Resistance to Imatinib: Mechanisms and Management

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Key Words
Chronic myelogenous leukemia; imatinib; BCR-ABL; kinase inhibitor; drug resistance

Abstract
Imatinib, a specific small molecule inhibitor of the Abl kinase, has become the standard drug therapy for chronic myelogenous leukemia in all phases. More than 80% of newly diagnosed patients with chronic phase attain a complete cytogenetic response (CCR). Although remissions in patients with early disease are generally durable, acquired resistance after an initial response is common in advanced disease. Reactivation of Bcr-Abl signaling is almost invariably present at the time of relapse, consistent with re-establishment of the initial pathogenetic mechanism. Mutations in the kinase domain (KD) of Bcr-Abl that impair drug binding and increased expression of Bcr-Abl have been identified as major mechanism of acquired drug resistance. The fact that Bcr-Abl remains central to disease pathogenesis at the time of relapse implies that it also remains the optimal drug target. Alternative Abl kinase inhibitors with increased potency and activity against most Bcr-Abl KD mutants are currently undergoing phase I/II clinical testing, with encouraging early results. Despite the high rates of CCR, persistence of residual leukemia as assessed by reverse transcription polymerase chain reaction is the rule even in patients with chronic phase, suggesting that even these patients may remain at risk of relapse. Understanding the mechanisms underlying disease persistence will be crucial for developing strategies to eradicate residual leukemia. (JNCCN 2005;3:757-768)

Definitions
Response to Therapy
Monitoring chronic myelogenous leukemia (CML) patients on treatment has become complex, and precise knowledge of response criteria and definitions is required for optimal patient management (Table 1). The criteria for complete hematologic response and the degrees of cytogenetic response have been in use for a long time. More recently, major molecular response (MMR) was defined as the reduction of BCR-ABL messenger RNA (mRNA) by at least 3 orders of magnitude. No universal consensus has been reached as to the definition of complete molecular response (CMR), but the emerging consensus may be negativity on two subsequent occasions with a polymerase chain reaction (PCR) test capable of detecting one leukemic cell in 10^5 or more normal cells. Usually this will require nested reverse transcriptase-polymerase chain reaction (RT-PCR).

Primary and Acquired Resistance
Acquired resistance must be distinguished from primary resistance or refractoriness. Acquired resistance implies an increase in disease activity after a response at the hematologic, cytogenetic, or molecular level, whereas primary resistance is the failure to achieve a defined level of response. This distinction is important, as the mechanisms underlying the two types of resistance may be different, and overcoming them may require different therapeutic strategies.

Resistance to Imatinib: Scope of the Problem
The incidence of primary resistance at the various levels of response is directly correlated to the stage of disease at which treatment is initiated. Thus, primary hematologic resistance is very rare in chronic phase but common in blast crisis. Conversely, refractoriness at the molecular level, that is, the persistence of residual disease as detected...
by RT-PCR, is the rule even in newly diagnosed patients with chronic phase, at least on standard doses (400 mg daily) of imatinib (Figure 1).

Not surprisingly, patients with advanced disease are at a higher risk of relapse (acquired resistance). Thus, at 36 months follow-up, estimated progression-free survival (PFS) in the patients treated in the phase II studies of imatinib was 7% for patients with blast crisis, 40% for patients with accelerated phase disease, and 80% for patients with late chronic phase disease (that is, for whom interferon-α failed). In newly diagnosed patients with chronic phase disease, arguably the most relevant group, estimated PFS was 84% at 42 months. The yearly rate of relapse in this cohort peaked in the second year at 7.5% but declined thereafter (Table 2). Interestingly, the rate of progression to accelerated phase or blast crisis was rather constant during the observation period. Whether this pattern will persist remains to be seen with longer follow up.

Patients conceivably could remain at risk of progression as long as residual disease exists. Consistent with this, rapid recurrence of disease was seen in most patients with complete cytogenetic response (CCR) or even CMR who stopped taking imatinib. The overwhelming majority of patients, even of those treated in early chronic phase, remain positive by RT-PCR. Although considerable heterogeneity is noted across various publications (Table 3), higher doses of imatinib appear to be capable of inducing higher rates of CMR. However, the differences may partially reflect differences in the sensitivity of the PCR assays. Several prospective trials are currently underway in Europe and the United States to clarify this issue.

