S, Hu Y, Swerdlow S, Duffy T, Weinmann R, Lee F. Targeting BCR-ABL kinase activity-independent signaling pathways and leukemia stem cells is essential for curative therapy of Philadelphia chromosome positive (Ph+) leukemia [Abstract]. *Blood*. 2005;106. Abstract no. 1990.
69. Ptasznik A, Nakata Y, Kalota A, Emerson SG, Gewirtz AM. Short interfering RNA (siRNA) targeting the Lyn kinase induces apoptosis in primary, and drug-resistant, BCR-ABL1(+) leukemia cells. *Nat Med*. 2004;10:1187-9. [PMID: 15502840]
70. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med*. 1996;2:561-6. [PMID: 8616716]
71. Donato N, Wu J, Kong L, Meng F, Lee F, Talpaz M. Constitutive activation of SRC-family kinases in chronic myelogenous leukemia patients resistant to imatinib mesylate in the absence of BCR-ABL mutations: a rationale for use of multi-targeted kinase inhibitor-based therapy [Abstract]. *Blood*. 2005;106. Abstract no. 1087.
72. La Rosée P, Corbin AS, Stoffregen EP, Deininger MW, Druker BJ. Activity of the Bcr-Abl kinase inhibitor PD180970 against clinically relevant Bcr-Abl isoforms that cause resistance to imatinib mesylate (Gleevec, STI571). *Cancer Res*. 2002;62:7149-53. [PMID: 12499247]
73. Dorsey JF, Jove R, Kraker AJ, Wu J. The pyrido[2, 3-d]pyrimidine derivative PD180970 inhibits p210Bcr-Abl tyrosine kinase and induces apoptosis of K562 leukemic cells. *Cancer Res*. 2000;60:3127-31. [PMID: 10866298]
74. Huron DR, Gorre ME, Kraker AJ, Sawyers CL, Rosen N, Moasser MM. A novel pyridopyrimidine inhibitor of abl kinase is a picomolar inhibitor of Bcr-abl-driven K562 cells and is effective against STI571-resistant Bcr-abl mutants. *Clin Cancer Res*. 2003;9:1267-73. [PMID: 12684394]
75. Wolff NC, Veach DR, Tong WP, Bornmann WG, Clarkson B, Ilaria RL Jr. PD166326, a novel tyrosine kinase inhibitor, has greater antileukemic activity than imatinib mesylate in a murine model of chronic myeloid leukemia. *Blood*. 2005;105:3995-4003. [PMID: 15657179]
76. Tipping AJ, Baluch S, Barnes DJ, Veach DR, Clarkson BM, Bornmann WG, et al. Efficacy of dual-specific Bcr-Abl and Src-family kinase inhibitors in cells sensitive and resistant to imatinib mesylate. *Leukemia*. 2004;18:1352-6. [PMID: 15201856]
77. Golas JM, Arndt K, Etienne C, Lucas J, Nardin D, Gibbons J, et al. SKI-606, a 4-anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. *Cancer*

- Res. 2003;63:375-81. [PMID: 12543790]
78. **Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL.** Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science*. 2004;305:399-401. [PMID: 15256671]
79. O'Hare T, Walters DK, Stoffregen EP, Jia T, Manley PW, Mestan J, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res*. 2005;65:4500-5. [PMID: 15930265]
80. **Weisberg E, Manley PW, Breitenstein W, Brügger J, Cowan-Jacob SW, Ray A, et al.** Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell*. 2005;7:129-41. [PMID: 15710326]
81. **Golemovic M, Verstovsek S, Giles F, Cortes J, Manshoury T, Manley PW, et al.** AMN107, a novel aminopyrimidine inhibitor of Bcr-Abl, has in vitro activity against imatinib-resistant chronic myeloid leukemia. *Clin Cancer Res*. 2005;11:4941-7. [PMID: 16000593]
82. **Verstovsek S, Golemovic M, Kantarjian H, Manshoury T, Estrov Z, Manley P, et al.** AMN107, a novel aminopyrimidine inhibitor of p190 Bcr-Abl activation and of in vitro proliferation of Philadelphia-positive acute lymphoblastic leukemia cells. *Cancer*. 2005;104:1230-6. [PMID: 16078266]
83. **Tokarski J, Newitt J, Lee F, Lombardo L, Borzilleri R, Kish K, et al.** The crystal structure of Abl kinase with BMS-354825, a dual SRC/ABL kinase inhibitor [Abstract]. *Blood*. 2004;104:160a. Abstract no. 553.
84. **Burgess MR, Skaggs BJ, Shah NP, Lee FY, Sawyers CL.** Comparative analysis of two clinically active BCR-ABL kinase inhibitors reveals the role of conformation-specific binding in resistance. *Proc Natl Acad Sci U S A*. 2005;102:3395-400. [PMID: 15705718]
85. **Fabian MA, Biggs WH 3rd, Treiber DK, Atteridge CE, Azimioara MD, Benedetti MG, et al.** A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat Biotechnol*. 2005;23:329-36. [PMID: 15711537]
86. **Carter TA, Wodicka LM, Shah NP, Velasco AM, Fabian MA, Treiber DK, et al.** Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc Natl Acad Sci U S A*. 2005;102:11011-6. [PMID: 16046538]
87. **Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, et al.** Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N Engl J Med*. 2006;354:2531-41. [PMID: 16775234]
88. **Talpaz M, Apperley JF, Kim DW, Silver RT, Bullorsky EO, Cheng S, et al.** Dasatinib (D) in patients with accelerated phase chronic myeloid leukemia (AP-CML) who are resistant or intolerant to imatinib: results of the CA180005 'Start-A' study [Abstract]. *J Clin Oncol*. 2006;24:343s. Abstract no. 6526.
