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

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**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 and molecularly gives rise to the BCR-ABL1 hybrid gene. 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 or retrovirally transduced BCR-ABL1–positive mouse cells, 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 phenylaminoimidazole 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). However, these initial results were tem-
pered by the realization that varying levels of BCR-ABL1 messenger RNA can be detected by polymerase chain reaction (PCR) in most patients receiving imatinib\(^1\) and by the fact that responses in patients with advanced-phase CML were rare and generally short-lived.\(^2\) 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.\(^3\) 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.\(^4\) In cell-based assays, imatinib inhibits BCR-ABL1 kinase with 50% inhibitory concentration (IC\(_{50}\)) values of 0.1 to 0.5 \(\mu M\).\(^5\)-\(^7\) 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-\(\alpha\) and low-dose cytarabine, the standard of care at the time, in patients with newly diagnosed CML-CP.\(^8\) 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.\(^9\) Moreover, the progression-free and event-free survival rates were 93% and 83%, respectively, with an estimated CML-related mortality of only 5%.\(^9\) 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.\(^9\) 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,\(^10\) 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\textsuperscript{18,19} and the development of highly sensitive and specific DNA-based methods for BCR-ABL1 detection\textsuperscript{20} 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{12} The realization that not all patients with CML respond

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Time After Diagnosis (mos) & Failure & Suboptimal Response & Warnings \\
\hline
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 & AGA in Ph+ cells, loss of MMR, mutation & Rise in transcript level; other chromosomal abnormalities in Ph– cells \\
\hline
\end{tabular}
\caption{Failure and Suboptimal Response for Patients With CML in Early Chronic Phase Receiving Imatinib Therapy at 400 mg Daily}
\label{table1}
\end{table}

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. 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. 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 α1-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. However, these results have not been borne out in other studies, 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. Several groups have shown that imatinib is a substrate for ABCB1. 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.

In contrast with this study, Galimberti et al 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, 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.

Inhibition of imatinib influx through the human organic cation transporter (hOCT1) has also been proposed as an important factor regulating intracellular imatinib availability. 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. 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.

**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. 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. Quiescent CML cells (Lin-CD34+) account for approximately 0.5% of the CD34+ population and are characterized by intrinsic resistance to imatinib therapy. 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 al 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 dasatinib and bosutinib 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. On the other hand, the development of BCR-ABL1 mutations is more prevalent in patients receiving imatinib therapy who exhibit clonal evolution. The most frequent cytogenetic abnormalities associated with clonal evolution are trisomy 8 (34%), isochromosome 17 (20%), and duplicate Ph chromosome (38%), which have been linked to c-Myc overexpression, loss of 17p, and BCR-ABL1 overexpression, respectively. Of note, the tumor suppressor p53 is located on 17p and is found mutated in 25% to 30% of patients with myeloid BP CML. Notably, p53 inactivation has been shown to block the response to imatinib in vitro and in vivo, thus leading to imatinib resistance. 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. 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. 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). Whereas some SFKs are expressed ubiquitously, others exhibit tissue-specificity. Experiments with SRC dominant-negative mutants suggest that SFKs induce proliferation of BCR ABL1–expressing cells. BCR-ABL1 kinase activates LYN, HCK, and FGR. Upon activation, HCK activates signal transducer and activator of transcription 5 (STAT5), which in turn activates gene transcription upon binding to cognate DNA sequences and upregulation of cyclin D1, which results in cell-cycle progression from G1 to S phase. 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. 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. 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. 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-ABL1, LAMA84, and AR230-r imatinib-resistant cell lines in the absence of mutations within the BCR-ABL1 kinase domain. 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. 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. 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. 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. 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.

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. Gorre et al. 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. 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. Imatinib extends deeply into the catalytic domain, and its pyridinyl group locates underneath helix αC in the NH2-terminal lobe of ABL1 kinase. 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. 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).

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. Many others have also been generated in vitro by random mutagenesis of BCR-ABL1. 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. While some studies have linked the development of P-loop mutations to poor clinical outcome during imatinib therapy, others have not confirmed this observation. 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. 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. It is worth emphasizing that the presence of BCR-ABL1 mutations does not always explain clinical resistance to imatinib 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. Not surprisingly, the development of E255K is associated with poorer prognosis among patients with CML receiving imatinib. Griswold et al. 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 resistance.

<table>
<thead>
<tr>
<th>BCR-ABL1 (construct)</th>
<th>Imatinib Autophosphorylation</th>
<th>Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL1 p210+IL-3</td>
<td>N/A</td>
<td>&gt; 7,700</td>
</tr>
<tr>
<td>WT p210</td>
<td>221 ± 31</td>
<td>678 ± 39</td>
</tr>
<tr>
<td>G250E</td>
<td>2,287 ± 826</td>
<td>3,329 ± 1,488</td>
</tr>
<tr>
<td>G250V</td>
<td>498</td>
<td>624</td>
</tr>
<tr>
<td>Q252H</td>
<td>1,080 ± 119</td>
<td>851 ± 436</td>
</tr>
<tr>
<td>Y253H</td>
<td>&gt; 10,000</td>
<td>&gt; 7,000</td>
</tr>
<tr>
<td>E255K</td>
<td>4,856 ± 482</td>
<td>5,567</td>
</tr>
<tr>
<td>E255K</td>
<td>2,455 ± 433</td>
<td>7,161 ± 970</td>
</tr>
<tr>
<td>E255V</td>
<td>6,353 ± 636</td>
<td>6,111 ± 854</td>
</tr>
<tr>
<td>D276G</td>
<td>1,284</td>
<td>2,486</td>
</tr>
<tr>
<td>E292K</td>
<td>275 ± 81</td>
<td>1,552</td>
</tr>
<tr>
<td>T315I</td>
<td>&gt; 10,000</td>
<td>&gt; 7,000</td>
</tr>
<tr>
<td>F317C</td>
<td>1,090</td>
<td>694</td>
</tr>
<tr>
<td>F317L</td>
<td>797 ± 92</td>
<td>1,528 ± 227</td>
</tr>
<tr>
<td>F317V</td>
<td>544 ± 47</td>
<td>549 ± 173</td>
</tr>
<tr>
<td>M351T</td>
<td>593 ± 57</td>
<td>1,682 ± 233</td>
</tr>
<tr>
<td>E355G</td>
<td>601</td>
<td>1,149</td>
</tr>
<tr>
<td>F359V</td>
<td>1,528</td>
<td>595</td>
</tr>
<tr>
<td>F486S</td>
<td>1,238 ± 110</td>
<td>3,050 ± 597</td>
</tr>
</tbody>
</table>

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. 2006;7(2):129-141. With permission from Elsevier.
potency. Analysis of the phosphotyrosine proteome by mass spectrometry 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

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

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

* Sensitivity refers to the smallest size of subclones carrying mutations each test can detect.

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

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