**Acquired Resistance**

**Binding of Imatinib to the Abl Kinase**

Analysis of the crystal structure of Abl in complex with an imatinib analogue was crucial to understanding imatinib’s mechanism of action. The catalytic domains of tyrosine kinases exhibit a universal bilobar structure. The smaller N-terminal lobe consists predominantly of antiparallel beta-sheets, whereas the C-lobe is largely alpha-helical. The cleft between the two lobes contains the amino acid residues that are critical for catalysis, including the ATP-binding loop (P-loop) and the activation loop (A-loop) with the highly conserved DFG (asparagine-phenylalanine-glycine) motif at its 5’ end. The A-loop is instrumental in regulating kinase activity.

In the closed (inactive) conformation, the A-loop occludes the catalytic center of the kinase, preventing adenosine triphosphate (ATP) and substrate binding. In the open (active) conformation, the A-loop swings away from the catalytic center and
provides a scaffold for substrates (Figure 2). The conformation of the A-loop is regulated by phosphorylation of critical tyrosine residues within the loop. Although the A-loop conformations of active kinases are very similar to each other, pronounced differences exist between the inactive conformations. Crystal structure analysis revealed that imatinib binds the inactive conformation of Abl, while it is sterically excluded from the active conformation.15,16 This explains the drug’s selectivity for Abl over structurally related kinases such as Src.

Imatinib tightly binds to Abl, penetrating almost the entire catalytic cleft of the kinase and engaging 21 amino acid residues in hydrophobic interactions and hydrogen bonds. An additional important observation from the crystal structure analysis was that the P-loop, a glycine-rich flexible structure, undergoes extensive positional rearrangements upon drug binding, much in the way of an induced fit.

Table 2  Progression in Patients on First-Line Imatinib

<table>
<thead>
<tr>
<th>Year</th>
<th>Progression (%)</th>
<th>Progression to accelerated phase or blast crisis (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3.4</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>4.6</td>
<td>1.6</td>
</tr>
<tr>
<td>4</td>
<td>2.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Mean</td>
<td>4.0</td>
<td>2.0</td>
</tr>
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</table>

Mechanisms of Imatinib Resistance

Fundamental to understanding imatinib resistance was the observation that phosphorylation of CrkL, a major substrate of Bcr-Abl, was restored in leukemia cells from patients with acquired resistance, consistent with reactivation of Bcr-Abl signaling (Figure 3). Thus, the genetic abnormality responsible for leukemic transformation remains central to disease pathogenesis at the time of relapse, implying that Bcr-Abl continues to be the optimal therapeutic target. Mechanisms responsible for acquired resistance to imatinib include mutations in the kinase domain (KD) of Bcr-Abl that impair drug binding, BCR-ABL gene amplification, and increased expression of BCR-ABL mRNA.15–21 Clonal cytogenetic evolution, although frequent in patients with acquired resistance, is not a resistance mechanism sensu strictu, but may be instead an epiphenomenon that correlates with a poor prognosis, possibly reflecting genomic instability.22,24 Researchers believe that gene amplification and increased mRNA expression lead to a rise in Bcr-Abl protein, although this has never been formally shown in patient cells. With more Bcr-Abl protein, more active kinase will remain at a given concentration of imatinib. Aside from these Bcr-Abl–dependent mechanisms of resistance, activation of Src kinases has been
described in rare patients. Cell lines established from these patients are sensitive to Src kinase inhibitors, implying that a Src kinase has “replaced” Bcr-Abl.

Point mutations in the KD of BCR-ABL were detected in 50% to 90% of patients with acquired resistance. Probably, the differences in incidence between the various studies reflect differences in the sensitivity of the methods used for mutation detection. More than 20 different amino acid substitutions have been described in the KD of Bcr-Abl. The mutations can be divided into 3 broad categories according to the mechanism by which they interfere with imatinib binding. Some mutations, such as T315I, affect residues that make direct contact with the drug in hydrophobic interactions or hydrogen bonds. The second group involves mutations that prevent the structural rearrangements required for imatinib binding. P-loop mutations such as Y253F and E255K fall in this category. The third group involves mutations that stabilize the active conformation of the kinase, to which imatinib is unable to bind. This may underlie the resistance of activation mutants loop such as H396P and of the rather common M351T at the base of the activation loop (Figure 4).

In cell proliferation and kinase assays, Bcr-Abl KD mutants exhibit various degrees of resistance to imatinib (Table 4). Some, like M351T, show only a moderate loss of drug sensitivity, which implies that dose escalation may be able to recapture a response in patients with this type of mutation. In contrast, T315I or E255K are highly resistant to imatinib, and the drug concentrations required for their inhibition are much higher than those that can be achieved in the plasma of patients.

