



Donna Duke Morrison. *All in the Family*. Watercolor on canvas, 16" × 20".

Several factors are associated with imatinib resistance, and some interventions have already been designed to reverse this clinical problem.

Mechanisms of Primary and Secondary Resistance to Imatinib in Chronic Myeloid Leukemia

Alfonso Quintás-Cardama, MD, Hagop M. Kantarjian, MD, and Jorge E. Cortes, MD

Background: Although the vast majority of patients with chronic myeloid leukemia (CML) respond to the tyrosine kinase inhibitor (TKI) imatinib mesylate, resistance might occur *de novo* or during treatment.

Methods: The authors reviewed the known mechanisms of primary and secondary resistance to imatinib and other TKIs used in the management of CML.

Results: Mutations within the kinase domain of BCR-ABL1 account for 30% to 40% of cases of imatinib resistance. Other mechanisms include BCR-ABL1 amplification, overexpression of the SRC family of kinases, and pharmacokinetic and pharmacodynamic factors.

Conclusions: Although not all resistance mechanisms have been identified and understood, several agents based on the known mechanisms have already been designed and developed and are beginning clinical trials.

Introduction

Chronic myeloid leukemia (CML) arises from the neoplastic transformation of a hematopoietic stem cell carrying the balanced translocation t(9;22)(q34;q11), which cytogenetically results in the Philadelphia chromosome^{1,2} and molecularly gives rise to the BCR-ABL1 hybrid gene.^{3,4} The protein kinase BCR-ABL1 encoded by the BCR-ABL1 oncogene is constitutively activated in CML. Several experimental models, such as BCR-

ABL1-expressing CD34+ cells in culture^{5,6} or retrovirally transduced BCR-ABL1-positive mouse cells,^{7,9} have demonstrated that BCR-ABL1 kinase is central to the pathogenesis of CML, which has provided the rationale for the targeted use of tyrosine kinase inhibitors (TKIs) for the treatment of CML. The unprecedented success of the first agent of this kind, imatinib mesylate, propelled the development of targeted therapies in multiple areas of cancer medicine. Imatinib, a phenylamino-pyrimidine TKI that specifically targets BCR-ABL1, KIT, and PDGFR kinases, has proven to be highly active and safe in patients with CML and has become standard front-line therapy for patients with this disorder. After a median follow-up of 72 months, the cumulative rates of complete hematologic response and cytogenetic response were 97% and 83%, respectively, for patients with CML in chronic phase (CML-CP) treated in the International Randomized Study of Interferon and STI571 (IRIS).¹⁰ However, these initial results were tem-

From the Department of Leukemia at The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Submitted July 3, 2008; accepted October 15, 2008.

Address correspondence to Alfonso Quintás-Cardama, MD, M. D. Anderson Cancer Center, Department of Leukemia, Unit 428, 1515 Holcombe Boulevard, Houston, TX 77030. E-mail: aquintas@mdanderson.org

Abbreviations used in this paper: TKI = tyrosine kinase inhibitor; CML = chronic myeloid leukemia, IRIS = International Randomized Study of Interferon and STI571, BP = blast phase.

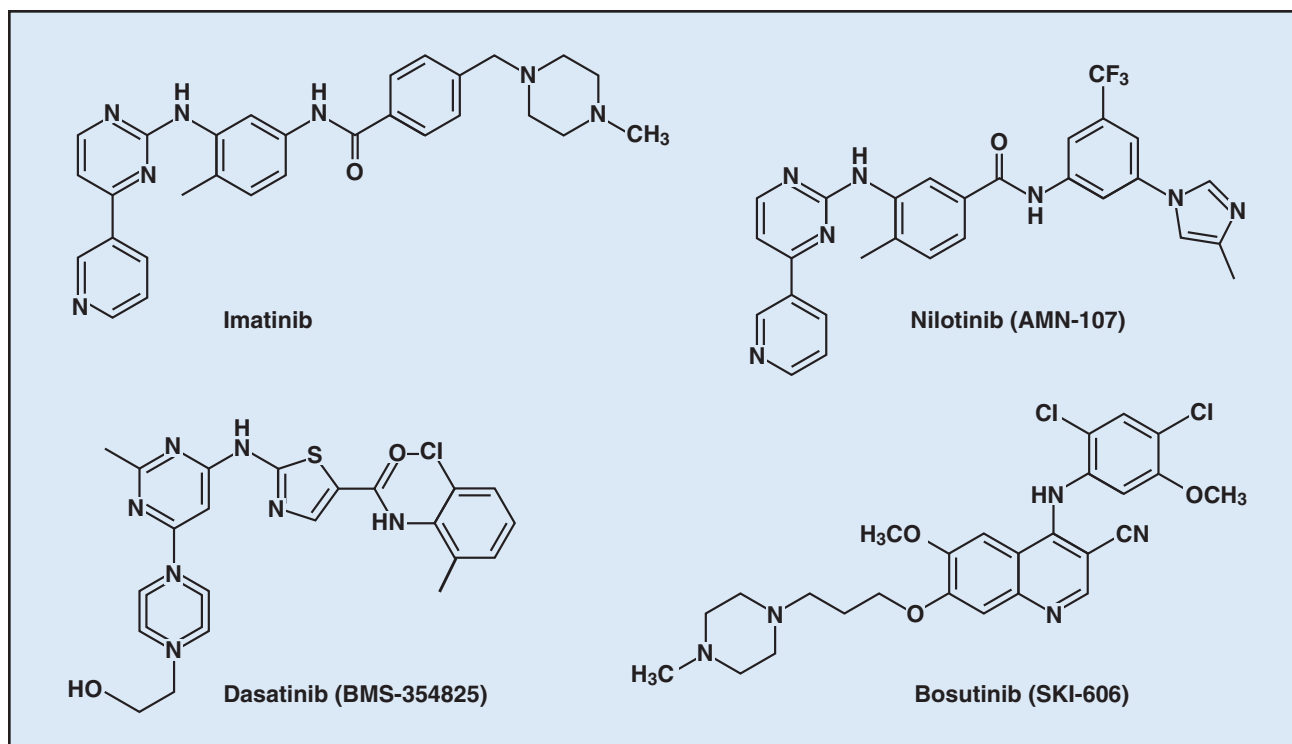


Figure. — Chemical structures of imatinib and second generation tyrosine kinase inhibitors currently under development in CML. This research was originally published in *Nat Rev Drug Discov.* Quintás-Cardama A, Kantarjian H, Cortes J. Flying under the radar: the new wave of BCR-ABL inhibitors. *Nat Rev Drug Discov.* 2007;6(10):834-848.

pered by the realization that varying levels of *BCR-ABL1* messenger RNA can be detected by polymerase chain reaction (PCR) in most patients receiving imatinib¹¹ and by the fact that responses in patients with advanced-phase CML were rare and generally short-lived.¹² These phenomena generated the concept of imatinib resistance.