89. **Cortes JE, Kim DW, Rosti G, Rousselot P, Bleickardt E, Zink R, et al.** Dasatinib (D) in patients (pts) with chronic myelogenous leukemia (CML) in myeloid blast crisis (MBC) who are resistant or intolerant to imatinib: results of the CA180006 'start B' study [Abstract]. *J Clin Oncol*. 2006;344s. Abstract no. 6529.
90. **Hochhaus A, Kantarjian H, Bacarani M, et al.** Dasatinib in patients with chronic phase chronic myeloid leukemia (CP-CML) who are resistant or intolerant to imatinib: results of the CA180013 'start C' study [Abstract]. *J Clin Oncol*. 2006;24:339s. Abstract no. 6508.
91. **Coutre S, Martinelli G, Dombret H, Hochhaus A, Larson R, Saglio G, et al.** Dasatinib in patients with chronic myelogenous leukemia (CML) in lymphoid blast crisis (LB-CML) or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL) who are imatinib (IM)-resistant (IM-R) or intolerant (IM-I): the CA180015 'start-L' study [Abstract]. *J Clin Oncol*. 2006;24:344s. Abstract no. 6528.
92. **Mestan J, Weisberg E, Cowan-Jacob S, Doriano F, Pascal F, Gabriele F, et al.** AMN107: in vitro profile of a new inhibitor of the tyrosine kinase activity of Bcr-Abl [Abstract]. *Blood*. 2004;104:546a. Abstract no. 1978.
93. **le Coutre P, Baskaynak G, Tamm I, Westermann J, Duyster J, Bonin M.** Activity and induction of apoptosis of the specific tyrosine kinase inhibitor AMN107 in Bcr-Abl+ cell lines and in imatinib resistant primary cells from CML patients [Abstract]. *Blood*. 2004;104:218a. Abstract no. 762.
94. **Kantarjian H, Giles F, Wunderle L, Bhalla K, O'Brien S, Wassmann B, et al.** Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. *N Engl J Med*. 2006;354:2542-51. [PMID: 16775235]
95. **Kantarjian H, Gattermann N, O'Brien S, et al.** A phase II study of AMN107, a novel inhibitor of Bcr-Abl, administered to imatinib resistant and intolerant patients (pts) with chronic myelogenous leukemia (CML) in chronic phase (CP) [Abstract]. *J Clin Oncol*. 2006;24:345s. Abstract no. 6534.
96. **Le Coutre P, Ottmann O, Gatterman N, et al.** A phase II study of AMN107, a novel inhibitor of Bcr-Abl, administered to imatinib-resistant or intolerant patients (pts) with chronic myelogenous leukemia (CML) in accelerated phase (AP) [Abstract]. *J Clin Oncol*. 2006;24:344s. Abstract no. 6531.
97. **Giles FJ, Larson R, Le Coutre P, et al.** A phase II study of AMN107, a novel inhibitor of Bcr-Abl, administered to imatinib-resistant or intolerant patients (pts) with Ph+ chronic myelogenous leukemia (CML) in blast crisis (BC) or relapsed/refractory Ph+ acute lymphoblastic leukemia (ALL) [Abstract]. *J Clin Oncol*. 2006;24:345s. Abstract no. 6536.
98. **Gumireddy K, Baker SJ, Cosenza SC, John P, Kang AD, Robell KA, et al.** A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance. *Proc Natl Acad Sci U S A*. 2005;102:1992-7. [PMID: 15677719]
99. **Gumireddy K, Reddy MV, Cosenza SC, Boominathan R, Boomi Nathan R, Baker SJ, et al.** ON01910, a non-ATP-competitive small molecule inhibitor of Plk1, is a potent anticancer agent. *Cancer Cell*. 2005;7:275-86. [PMID: 15766665]
100. **Mow BM, Chandra J, Svingen PA, Hallgren CG, Weisberg E, Kottke TJ, et al.** Effects of the Bcr/abl kinase inhibitors STI571 and adaphostin (NSC 680410) on chronic myelogenous leukemia cells in vitro. *Blood*. 2002;99:664-71. [PMID: 11781252]
101. **Chandra J, Hackbarth J, Le S, Loegering D, Bone N, Bruzek LM, et al.** Involvement of reactive oxygen species in adaphostin-induced cytotoxicity in human leukemia cells. *Blood*. 2003;102:4512-9. [PMID: 12920036]
102. **Deininger MW, O'Brien SG, Ford JM, Druker BJ.** Practical management of patients with chronic myeloid leukemia receiving imatinib. *J Clin Oncol*. 2003;21:1637-47. [PMID: 12668652]
103. **Peggs K, Mackinnon S.** Imatinib mesylate—the new gold standard for treatment of chronic myeloid leukemia. *N Engl J Med*. 2003;348:1048-50. [PMID: 12637616]

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