Current thinking holds that KD mutations may be present before therapy and selected in the presence of drug, much like the selection of drug-resistant bacteria under the selective pressure of antibiotics. In agreement with this, mutations that were detected at the time of relapse were found in pretherapeutic samples from some patients. In most instances, detection of the mutant clones before therapy required sensitive techniques such as allele-specific PCR. However, in some cases the mutant clones were detected at a much higher ratio. This raises the question of whether certain mutations may confer a growth or survival advantage even in the absence of imatinib, which would explain the expansion of the mutant clone before therapy. Alternatively, a mutation may

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**Figure 3** Reactivation of Bcr-Abl signaling in a patient with chronic myelogenous leukemia with acquired resistance to imatinib. Protein lysates from white blood cells were resolved using SDS-polyacrylamide gel electrophoresis and probed with an anti-CrkL antibody. Phosphorylated (P-CrkL) and unphosphorylated (CrkL) forms of CrkL can be distinguished by their different migration. The histogram shows the ratio between phosphorylated and unphosphorylated forms as assessed by densitometry. The red line shows the reduction of phosphorylated CrkL on days 27 and 83, consistent with inhibition of Bcr-Abl signaling, and the reactivation on day 166. Sequencing showed the presence of an E255K mutation.


**Figure 4** Relative frequencies of kinase domain mutations in patients with imatinib resistance. Mutations cluster the P-loop (green), on residues T315 (red) and M351 (blue) and in the activation loop (yellow). A number of additional mutations have been described. Mutations may directly interfere with imatinib binding (T315I), favor the active conformation of the activation loop (H396P and probably M351T), or prevent the conformation changes required for imatinib binding (P-loop mutations).
<table>
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<tr>
<th>Numbering according to ABL exon 1a</th>
<th>Numbering according to ABL exon 1b</th>
<th>Exchange</th>
<th>N = 177</th>
<th>IC50 [mcM] - BCR-ABL autophosphorylation (in vitro kinase assay)</th>
<th>IC50 [mcM] - ABL autophosphorylation (in vitro assay)</th>
<th>IC50 [mcM] - cell proliferation</th>
<th>Comments</th>
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<td>M244 M263</td>
<td>L248 L267</td>
<td>V</td>
<td>3</td>
<td>1.69</td>
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<tr>
<td>G250 G269</td>
<td>Q252 Q271</td>
<td>E</td>
<td>6</td>
<td>3.39</td>
<td>7.4 to &gt;10</td>
<td>1.6, 4.5 to &gt;20</td>
<td>2-fold increased resistance in proliferation assays</td>
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<tr>
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<td>G250 G269</td>
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<td>1</td>
<td>0.56</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>Q252 Q271</td>
<td>Q252 Q271</td>
<td>H</td>
<td>8</td>
<td>4.52</td>
<td>2.9, 10.4</td>
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<td>NR</td>
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<tr>
<td>Y253 Y272</td>
<td>Y253 Y272</td>
<td>F</td>
<td>6</td>
<td>3.39</td>
<td>5.9, 21.4</td>
<td>72</td>
<td>3 to 8.9</td>
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<tr>
<td>E255 E274</td>
<td>E255 E274</td>
<td>K</td>
<td>34</td>
<td>19.21</td>
<td>27.9</td>
<td>&gt;200</td>
<td>15 to 33</td>
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<tr>
<td>D276 D295</td>
<td>D276 D295</td>
<td>G</td>
<td>1</td>
<td>0.56</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<tr>
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<td>T315 T334</td>
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<tr>
<td>M343 M362</td>
<td>M351 M370</td>
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<td>NR</td>
<td>NR</td>
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<tr>
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<tr>
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<td>V379 V398</td>
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<td>NR</td>
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<tr>
<td>L387 L406</td>
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<td>1.1, 2.2</td>
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<tr>
<td>H396 H415</td>
<td>H396 H415</td>
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<td>1.4 to 4.3</td>
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<td>L406 L408</td>
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<td>3.39</td>
<td>NR</td>
<td>0.22, 7.3</td>
<td>5.4, 11</td>
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<tr>
<td>S417 S436</td>
<td>E459 E478</td>
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<td>1</td>
<td>0.56</td>
<td>NR</td>
<td>NR</td>
<td>1.8</td>
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<tr>
<td>E459 E478</td>
<td>F486 F505</td>
<td>K</td>
<td>1</td>
<td>0.56</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>F486 F505</td>
<td>F486 F505</td>
<td>S</td>
<td>1</td>
<td>0.56</td>
<td>1.1</td>
<td>0.67</td>
<td>1.4, 9.1</td>
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</tbody>
</table>