Over the last 5 years, multiple studies have addressed the problem of imatinib resistance and helped to define the major elements contributing to this occurrence. Major inroads made in our understanding of the mechanisms driving imatinib resistance have resulted in the design of novel targeted agents to overcome the limitations of imatinib therapy. Some of these agents have reached advanced stages of clinical development, whereas many others are undergoing preclinical testing (Figure).

Imatinib Therapy in Patients With CML

BCR-ABL1 kinase is a pivotal driver of the pathogenesis of CML through phosphorylation and activation of a broad range of downstream substrates that modulate signal transduction and transformation.¹³ Thus, BCR-ABL1 kinase represents an obvious therapeutic target. Imatinib mesylate, an orally bioavailable 2-phenylaminopyrimidine, was the first compound described to target BCR-ABL1 kinase in a robust and efficacious manner.¹⁴ In cell-based assays, imatinib inhibits BCR-ABL1 kinase with 50% inhibitory concentration (IC_{50}) values

of 0.1 to 0.5 μM .¹⁴⁻¹⁶ Although only moderate, the potency of imatinib against BCR-ABL1 suffices to render important clinical benefits. In the phase III randomized IRIS trial, the efficacy of imatinib was compared to the combination of IFN- α and low-dose cytarabine, the standard of care at the time, in patients with newly diagnosed CML-CP.¹⁷ A recently published update of the IRIS trials indicates that 89% and 83% of patients have achieved a major cytogenetic response and a complete cytogenetic response, respectively, after a median follow-up of 72 months.¹⁰ Moreover, the progression-free and event-free survival rates were 93% and 83%, respectively, with an estimated CML-related mortality of only 5%.¹⁰ The rates of progression to accelerated phase or blast phase (BP) have steadily decreased after the second year of imatinib therapy, being 0% during the sixth year of therapy.¹⁰ As a consequence, imatinib is widely considered standard front-line therapy for patients with CML. Despite these unprecedented results, leukemic residual cells are detectable in most patients with CML receiving imatinib,¹¹ and some of these patients will eventually develop resistance to imatinib therapy, especially those with advanced-phase CML.

Clinical Resistance to Imatinib

Resistance to imatinib can be divided into primary (also referred to as “refractoriness”), in which patients exhibit lack of efficacy to this TKI from the start of therapy, and secondary (also referred to as “acquired resistance”),

which ensues upon the initial achievement of some degree of response to imatinib lasting for a period of time of variable length. Acquired resistance can be precisely characterized in the event of loss of a major cytogenetic response and complete hematologic response. Resistance can be further segregated into hematologic (lack of normalization of peripheral blood counts), cytogenetic (persistence of Ph chromosome), and molecular (persistence of *BCR-ABL1* transcripts by reverse transcriptase polymerase chain reaction [RT-PCR]). However, there is no consensus as to how to define acquired resistance based on loss of molecular response. The reason for this lack of agreement relates to the fact that quantitative RT-PCR techniques currently in use to monitor *BCR-ABL1* transcript levels are not standardized, and it also relates to the lack of a precise definition regarding what increment of *BCR-ABL1* transcript levels constitutes loss of molecular response. On the other hand, *BCR-ABL1* transcript levels undergo variations over time, which mandates serial determinations to confirm any actual upward trend. Other limitations regarding current RNA-based methods for quantification of *BCR-ABL1* transcript levels include the potential for RNA degradation, a requirement for a reverse transcription step, and inter-laboratory differences regarding sensitivity and specificity. Certainly, current efforts to standardize and optimize procedural aspects of the quantitative RT-PCR technique for measuring *BCR-ABL1* transcripts^{18,19} and the development of highly sensitive and specific DNA-based methods for *BCR-ABL1* detection²⁰ will facilitate the correlation between loss of molecular response and emergence of acquired resistance.

The direct consequence of acquired resistance is clinical failure of imatinib therapy. A consensus panel of experts on behalf of the European LeukemiaNet has

set forth recommendations for the clinical management of patients who develop imatinib failure (Table 1). The latter are defined based on the lack of achievement of a predefined level of response at specific chronologic milestones.²¹ In addition to defining imatinib failure, these guidelines define suboptimal response and alert clinicians about patients exhibiting certain “warning signs” who may require closer monitoring. The panel of experts recommended a dose increase of imatinib from 400 mg daily to 600–800 mg daily, allogeneic stem cell transplantation, or investigational therapies in the event of imatinib failure.²¹ Given the lack of evidence at the time of the issuance of these guidelines, a major limitation is the absence of specific recommendations as to when and in whom to switch therapy from imatinib to a second-generation TKI. At present, a change in therapy to a second-generation TKI is recommended for patients who meet criteria for imatinib failure. Patients with suboptimal response need close monitoring, and a dose escalation from 400 mg to 800 mg is justified. Ongoing studies are investigating the role of change of therapy to a second-generation TKI in this setting compared with the benefit of dose escalation.

Mechanisms of Resistance to Imatinib

Imatinib has become the standard front-line therapy for CML. However, the initial enthusiasm caused by the impressive results obtained with imatinib was partially tempered by the fact that *BCR-ABL1* transcripts were rendered undetectable in only a small fraction of treated patients.¹¹ It is estimated that approximately 20% to 30% of patients will eventually develop resistance to imatinib. Moreover, the responses obtained in patients with advanced CML are low and typically short-lived.¹² The realization that not all patients with CML respond

Table 1. — Failure and Suboptimal Response for Patients With CML in Early Chronic Phase Receiving Imatinib Therapy at 400 mg Daily

Time After Diagnosis (mos)	Failure	Suboptimal Response	Warnings
0	N/A	N/A	High risk, del der(9), ACAs in Ph+ cells
3	No HR (stable disease or progression)	Less than CHR	N/A
6	Less than CHR, no cytogenetic response	Less than PCyR	N/A
12	Less than PCyR	Less than CCyR	Less than MMR
18	Less than CCyR	Less than MMR	N/A
Any time	Loss of CHR or CCyR, mutation	ACA in Ph+ cells, loss of MMR, mutation	Rise in transcript level; other chromosomal abnormalities in Ph- cells

Failure denotes that imatinib therapy must be switched whenever available. Suboptimal response denotes that further therapeutic benefit may still be attained with continuation of imatinib therapy although long-term outcome is not likely to be optimal. Warnings indicate that patients must be closely monitored and may be eligible for other therapies. High risk is defined according to the Sokal or Hasford scores. N/A = not applicable, HR = hematologic response, CHR = complete hematologic response, CCyR = complete cytogenetic response, PCyR = partial cytogenetic response, MMR = major molecular response, ACA = additional chromosomal abnormality, del der(9) = deletion of derivative chromosome 9. This research was originally published in *Blood*. Baccarani M, Saglio G, Goldman J, 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(6):1809-1820. Epub 2006 May 18. © the American Society of Hematology.

to imatinib therapy prompted the development of laboratory models to understand the basis of imatinib resistance. Currently, resistance to imatinib and other TKIs is believed to be a consequence of the interaction of multiple factors including (but not limited to) treatment compliance, bioavailability, pharmacodynamics, genetic changes, *BCR-ABL1* kinase domain mutations, or combinations thereof. In simple terms, the mechanisms of imatinib resistance can be subdivided in BCR-ABL1-dependent and -independent.

BCR-ABL1-Independent Mechanisms of Resistance

Pharmacokinetic Considerations: Several studies have shown significant variability regarding imatinib plasma levels among patients receiving imatinib, which suggests that oral doses of imatinib administered at 400 mg daily do not guarantee the delivery of effective concentrations to the target cells.^{22,23} Since imatinib is metabolized largely by the cytochrome p450 isoenzymes P3A4 (CYP3A4) and P3A5 (CYP3A5), differences in the concentrations of CYP3A4/A5 or drugs than can inhibit or induce said enzymes have the potential to greatly affect the levels of imatinib in plasma. This is important given that trough imatinib plasma levels are associated with cytogenetic and molecular responses to standard-dose imatinib.²⁴ In that regard, administration of imatinib at doses of 400 mg daily resulted in peak plasma concentrations ranging from 2 to 3 µg/mL, but these were 4 to 5 µg/mL when doses of 600 mg daily were used.

It has been proposed that excessive binding of imatinib to the plasma protein α 1-acid glycoprotein-1 (AGP1), an acute-phase reactant that binds cationic drugs at a 1:1 molar ratio, may result in diminished levels of active drug, limited therapeutic activity, and development of resistance.²⁵ However, these results have not been borne out in other studies,²⁶ and the impact of AGP1 as a cause of imatinib resistance remains controversial.

Intracellular Uptake of Imatinib: The amount of imatinib that actually enters the target cell is a direct function of the balance between influx and efflux. The adenosine triphosphate-binding cassette (ABC) transporter ABCB1 (or MDR-1) is a transmembrane protein that mediates multidrug resistance in multiple neoplasias through regulation of the efflux of different chemotherapeutic agents. Interestingly, ABCB1 is overexpressed in cells from patients with BP CML and has been linked to the development of imatinib resistance.²⁷ Several groups have shown that imatinib is a substrate for ABCB1.²⁸⁻³⁰ However, the imatinib efflux activity of ABCB1 is relatively small compared with the activity of this protein on classical cytotoxic agents. The role of ABCB1 in imatinib resistance remains unclear as a recent study has shown that overexpression of ABCB1 in K562 cells does not confer resistance to imatinib in vitro.³¹ In contrast with this study, Galimberti et al³² showed that those patients who failed to

attain a major cytogenetic response or progressed exhibited ABCB1 overexpression. ABCB1, as well as the ABC transporter breast-cancer resistance protein (ABCG2), is expressed on the surface of the epithelial cells of the gastrointestinal and biliary tracts and also in primitive normal hematopoietic stem cells,^{33,34} suggesting that imatinib efflux at this level may result in suboptimal imatinib bioavailability and perhaps CML stem cell insensitivity to imatinib. However, it has been recently shown that imatinib is an inhibitor of, but not a substrate for, ABCG2 and that, therefore, ABCG2 does not modulate intracellular concentrations of imatinib in CML stem cells.³⁴

Inhibition of imatinib influx through the human organic cation transporter (hOCT1) has also been proposed as an important factor regulating intracellular imatinib availability.^{30,35} Polymorphisms of this carrier protein may alter the entry of imatinib in the cell. However, although hOCT1 may regulate imatinib influx, a definite connection with response in patients with CML has not been clearly identified. Since all the above-mentioned transporters regulate in concert the active transport of imatinib in and out of the cell and may potentially play an important role in the pharmacogenetics of imatinib, the impact of a variety of single nucleotide polymorphisms in the genes encoding for these proteins has been recently reported.³⁶ Notably, the GG allele in *ABCG2* (rs2231137) and advanced-phase CML was significantly associated with poor response to imatinib, whereas the GG allele at *HOCT1* (rs683369) and advanced-phase correlated with a high rate of loss of response or treatment failure. The CC allele in *ABCG2* (rs2231142) was also found to be an independent predictor of more frequent need for imatinib dose escalation.³⁶

CML Stem Cell Quiescence: The clearance of *BCR-ABL1* transcripts during imatinib therapy follows a biphasic decline characterized by an initial phase during which transcripts are rapidly cleared, followed by a second phase that is characterized by a slower rate of clearance.³⁷⁻³⁹ This biphasic decline is likely due to a differential susceptibility of CML cell subpopulations to imatinib. In this model, differentiated cells are readily cleared by the drug, while CML stem cells are not affected by virtue of their quiescent status, leading to persistence of residual disease.^{38,39} Quiescent CML cells (Lin-CD34+) account for approximately 0.5% of the CD34+ population and are characterized by intrinsic resistance to imatinib therapy.⁴⁰⁻⁴² The insensitivity of quiescent CML stem cells is likely multifactorial and related to reduced imatinib exposure due to alterations in drug uptake or efflux and mutations within the tyrosine kinase domain of *BCR-ABL1*. Moreover, *BCR-ABL1* is overexpressed in primitive CML cells. In fact, Copland et al⁴³ showed that primitive imatinib-resistant CML CD34+CD38- cells carried a single copy of *BCR-*

ABL1 but expressed significantly higher *BCR-ABL1* transcript levels and BCR-ABL1 protein kinase than more mature CML cells. In addition, CrKL phosphorylation was higher in primitive CD34+CD38- cells than in the total CD34+ population. Albeit more effective than imatinib within the CML stem cell compartment, other TKIs such as dasatinib⁴³ and bosutinib⁴⁴ have shown limited activity against the most primitive quiescent CML cells and appear to be resistant to both drugs.

The resistance exhibited by the small subpopulation of quiescent CML cells will preclude the ultimate eradication of *BCR-ABL1*-positive cells. To attain this objective, combination therapies involving a TKI and agents with activity against CML acting through non-*BCR-ABL1* kinase mechanisms will be required. To this end, the ability to eradicate residual disease with several immune approaches is currently being tested in clinical trials.