The values are given as fold changes over wildtype. Data are compiled from several studies.17–21,23,27,28,30

†Immunoblots were published but IC50 values were not given.29

NR, not reported.
be an epiphenomenon that co-migrates with the resistant phenotype. A recent study that analyzed the frequency of mutations in samples from untreated patients irrespective of their subsequent response to drug detected mutations in approximately 20% of patients and found a significant association with disease phase and the presence of clonal cytogenetic evolution. Surprisingly, however, not all mutant clones detected before therapy were subsequently selected in the presence of imatinib, suggesting that additional factors are required to fully establish a drug-resistant phenotype. For example, mutations can possibly occur in cell clones destined to undergo terminal differentiation or in clones that lack sufficiently high expression of Bcr-Abl. Whatever the precise mechanism, the occurrence of a KD mutation in a patient on therapy must be interpreted within the clinical context.

Overcoming Resistance to Imatinib

Restoring Inhibition of Bcr-Abl Signaling

Because of its reactivation at the time of relapse, Bcr-Abl remains the optimal therapeutic target. Several approaches to restoring inhibition of Bcr-Abl are possible. Their efficacy depends on the underlying mechanism of resistance.

Dose Escalation: Common practice in the clinic is to escalate the dose of imatinib if this is still an option. This strategy may recapture responses in cases of weakly resistant mutations, such as M351T, or in patients with BCR-ABL gene amplification and moderate or increased protein levels. In pharmacokinetic studies performed during phase I trials, the steady-state trough levels at 400 mg daily were 1.43 mcg/mL compared with 2.66 mcg/mL for 800 mg, a difference that may be clinically relevant. Overall, between 30% and 50% of patients with acquired resistance will respond to dose escalation from 300 to 600 mg or 400 to 800 mg daily. No correlative studies have been published thus far, and so whether these predictions from the laboratory are correct remains unknown. Unfortunately, responses are frequently not maintained, which indicates that dose escalation should not be considered a long-term strategy for patients with acquired resistance.

Conventional Agents: Another common approach is to add conventional agents such as cytarabine. In vitro studies, mainly in cell lines, have shown that most combinations between imatinib and conventional cytotoxic drugs are synergistic or at least additive. No systematic study has been performed to assess the clinical efficacy of this intervention. Another possibility is to replace imatinib with a conventional agent. Disappearance of an imatinib-resistant KD mutant clone has been documented in a patient who was switched to hydrea. This result suggests that some mutant resistant clones exhibit a proliferation and survival advantage in the presence of imatinib but may be more sensitive to hydrea or other conventional agents. This strategy will not lead to a genuine remission but may nonetheless help to buy time.

Alternative Abl Kinase Inhibitors: The most promising approach to restoring suppression of Bcr-Abl kinase activity is using alternative Abl inhibitors that retain activity against mutant Bcr-Abl. Most of these structurally diverse compounds were originally developed as ATP-competitive Src kinase inhibitors and are therefore collectively referred to as Abl/Src inhibitors. Examples include PD180970 and BMS-354825 (dasatinib). The crystal structures of several compounds in complex with Abl have been determined and have shown significant differences compared with imatinib. Most importantly, Abl/Src inhibitors appear with bind both active and inactive conformations of Abl. In addition, binding to the P-loop does not require the extensive structural rearrangements that are necessary for accommodating imatinib. The activity profiles of the various Abl/Src inhibitors in cell proliferation and in vitro kinase assays are similar. Most of the common Bcr-Abl kinase mutants are inhibited at drug concentrations that are equal to or only slightly higher than for unmutated Bcr-Abl (Table 5). The exception is the T315I mutant, which is completely resistant to all Abl/Src inhibitors described thus far.

The major differences between the individual Abl/Src inhibitors relate to their pharmacokinetics. Dasatinib has excellent oral bioavailability and is currently in phase I and II clinical trials. Preliminary results of these trials show activity in relapsed CML in all phases. In particular, a considerable rate of complete cytogenetic responses was seen, even in patients with imatinib-resistant accelerated phase disease or blast crisis.