Clonal Evolution: The acquisition of additional nonrandom cytogenetic aberrancies in Ph+ metaphases, also known as “clonal evolution,” has been observed in most patients with CML during transition to BP. In some reports, clonal evolution has been reported in advanced-phase CML at higher frequencies than in *BCR-ABL1* mutations.⁴⁵ On the other hand, the development of *BCR-ABL1* mutations is more prevalent in patients receiving imatinib therapy who exhibit clonal evolution.⁴⁶ The most frequent cytogenetic abnormalities associated with clonal evolution are trisomy 8 (34%), isochromosome 17 (20%), and duplicate Ph chromosome (38%),⁴⁷ which have been linked to *c-Myc* overexpression, loss of 17p, and *BCR-ABL1* overexpression, respectively.⁴⁸⁻⁵⁰ Of note, the tumor suppressor *p53* is located on 17p and is found mutated in 25% to 30% of patients with myeloid BP CML.⁴⁹ Notably, *p53* inactivation has been shown to block the response to imatinib in vitro and in vivo, thus leading to imatinib resistance.⁵¹ Other cytogenetic aberrancies, such as trisomy 19, trisomy 21, trisomy 17, and deletion 7, have been identified in less than 10% of cases of clonal evolution.⁵² Also, 10% to 15% of patients with CML present with deletions of the derivative chromosome 9, which may lead to more rapid progression to BP than those lacking this abnormality.⁵³ Overall, clonal evolution is a phenomenon that reflects a state of genetic instability that is frequently associated with advanced stages of CML and appears to play a pivotal role in CML progression.

SRC Overexpression: The SRC family kinases (SFKs) encompass 9 cytoplasmic nonreceptor homologous nonreceptor protein kinases (SRC, FYN, YES, BLK, YRK, FGR, HCK, LCK, and LYN).⁵⁴ Whereas some SFKs are expressed ubiquitously, others exhibit tissue-specificity.⁵⁴ Experiments with SRC dominant-negative mutants suggest that SFKs induce proliferation of BCR-ABL1-expressing cells.⁵⁴⁻⁵⁶ BCR-ABL1 kinase activates LYN, HCK, and FGR.⁵⁷ Upon activation, HCK activates

signal transducer and activator of transcription 5 (STAT5), which in turn activates gene transcription upon binding to cognate DNA sequences^{57,58} and upregulation of cyclin D1, which results in cell-cycle progression from G₁ to S phase.^{59,60} However, several lines of evidence have questioned the role of SFKs in the pathogenesis of CML. First, transduction of *BCR-ABL1* into bone marrow cells from mice lacking HCK, LYN, and FGR — the 3 main SFKs expressed in early hematopoietic progenitor and myeloid cells — can efficiently induce a CML-like myeloproliferative disorder.⁵⁷ Second, patients rendered insensitive to imatinib upon development of the *BCR-ABL1 Thr315Ile (T315I)* mutation are also resistant to the potent ABL1/SFK inhibitor dasatinib, suggesting that the role of SFKs in the maintenance of CML is at least limited. Yet, overexpression and/or activation of HCK and LYN has been implicated in CML progression to BP and imatinib resistance.⁶¹⁻⁶⁴ In fact, LYN kinase has been shown to function as a regulator of imatinib sensitivity in CML, and it is found persistently activated in patients after failure of imatinib therapy who carry no *BCR-ABL1* mutations.⁶⁵ These observations indicate that in cells with high LYN expression, a combined approach targeting both BCR-ABL1 and LYN kinases may be necessary to overcome this form of imatinib resistance.

BCR-ABL1-Related Mechanisms of Resistance

BCR-ABL1 Overexpression: Upregulation of the BCR-ABL1 kinase in association with amplification of the *BCR-ABL1* gene was first reported in the Ba/F3 BCR-ABL-r, LAMA84-r, and AR230-r imatinib-resistant cell lines in the absence of mutations within the *BCR-ABL1* kinase domain.²⁷ *BCR-ABL1* amplification was first documented in the clinic in 3 of 11 patients with BP CML or Ph+ acute lymphoblastic leukemia (ALL) who developed resistance to imatinib therapy.⁶⁶ However, a subsequent screening in 66 patients with imatinib-resistant CML (33 in myeloid BP, 2 in lymphoid BP, 16 in accelerated phase, 13 in chronic phase, and 2 with Ph+ ALL) showed that only 2 patients had *BCR-ABL1* gene amplification evaluated by fluorescence in situ hybridization (FISH), suggesting that imatinib resistance due to point mutations is a far more common mechanism of resistance.⁶⁷ That said, a connection between *BCR-ABL1* gene amplification and *BCR-ABL1* mutations may exist since CD34+ CML cells expressing high amounts of *BCR-ABL1* are much less sensitive to imatinib, yield mutant subclones resistant to imatinib, and develop mutations much faster than those with low levels of *BCR-ABL1* expression.⁶⁸ Therefore, the levels of BCR-ABL1 protein dictate, at least partially, the rate at which imatinib-resistant BCR-ABL1-positive clones emerge and become dominant.⁶⁸ Of note, granulocyte-macrophage progenitor (GMP) cells with a CD3+CD38+Lin- phenotype obtained from patients

with BP CML express higher numbers of *BCR-ABL1* transcripts than their counterparts obtained from patients with CML-CP.⁶⁹

Point Mutations in the Kinase Domain of *BCR-ABL1*: The development of mutations within the kinase domain of *BCR-ABL1* has constituted a pervading theme regarding TKI resistance in CML. The frequency of *BCR-ABL1* mutations in patients resistant to imatinib ranges from 40% to 90%, depending on the definition of resistance, the methodology of detection, and CML phase.^{66,70-75} Gorre et al⁶⁶ were the first to document the development of *BCR-ABL1* mutations in 11 patients with advanced-phase CML or Ph+ ALL who relapsed on imatinib. In 6 of 9 assessable patients, resistance was associated with the presence of the T315I mutation. In a more recent and extensive analysis, the T315I mutation was detected in 15% of 112 patients with CML who failed imatinib therapy.⁷⁶ Imatinib binds to a catalytically inactive conformation of ABL1 kinase, often referred to as the “DFG-out” conformation, in which the highly conserved Asp-Phe-Gly (DFG) residues are swung out of their position in the active kinase conformation.⁷⁷ Imatinib extends deeply into the catalytic domain, and its pyridinyl group locates underneath helix α C in the NH2-terminal lobe of ABL1 kinase.⁷⁷ Thr315, also known as the gatekeeper residue, locates at the periphery of the nucleotide-

binding site of ABL1 and forms a key H-bond interaction with imatinib.⁷⁸ The T315I mutation disrupts this H-bond interaction, which, in addition to the bulk of the isoleucine side-chain, sterically impairs imatinib binding, resulting in complete insensitivity to imatinib and the second-generation TKIs dasatinib, nilotinib, and bosutinib at clinically achievable concentrations (Figure).^{12,66,79-84}

To date, more than 100 distinct point mutations encoding for single amino acid substitutions in the kinase domain of *BCR-ABL1* have been detected in patients with CML resistant to imatinib therapy.^{71,77,78,85-88} Many others have also been generated in vitro by random mutagenesis of *BCR-ABL1*.^{67,86} Notably, different mutations can occur at the same position, resulting in a different amino acid substitution, and different substitutions confer distinct degrees of resistance to imatinib (Table 2). The most frequently reported mutations in clinical specimens are those that map to the P-loop region (residues 244 to 255) of the kinase domain, which serves as a docking site for phosphate moieties of ATP.^{89,91} While some studies have linked the development of P-loop mutations to poor clinical outcome during imatinib therapy,^{92,93} others have not confirmed this observation.⁴⁶ In addition, a subset of mutations occur at the activation (A) loop (residues 381 to 402), which is a key regulatory element of the ABL1 kinase. Mutations within the A loop prevent the kinase from adopting the inactive conformation to which imatinib binds. *BCR-ABL1* mutations have also been described mapping to the catalytic (C) domain (residues 350 to 363). In advanced-phase CML, some mutations appear to map to specific areas of the kinase domain affecting a selected group of residues such as Q253, Y253, E255, T315, E459, and F486.⁹⁴ Despite the wide variety of point mutations found in *BCR-ABL1*, most mutants are rare. In fact, mutations involving residues Gly250, Tyr253, Glu255, Thr315, Met351, and Phe359 account for 60% to 70% of all mutations.⁹⁵

It is worth emphasizing that the presence of *BCR-ABL1* mutations does not always explain clinical resistance to imatinib⁹⁶ and that different mutations are endowed with different transforming capability. In a pre-B-cell transformation assay, T315I (which has weaker kinase activity than p210BCR-ABL1) and E255K consistently showed a 10% to 20% increase in oncogenic potency relative to p210BCR-ABL1, whereas Y253F and E255V displayed potencies similar to p210BCR-ABL1 and Y253H, T315A, F317L, and M351T were markedly weaker.⁹⁷ Not surprisingly, the development of E255K is associated with poorer prognosis among patients with CML receiving imatinib.⁹³ Griswold et al⁹⁸ reported that relative to unmutated BCR-ABL1, the P-loop mutations Y253F and E255K exhibited increased transformation potency, whereas M351T and H396P were less potent. Notably, the kinase activity of E255K, H396P, and T315I did not correlate with transforming

Table 2. — Activity of Imatinib on Kinase- and Cell-Based (Ba/F3) Assays Against a Selection of *BCR-ABL1* Mutants Found in Patients With CML

<i>BCR-ABL1</i> (construct)	Imatinib	
	Autophosphorylation	Proliferation
BCR-ABL1 p210+IL-3	N/A	> 7,700
WT p210	221 ± 31	678 ± 39
G250E	2,287 ± 826	3,329 ± 1,488
G250V	489	624
Q252H	1,080 ± 119	851 ± 436
Y253H	> 10,000	> 7,000
E255K	4,856 ± 482	5,567
E255K	2,455 ± 433	7,161 ± 970
E255V	6,353 ± 636	6,111 ± 854
D276G	1,284	2,486
E292K	275 ± 81	1,552
T315I	> 10,000	> 7,000
F317C	1,090	694
F317L	797 ± 92	1,528 ± 227
F317V	544 ± 47	549 ± 173
M351T	593 ± 57	1,682 ± 233
E355G	601	1,149
F359V	1,528	595
F486S	1,238 ± 110	3,050 ± 597

All concentrations are shown in nmol/mL. IL-3 = interleukin-3. Adapted from Weisberg E, Manley PW, Breitenstein W, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell*. 2005;7(2):129-141. With permission from Elsevier.

Table 3. — Methodologies for the Detection of Mutations Within the Kinase Domain of *BCR-ABL1*

Technique	Sensitivity (%)*	Biased**	Quantitative	Availability
Direct sequencing	15–25	No	No	+++
Subcloning and sequencing	5–10	No	Yes	+
D-HPLC	0.1–10	No	No	++
Pyrosequencing	5	No	Semi-quantitative	++
Double-gradient denaturing electrophoresis	5	No	No	+
Multiplex SNP and mass spectrometry	1.5–3	No	Yes	+
Fluorescence PCR and PNA clamping	0.2	Yes	Semi-quantitative	+
TaqMan-based RQ-PCR	0.1	Yes	Yes	+
Polymerase colony assay	0.01	No	Yes	+
Allele-specific oligonucleotide PCR	0.001	Yes	No	++

* Sensitivity refers to the smallest size of subclones carrying mutations each test can detect.
 ** Biased denotes that the technique was designed to detect specific mutations. D-HPLC = denaturing high-performance liquid chromatography, SNP = single nucleotide polymorphism, PNA = peptide nucleic acid, PCR = polymerase chain reaction, RQ-PCR = real-time quantitative PCR. Adapted from Hughes T, Deininger M, Hochhaus A, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. *Blood*. 2006; 108(1):28-37. © the American Society of Hematology.

potency. Analysis of the phosphotyrosine proteome by mass spectroscopy confirmed the presence of different phosphorylation signatures among the different mutants, confirming that different mutations determine substrate specificity leading to activation of different downstream pathways.

By *BCR-ABL1* genotype analysis, some patients with CML after failure of sequential therapy with imatinib and dasatinib were found to carry more than one mutation within the same *BCR-ABL1* molecule (polymutants), which was associated with increased oncogenic potency compared with each individual mutation in transformation assays.⁹⁹ Recently, 61 patients with CML after imatinib intolerance (n = 10) or imatinib resistance (n = 51) who received therapy with dasatinib were studied by DNA expansion of specific clones followed by DNA sequencing of at least 10 clones. This study demonstrated the presence of 118 distinct mutations (77 previously not reported) at 112 amino acids.⁸⁷ More than 90% of patients harbored BCR-ABL1 kinase domain mutations prior to the start of dasatinib, and polymutants were detected in 57% of patients.⁸⁷ The treatment of patients carrying polymutants may require the use of a combination of TKIs with activity against all single-point mutations contained in the compound mutation.