In contrast to the Abl/Src inhibitors described previously, AMN107, another compound in early clinical development, has been developed from an imatinib backbone. Compared with imatinib, the piperezinyl group has been modified to eliminate two energetically unfavorable hydrogen bonds with the
kinase. As a result, although it still binds only the inactive conformation of Abl, AMN107 has much greater potency compared with imatinib. Thus, although the differential of activity against the various mutants compared with unmutated Abl is largely maintained, inhibition occurs at much lower concentrations. Phase I and II studies are underway, and preliminary results show that plasma concentrations are achievable that inhibit most common mutations.47

As with the Abl/Src inhibitors, the exception to the rule is T315I, which is completely resistant.

One obvious concern is that T315I may be selected as the default mutant in the presence of alternative Abl/Src inhibitors or AMN107. Efforts are being made to develop inhibitors with activity against T315I. In addition to eliminating a hydrogen bond to imatinib, the substitution of threonine with the bulky isoleucine at position 315 leads to a sterical clash with hitherto published ATP-competitive inhibitors. Because T315 has a gatekeeper function for the kinase, synthesis of an ATP-competitive inhibitor with activity against T315I may be challenging, and no clinically viable compound has been noted yet. An alternative approach would be to use inhibitors that are substrate- rather than ATP-competitive. One such compound, termed ON012380, was recently described,

Table 5  Sensitivity of Common Abl Kinase Domain Mutants to Imatinib, BMS-453825 and AMN107 in Kinase and Cell Proliferation Assays

<table>
<thead>
<tr>
<th>Cellular Proliferation</th>
<th>Bcr-Abl tyrosine phosphorylation</th>
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</thead>
<tbody>
<tr>
<td><strong>IC50 (nM)</strong></td>
<td><strong>Fold Change</strong></td>
</tr>
<tr>
<td><strong>WT Bcr-Abl</strong></td>
<td>260</td>
</tr>
<tr>
<td><strong>M244V</strong></td>
<td>2000</td>
</tr>
<tr>
<td><strong>G250E</strong></td>
<td>1350</td>
</tr>
<tr>
<td><strong>Q252H</strong></td>
<td>1325</td>
</tr>
<tr>
<td><strong>Y253F</strong></td>
<td>3475</td>
</tr>
<tr>
<td><strong>Y253H</strong></td>
<td>&gt;6400</td>
</tr>
<tr>
<td><strong>E255K</strong></td>
<td>5200</td>
</tr>
<tr>
<td><strong>E255V</strong></td>
<td>&gt;6400</td>
</tr>
<tr>
<td><strong>F311L</strong></td>
<td>480</td>
</tr>
<tr>
<td><strong>T315I</strong></td>
<td>&gt;6400</td>
</tr>
<tr>
<td><strong>F317L</strong></td>
<td>1050</td>
</tr>
<tr>
<td><strong>M351T</strong></td>
<td>880</td>
</tr>
<tr>
<td><strong>F359V</strong></td>
<td>1825</td>
</tr>
<tr>
<td><strong>V379I</strong></td>
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</tr>
<tr>
<td><strong>L387M</strong></td>
<td>1000</td>
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<tr>
<td><strong>H396P</strong></td>
<td>850</td>
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<td><strong>H396R</strong></td>
<td>1750</td>
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<tr>
<td><strong>Parental Ba/F3</strong></td>
<td>&gt;6400</td>
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Note: Fold Change refers to the fold difference in the IC50, relative to WT, which is set to 1.

Abbreviations: NS, no signal detected; Bcr-Abl not expressed; ND, not determined.


† Values in parentheses are IC90 values, given for AMN107 and BMS-354825 only.
which inhibits the T315I mutant at nanomolar concentrations. The clinical development of this compound is currently uncertain.

**Agents That Down-Regulate Bcr-Abl Expression:**
Another option to target Bcr-Abl is to reduce its level of expression. These include inhibitors of heat shock protein 90 (HSP90), a chaperone required for Bcr-Abl stability. Examples are 17-allylamino-17-demethoxygeldanamycin (17-AAG) and LAQ824, a histone deacetylase inhibitor that also interferes with HSP90 function. A recent study showed that Bcr-Abl proteins with E255K and T315I mutations may be even more sensitive to 17-AAG than unmutated Bcr-Abl. Phase I studies with 17-AAG are in progress.

Finally, arsenic trioxide has been shown to induce down-regulation of Bcr-Abl, although the precise mechanism is not known. In a small phase I study, the combination of imatinib and arsenic trioxide was well tolerated, but no therapeutic activity was seen. This may be because the imatinib dose had been reduced at study entry because of concerns about toxicity.