Most discrepancies concerning the incidence and number of reported BCR-ABL1 mutations relate to remarkable differences in sensitivities of the methodologies of detection employed in different studies (Table 3). Most studies employ the use of direct sequencing for mutation detection. The ease of use of this technique has made it readily available to most clinical laboratories. However, its sensitivity is approximately 10% to 20%. Therefore, the mutant allele must be present in a significant proportion of screened

clones for it to be detected. This results in high rates of false negatives.^{71,87} A significant improvement over direct sequencing in terms of sensitivity is the use of DNA expansion of specific clones followed by DNA sequencing of a significant number of clones, which in most studies is set arbitrarily at 10. Although more sensitive, subcloning techniques are cumbersome, time-consuming, and expensive.^{71,87} Several studies have shown that denaturing high-performance liquid chromatography, with a sensitivity of 1% to 10%,¹⁰⁰⁻¹⁰² or pyrosequencing, with a sensitivity of 5% and the possibility of quantifying the mutant clone,⁹⁶ may be successfully used for clinical purposes. Other highly sensitive techniques that still require more extensive validation prior to widespread use in clinical laboratories include allele-specific oligonucleotide PCR¹⁰³ and the PCR colony assay.¹⁰⁴

Conclusions

While virtually all patients reach some degree of response to imatinib therapy, the depth, quality, and duration of this response are suboptimal in a subset of them, and this occurrence underlies the development of resistance to imatinib therapy. Multiple mechanisms of resistance have been invoked to explain why some patients fail to achieve the desired response to this TKI. More frequently, imatinib resistance is linked to the presence of *BCR-ABL1* mutations. In recent years, important inroads have been made in the understanding of these mechanisms of resistance that have resulted in the development of therapeutics that, like the second generation of BCR-ABL1 TKIs, are capable of overcoming imatinib resistance in many patients. Yet, in some patients, imatinib resistance is far from being completely understood, and in other patients, like those carrying

the T315I mutation, it is far from being satisfactorily addressed from a therapeutic standpoint. Thus, research efforts must continue to improve the management and outcome of this problematic subset of patients.

Disclosures

Dr Cortes and Dr Kantarjian report grants/research support from Novartis Pharmaceuticals Corp, Bristol-Myers Squibb Company, and Wyeth.

Dr Quintás-Cardama reports no significant relationship with the companies/organizations whose products or services may be referenced in this article.

References

1. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. *Science*. 1960;132:1497. Abstract.
2. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 1973;243(5405):290-293.
3. Bartram CR, de Klein A, Hagemeijer A, et al. Translocation of c-abl1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. *Nature*. 1983;306(5940):277-280.
4. Groffen J, Stephenson JR, Heisterkamp N, et al. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell*. 1984;36(1):93-99.
5. Ramaraj P, Singh H, Niu N, et al. Effect of mutational inactivation of tyrosine kinase activity on BCR/ABL-induced abnormalities in cell growth and adhesion in human hematopoietic progenitors. *Cancer Res*. 2004;64(15):5322-5331.
6. Zhao RC, Jiang Y, Verfaillie CM. A model of human p210(bcr/ABL)-mediated chronic myelogenous leukemia by transduction of primary normal human CD34(+) cells with a BCR/ABL-containing retroviral vector. *Blood*. 2001;97(8):2406-2412.
7. 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(4944):824-830.
8. Kelliher MA, McLaughlin J, Witte ON, et al. Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. *Proc Natl Acad Sci U S A*. 1990;87(17):6649-6653.
9. Zhang X, Ren R. Bcr-Abl efficiently induces a myeloproliferative disease and production of excess interleukin-3 and granulocyte-macrophage colony-stimulating factor in mice: a novel model for chronic myelogenous leukemia. *Blood*. 1998;92(10):3829-3840.
10. Hochhaus A, Druker BJ, Larson RA, et al. IRIS 6-year follow-up: sustained survival and declining annual rate of transformation in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. *Blood (ASH Annual Meeting Abstracts)*. 2007;110. Abstract 25.
11. Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*. 2006;355(23):2408-2417.
12. Druker BJ, Sawyers CL, Kantarjian H, 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(14):1038-1042. Erratum in: *N Engl J Med*. 2001;345(3):232.
13. Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer*. 2005;5(3):172-183.
14. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med*. 1996;2(5):561-566.
15. Beran M, Cao X, Estrov Z, et al. 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*. 1998;4(7):1661-1672.
16. Gambacorti-Passerini C, le Coutre P, Mologni L, et al. Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis. *Blood Cells Mol Dis*. 1997;23(3):380-394.
17. O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2003;348(11):994-1004.
18. Saldanha J, Silvy M, Beaufils N, et al. Characterization of a reference material for BCR-ABL (M-BCR) mRNA quantitation by real-time amplification assays: towards new standards for gene expression measurements. *Leukemia*. 2007;21(7):1481-1487. Epub 2007 May 3.
19. Branford S, Cross NC, Hochhaus A, et al. Rationale for the recommendations for harmonizing current methodology for detecting BCR-ABL transcripts in patients with chronic myeloid leukaemia. *Leukemia*. 2006;20(11):1925-1930. Epub 2006 Sep 14.
20. Morley A, Bartley P, Martin-Harris H, et al. DNA-based measurement of BCR-ABL in chronic myeloid leukemia (CML). *Blood (ASH Annual Meeting Abstracts)*. 2007;110. Abstract 2946.
21. Baccarani M, Saglio G, Goldman J, 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(6):1809-1820. Epub 2006 May 18.
22. le Coutre P, Kreuzer KA, Pursche S, et al. Pharmacokinetics and cellular uptake of imatinib and its main metabolite CGP74588. *Cancer Chemother Pharmacol*. 2004;53(4):313-323. Epub 2003 Dec 5.
23. Peng B, Hayes M, Resta D, et al. Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. *J Clin Oncol*. 2004;22(5):935-942.
24. Picard S, Titier K, Etienne G, et al. Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood*. 2007;109(8):3496-3499. Epub 2006 Dec 27.
25. Gambacorti-Passerini C, Barni R, le Coutre P, et al. Role of alpha1 acid glycoprotein in the in vivo resistance of human BCR-ABL(+) leukemic cells to the abl inhibitor STI571. *J Natl Cancer Inst*. 2000;92(20):1641-1650.
26. Jørgensen HG, Elliott MA, Allan EK, et al. Alpha1-acid glycoprotein expressed in the plasma of chronic myeloid leukemia patients does not mediate significant in vitro resistance to STI571. *Blood*. 2002;99(2):713-715.
27. Mahon FX, Deininger MW, Schultheis B, et al. Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. *Blood*. 2000;96(3):1070-1079.
28. Dai H, Marbach P, Lemaire M, et al. Distribution of STI-571 to the brain is limited by P-glycoprotein-mediated efflux. *J Pharmacol Exp Ther*. 2003;304(3):1085-1092.
29. Mahon FX, Belloc F, Lagarde V, et al. MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. *Blood*. 2003;101(6):2368-2373.
30. Thomas J, Wang L, Clark RE, et al. Active transport of imatinib into and out of cells: implications for drug resistance. *Blood*. 2004;104(12):3739-3745. Epub 2004 Aug 17.
31. Ferrao PT, Frost MJ, Siah SP, et al. Overexpression of P-glycoprotein in K562 cells does not confer resistance to the growth inhibitory effects of imatinib (STI571) in vitro. *Blood*. 2003;102(13):4499-4503. Epub 2003 Jul 24.
32. Galimberti S, Cervetti G, Guerrini F, et al. Quantitative molecular monitoring of BCR-ABL and MDR1 transcripts in patients with chronic myeloid leukemia during Imatinib treatment. *Cancer Genet Cytogenet*. 2005;162(1):57-62.
33. Zhou S, Schuetz JD, Bunting KD, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med*. 2001;7(9):1028-1034.
34. Jordanides NE, Jørgensen HG, Holyoake TL, et al. Functional ABCG2 is overexpressed on primary CML CD34+ cells and is inhibited by imatinib mesylate. *Blood*. 2006;108(4):1370-1373. Epub 2006 Apr 20.
35. Crossman LC, Druker BJ, Deininger MW, et al. hOCT 1 and resistance to imatinib. *Blood*. 2005;106(3):1133-1134; author reply 1134.
36. Kim DH, Sriharsha L, Xu W, et al. Clinical relevance of a pharmacogenetic approach using multiple candidate gene polymorphisms to predict response and resistance to imatinib mesylate therapy in chronic myeloid leukemia. *Blood (ASH Annual Meeting Abstracts)*. 2007;110. Abstract 737.
37. Komarova NL, Wodarz D. Effect of cellular quiescence on the success of targeted CML therapy. *PLoS ONE*. 2007;2(10):e990.
38. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. *Nature*. 2005;435(7046):1267-1270.
39. Roeder I, Horn M, Glauche I, et al. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. *Nat Med*. 2006;12(10):1181-1184. Epub 2006 Oct 1.
40. Holtz MS, Forman SJ, Bhatia R. Nonproliferating CML CD34+ progenitors are resistant to apoptosis induced by a wide range of proapoptotic stimuli. *Leukemia*. 2005;19(6):1034-1041.
41. Holtz MS, Slovak ML, Zhang F, et al. Imatinib mesylate (STI571) inhibits growth of primitive malignant progenitors in chronic myelogenous leukemia through reversal of abnormally increased proliferation. *Blood*. 2002;99(10):3792-3800.
42. Graham SM, Jørgensen HG, Allan E, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood*. 2002;99(1):319-325.
43. Copland M, Hamilton A, Elrick LJ, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood*. 2006;107(11):4532-4539. Epub 2006 Feb 9.
44. König H, Holyoake TL, Bhatia R. Effective and selective inhibition of chronic myeloid leukemia primitive hematopoietic progenitors by the dual Src/Abl kinase inhibitor SKI-606. *Blood*. 2008;111(4):2329-2338. Epub 2007 Dec 4.
45. Lahaye T, Riehm B, Berger U, et al. Response and resistance in 300

patients with BCR-ABL-positive leukemias treated with imatinib in a single center: a 4.5-year follow-up. *Cancer*. 2005;103(8):1659-1669.

46. Jabbour E, Kantarjian H, Jones D, et al. Frequency and clinical significance of BCR-ABL mutations in patients with chronic myeloid leukemia treated with imatinib mesylate. *Leukemia*. 2006;20(10):1767-1773. Epub 2006 Jul 20.

47. Johansson B, Fioretos T, Mitelman F. Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. *Acta Haematol*. 2002;107(2):76-94.

48. Jennings BA, Mills KI. c-myc locus amplification and the acquisition of trisomy 8 in the evolution of chronic myeloid leukaemia. *Leuk Res*. 1998;22(10):899-903.

49. Calabretta B, Perrotti D. The biology of CML blast crisis. *Blood*. 2004;103(11):4010-4022. Epub 2004 Feb 24.

50. Gaiger A, Henn T, Hörth E, et al. Increase of bcr-abl chimeric mRNA expression in tumor cells of patients with chronic myeloid leukemia precedes disease progression. *Blood*. 1995;86(6):2371-2378.

51. Wendel HG, de Stanchina E, Cepero E, et al. Loss of p53 impedes the antileukemic response to BCR-ABL inhibition. *Proc Natl Acad Sci U S A*. 2006;103(19):7444-7449. Epub 2006 May 1.

52. Quintás-Cardama A, Cortes JE. Chronic myeloid leukemia: diagnosis and treatment. *Mayo Clin Proc*. 2006;81(7):973-988.

53. Huntly BJ, Bench A, Green AR. Double jeopardy from a single translocation: deletions of the derivative chromosome 9 in chronic myeloid leukemia. *Blood*. 2003;102(4):1160-1168. Epub 2003 May 1.

54. Abram CL, Courtneidge SA. Src family tyrosine kinases and growth factor signaling. *Exp Cell Res*. 2000;254(1):1-13.

55. Stanglmaier M, Warmuth M, Kleinlein I, et al. The interaction of the Bcr-Abl tyrosine kinase with the Src kinase Hck is mediated by multiple binding domains. *Leukemia*. 2003;17(2):283-289.

56. Danhauser-Riedel S, Warmuth M, Druker BJ, et al. Activation of Src kinases p53/56lyn and p59hck by p210bcr/abl in myeloid cells. *Cancer Res*. 1996;56(1):3589-3596.

57. Hu Y, Liu Y, Pelletier S, 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(5):453-461. Epub 2004 Apr 18.

58. Bhatia R, Holtz M, Niu N, et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood*. 2003;101(12):4701-4707. Epub 2003 Feb 6.

59. Kerkhoff E, Rapp UR. Cell cycle targets of Ras/Raf signalling. *Oncogene*. 1998;17(11 Reviews):1457-1462.

60. Nosaka T, Kawashima T, Misawa K, et al. STAT5 as a molecular regulator of proliferation, differentiation and apoptosis in hematopoietic cells. *EMBO J*. 1999;18(17):4754-4765.

61. Dai Y, Rahmani M, Corey SJ, et al. 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(33):34227-34239. Epub 2004 Jun 2.

62. Donato NJ, Wu JY, Stapley J, et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. *Blood*. 2003;101(2):690-698.

63. Donato NJ, Wu JY, Stapley J, et al. Imatinib mesylate resistance through BCR-ABL independence in chronic myelogenous leukemia. *Cancer Res*. 2004;64(2):672-677.

64. Hofmann WK, de Vos S, Elashoff D, et al. Relation between resistance of Philadelphia-chromosome-positive acute lymphoblastic leukaemia to the tyrosine kinase inhibitor STI571 and gene-expression profiles: a gene-expression study. *Lancet*. 2002;359(9305):481-486.

65. Wu J, Meng F, Lu H, et al. Lyn regulates BCR-ABL and Gab2 tyrosine phosphorylation and c-Cbl protein stability in imatinib-resistant chronic myelogenous leukemia cells. *Blood*. 2008;111(7):3821-3829. Epub 2008 Jan 30.

66. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science*. 2001;293(5531):876-880. Epub 2001 Jun 21.

67. Hochhaus A, Kreil S, Corbin AS, et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia*. 2002;16(11):2190-2196.

68. Barnes DJ, Palaiologou D, Panousopoulou E, et al. Bcr-Abl expression levels determine the rate of development of resistance to imatinib mesylate in chronic myeloid leukemia. *Cancer Res*. 2005;65(19):8912-8919.

69. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med*. 2004;351(7):657-667.

70. Hochhaus A, La Rosée P. Imatinib therapy in chronic myelogenous leukemia: strategies to avoid and overcome resistance. *Leukemia*. 2004;18(8):1321-1331.

71. Shah NP, Nicoll JM, Nagar B, 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(2):117-125.

72. Löwenberg B. Minimal residual disease in chronic myeloid leukemia.

N Engl J Med. 2003;349(15):1399-1401.

73. Corbin AS, La Rosée P, Stoffregen EP, et al. Several Bcr-Abl kinase domain mutants associated with imatinib resistance remain sensitive to imatinib. *Blood*. 2003;101(11):4611-4614. Epub 2003 Feb 6.

74. Gambacorti-Passerini CB, Gunby RH, Piazza R, et al. Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. *Lancet Oncol*. 2003;4(2):75-85.

75. Hughes T, Branford S. Molecular monitoring of BCR-ABL as a guide to clinical management in chronic myeloid leukaemia. *Blood Rev*. 2006;20(1):29-41. Epub 2005 Mar 2.

76. Cortes J, Jabbour E, Kantarjian H, et al. Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with multiple tyrosine kinase inhibitors. *Blood*. 2007;110(12):4005-4011. Epub 2007 Sep 4.

77. Schindler T, Bornmann W, Pellicena P, et al. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science*. 2000;289(5486):1938-1942.

78. Nagar B, Bornmann WG, Pellicena P, et al. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res*. 2002;62(15):4236-4243.

79. Weisberg E, Manley PW, Breitenstein W, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell*. 2005;7(2):129-141.

80. Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. *N Engl J Med*. 2006;354(24):2542-2551.

81. Talpaz M, Shah NP, Kantarjian H, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N Engl J Med*. 2006;354(24):2531-2541.

82. Lombardo LJ, Lee FY, Chen P, et al. Discovery of N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem*. 2004;47(27):6658-6661.

83. Shah NP, Tran C, Lee FY, et al. Overriding imatinib resistance with a novel Abl kinase inhibitor. *Science*. 2004;305(5682):399-401.

84. Weisberg E, Manley P, Mestan J, et al. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br J Cancer*. 2006;94(12):1765-1769. Epub 2006 May 23.

85. Hantschel O, Nagar B, Guettler S, et al. A myristoyl/phosphotyrosine switch regulates c-Abl. *Cell*. 2003;112(6):845-857.

86. Azam M, Latek RR, Daley GQ. Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR-ABL. *Cell*. 2003;112(6):831-843.

87. Quintás-Cardama A, Gibbons DL, Kantarjian H, et al. Mutational analysis of chronic myeloid leukemia (CML) clones reveals heightened BCR-ABL1 genetic instability and wild-type BCR-ABL1 exhaustion in patients failing sequential imatinib and dasatinib therapy. *Blood (ASH Annual Meeting Abstracts)*. 2007;110. Abstract 1938.

88. Hughes T, Deininger M, Hochhaus A, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. *Blood*. 2006;108(1):28-37. Epub 2006 Mar 7.

89. O'Hare T, Walters DK, Stoffregen EP, 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(11):4500-4505.

90. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. *Blood*. 2005;105(7):2640-2653. Epub 2004 Dec 23.

91. Carter TA, Wodicka LM, Shah NP, et al. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc Natl Acad Sci U S A*. 2005;102(31):11011-11016. Epub 2005 Jul 26.

92. Branford S, Rudzki Z, Walsh S, 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(1):276-283. Epub 2003 Mar 6.

93. Soverini S, Martinelli G, Rosti G, et al. ABL mutations in late chronic phase chronic myeloid leukemia patients with up-front cytogenetic resistance to imatinib are associated with a greater likelihood of progression to blast crisis and shorter survival: a study by the GIMEMA Working Party on Chronic Myeloid Leukemia. *J Clin Oncol*. 2005;23(18):4100-4109. Epub 2005 May 2.

94. Apperley JF. Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. *Lancet Oncol*. 2007;8(11):1018-1029.

95. Weisberg E, Manley PW, Cowan-Jacob SW, et al. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. *Nat Rev Cancer*. 2007;7(5):345-356.

96. Khorashad JS, Anand M, Marin D, et al. The presence of a BCR-ABL mutant allele in CML does not always explain clinical resistance to imatinib. *Leukemia*. 2006;20(4):658-663.

97. Skaggs BJ, Gorre ME, Ryzkin A, et al. Phosphorylation of the ATP-binding loop directs oncogenicity of drug-resistant BCR-ABL mutants. *Proc*

Natl Acad Sci U S A. 2006;103(51):19466-19471. Epub 2006 Dec 12.

98. Griswold IJ, MacPartlin M, Bumm T, et al. Kinase domain mutants of Bcr-Abl exhibit altered transformation potency, kinase activity, and substrate utilization, irrespective of sensitivity to imatinib. *Mol Cell Biol.* 2006;26(16):6082-6093.

99. Shah NP, Skaggs BJ, Branford S, et al. Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCR-ABL mutations with altered oncogenic potency. *J Clin Invest.* 2007;117(9):2562-2569.

100. Deininger MW, McGreevey L, Willis S, et al. Detection of ABL kinase domain mutations with denaturing high-performance liquid chromatography. *Leukemia.* 2004;18(4):864-871.

101. Soverini S, Martinelli G, Amabile M, et al. Denaturing-HPLC-based assay for detection of ABL mutations in chronic myeloid leukemia patients resistant to Imatinib. *Clin Chem.* 2004;50(7):1205-1213. Epub 2004 Apr 23.

102. Ernst T, Erben P, Muller MC, et al. Dynamics of BCR-ABL mutated clones prior to hematologic or cytogenetic resistance to imatinib. *Haematologica.* 2008;93(2):186-192. Epub 2008 Jan 26.

103. Willis SG, Lange T, Demehri S, et al. High-sensitivity detection of BCR-ABL kinase domain mutations in imatinib-naive patients: correlation with clonal cytogenetic evolution but not response to therapy. *Blood.* 2005;106(6):2128-2137. Epub 2005 May 24.

104. Nardi V, Raz T, Cao X, et al. Quantitative monitoring by polymerase colony assay of known mutations resistant to ABL kinase inhibitors. *Oncogene.* 2008;27(6):775-782. Epub 2007 Aug 6.