**Other Signal Transduction Inhibitors**
Given that Bcr-Abl is reactivated at relapse in most patients, it remains the logical target, with signal transduction inhibitors that interfere with pathways downstream of Bcr-Abl clearly second choice. In vitro studies showed synergistic activity for a variety of agents, including inhibitors of mitogen-activated protein kinases, mTOR, and phosphatidyl inositol 3′ kinase (Table 6). Several of these agents are currently being evaluated in phase I trials as single agents or in combination with imatinib.

**Allogeneic Stem Cell Transplantation**
The indications for stem cell transplantation (SCT) in CML patients are a matter of considerable debate, and a full discussion of this subject would go beyond the scope of this review. Despite the promising activity of alternative Abl inhibitors in patients with acquired imatinib resistance, researchers doubt that these responses will be durable after the disease has progressed to accelerated phase or blast crisis. A consensus appears to exist that patients at this point should be offered SCT if they are eligible, with Dasatinib or AMN107 used for induction of a second chronic phase. The issue is more complicated in patients with a good response to imatinib but a high risk of relapse (e.g., individuals with high-risk chronic phase disease who do not experience CCR). In such patients, the decision whether or not to proceed to SCT will depend on the projected risk of transplant-related mortality and the patient’s personal preferences.

**Disease Persistence**
Residual disease may persist at any level. Because most patients (at least in developed countries) are diagnosed in the chronic phase and more than 80% of patients with chronic phase disease achieve CCR, disease persistence at the molecular level is the most common scenario. Although the rates of CMR vary among different studies (Table 3), clearly imatinib fails to eradicate minimal residual disease in most patients. Discontinuation of imatinib in patients with CCR or even CMR is almost invariably followed by relapse, except in those who have had SCT. This is in contrast to patients in CCR after interferon-α, a significant proportion of whom maintain CCR after discontinuation of drug. The mechanisms underlying disease persistence are not well understood and may be variable. Quiescent, imatinib-resistant primitive cells have been shown in CML patients. These cells may represent the persistent population, because they are capable of stably engrafting in immunodeficient mice.
an indication that they are capable of maintaining long-term (leukemic) hematopoiesis. Because the degree to which imatinib inhibits Bcr-Abl kinase activity in CML progenitor cells is unknown, the question of whether persistent cells are dependent on Bcr-Abl is currently unclear. A small study described KD mutations in CD34+ cells of 9 of 13 patients with CCR. Some of the mutations in this study confer only a minor degree of imatinib resistance in cellular and biochemical assays, suggesting that cells with these mutations may be able to maintain viability in the presence of drug but are unable to expand. Given that most of the patients in this cohort were fluorescent in situ hybridization–positive and that there was a high rate of relapse, it is somewhat doubtful whether this group is representative of the average patient with CCR.

As an alternative explanation, lineage-negative CML progenitor cells have been shown to express much higher levels of BCR-ABL mRNA than committed progenitor cells, which could render them drug resistant, similar to cells with BCR-ABL amplification. A third possibility is drug efflux. Imatinib is a substrate for P-glycoprotein and breast cancer resistant protein, two transporters that are highly expressed in hematopoietic stem cells. Thus, drug levels in the target cells could be much lower than plasma levels suggest.

All three mechanisms may be overcome with alternative Abl kinase inhibitors that are more potent than imatinib, have activity against kinase domain mutants, and may not be substrates for drug efflux pumps. However, that persistence is Bcr-Abl independent is also conceivable. CML progenitor cells are not completely growth factor–independent. Therefore, if Bcr-Abl kinase activity is inhibited, these cells may still be able to respond to physiologic growth and survival signals. If this is the case, then stem cell–rather than Bcr-Abl–targeted therapy would be required to eradicate the disease.

Conclusions
Imatinib is effective in all phases of CML. However, primary and acquired resistance is common in patients with advanced disease, and persistence of minimal residual disease is the rule in all patients. The most common mechanisms of acquired resistance are mutations in the KD of Bcr-Abl that impair drug binding and lead to reactivation of Bcr-Abl signaling. Alternative Abl kinase inhibitors such as dasatinib and AMN107, with activity against most KD mutants, are emerging as the most promising strategy to overcome resistance. However, whether these more potent inhibitors will also be capable of eradicating residual disease in a significant proportion of patients remains to be seen.

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