New Agents in the Treatment of Chronic Myelogenous Leukemia

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Key Words
Chronic myeloid leukemia, imatinib resistance, imatinib intolerant, second generation tyrosine kinase inhibitors

Abstract
The discovery of molecularly targeted agents that selectively inhibit bcr-abl tyrosine kinase activity, such as imatinib, has revolutionized the treatment and natural history of chronic myelogenous leukemia (CML). Treatment of chronic-phase CML with imatinib showed complete cytogenetic response rates of more than 40% in patients after failure of interferon-α, and more than 80% in patients with newly diagnosed CML. Patients with CML can now expect excellent long-term survival, often without major side effects. In most patients, however, residual leukemic burden remains detectable using a sensitive reverse transcription-polymerase chain reaction method. In addition, many patients undergoing imatinib therapy will either not respond or lose their response over time because of resistance or intolerance. The introduction of second-generation tyrosine kinase inhibitors (TKIs) re-establishes response in approximately half of these patients. Several agents are being developed for treating patients who experience suboptimal response to second-generation TKIs and for those who develop resistance caused by the emergence of highly resistant BCR-ABL1 mutations. This article provides an overview of novel targeted agents available for CML. (JNCCN 2009;7:1028–1037)

Imatinib is a selective inhibitor at the ATP binding site of the BCR-ABL protein, blocking downstream signaling pathways involved in the proliferation of the malignant clone. Several other tyrosine kinases, including c-KIT and platelet-derived growth factor receptor (PDGFR), are also inhibited by imatinib. This drug has been shown to induce complete hematologic and cytogenetic remissions in a high percentage of patients with chronic-phase chronic myelogenous leukemia (CML).1–5 These high response rates and duration translate into a remarkable overall survival; among the highest in the history of CML. However, recent studies, including an intention-to-treat analysis in a more “real-life” setting, showed that after 5 years of treatment, 37% to 49% of patients require further therapy after exhibiting resistance or intolerance.6–8

Primary resistance to imatinib is defined as experiencing 1) no complete hematologic response (CHR) at 3 months, 2) any cytogenetic response at 6 months, 3) a major cytogenetic response (MCyR) at 12 months, or 4) no complete cytogenetic response (CCyR) at 18 months.9 According to data from the International Randomized Interferon versus STI571 (IRIS) trial, these milestones are not achieved in 5%, 22%, 23%, and 24% of patients, respectively.2 A subset of patients exhibit secondary resistance; that is, the loss of a previously achieved response.10

After 5 years of follow-up in the IRIS trial, the estimated relapse rate was 17%, with a 7% progression to a more advanced phase. Various mechanisms are likely to contribute to imatinib resistance, including increased efflux or influx of the drug from the cancer cell, mediated by membrane transporters, such as the multidrug-resistance gene 1 protein (MDR-1) or human organic cation transporter protein (hOCT-1);11,12 increased expression of BCR-ABL kinase through gene amplification;13,14 increased imatinib binding by plasma proteins; and clonal evolution.10,15

One of the best established causes of imatinib resistance is the acquisition of point mutations within the
ABL kinase domain, which are detected in 22% to 55% of patients. These point mutations result in destabilization of the inactive conformation of the enzyme that is required for the binding of imatinib. More than 40 different mutations have been described, most of them mapping at contact residues (e.g., T315I, F317L) or the ATP binding loop (P-loop). P-loop mutations can confer high levels of resistance to imatinib and have been associated with poor prognosis. The T315I mutation renders BCR-ABL cells insensitive to imatinib and other clinically available tyrosine kinase inhibitors (TKIs) but does not always predict the clinical course. Other described mutations can be overcome by higher levels of imatinib. Overexpression of SRC family kinases, such as HCK, LYN, and, more recently, FYN, has also been implicated as a BCR-ABL–independent imatinib-resistant mechanism.

The response to imatinib therapy should be carefully monitored according to defined milestones. The 2009 NCCN Clinical Practice Guidelines in Oncology: Chronic Myelogenous Leukemia (in this issue; to view the most recent version of these guidelines, visit the NCCN Web site at www.nccn.org) and European LeukemiaNet recommend time-based milestone responses that should be achieved for continuing the same imatinib dosing schedule. Emerging evidence suggests that earlier milestones than those proposed by NCCN are warning signs in patients unlikely to obtain long-term benefits from imatinib. Inability to achieve a CCyR at 12 months of imatinib treatment has been associated with a higher risk for disease progression. Another recent retrospective study showed that inability to achieve a MCyR by 6 months predicted decreased overall survival. A recent publication using mainly high-dose imatinib showed that patients unable to achieve a 1-log reduction in BCR-ABL transcripts at 3 months, or more than a 2-log at 6 months, are unlikely to obtain a substantial response and are at high risk for disease progression.

**Treatment Options for Patients Resistant to Imatinib Therapy**

Loss of response or disease progression should prompt a change in treatment. Two major treatment options are clinically available for managing patients resistant to imatinib therapy: high-dose imatinib and second-generation TKIs (e.g., nilotinib, dasatinib). The phase III Tyrosine Kinase Inhibitor Optimization and Selectivity (TOPS) study reported that high-dose imatinib was not superior to standard-dose in terms of major molecular response (MMR) rates at 12 months among patients with early chronic-phase CML. However, longer follow-up may be required to show a potential benefit of high-dose imatinib therapy.

**Second-Generation TKIs**

**Nilotinib:** Nilotinib (Tasigna, AMN-107, Novartis International AG, Basel, Switzerland), is an aminopyrimidine-based imatinib analog that disrupts the ATP-phosphate–binding pocket of the ABL tyrosine kinase but also has important activity against c-KIT, PDGFR, and ephrin receptor kinase. Nilotinib is 30-fold more potent than imatinib against unmutated BCR-ABL in vitro and effectively inhibits 32 of 33 imatinib-resistant BCR-ABL mutations but not the T315I mutant. Certain P-loop mutations pose intermediate/high levels of resistance to nilotinib.

In October 2007, the FDA approved nilotinib at a dose of 400 mg twice a day for treating patients with imatinib-resistant or -intolerant CML in chronic or accelerated phase.

The pivotal phase II study involved 321 patients with CML in chronic phase (71% imatinib-resistant, 29% imatinib-intolerant; Table 1). Most patients were heavily pretreated; 72% received more than 600 mg daily of imatinib before study entry. Furthermore, patients intolerant to imatinib could not have experienced prior MCyR while on imatinib. After 24 months of follow-up, the overall MCyR rate was 58% and the CCyR was 42%. Responses were durable, with 84% of patients maintaining the MCyR at 18 months. The time-to-progression analysis showed that 64% of patients had not experienced progression at 18 months, with an overall survival rate of 91%.

The most frequently reported grade 3 to 4 biochemical laboratory abnormalities were transient and clinically asymptomatic, including elevated lipase (16%), hypophosphatemia (15%), hyperglycemia (12%), and elevated total bilirubin (7%). Grade 3 to 4 nonhematologic adverse events were infrequent, with rash, headache, and diarrhea occurring in only 2% of patients. The most common grade 3 to 4 hematologic laboratory abnormalities included neutropenia (30%), thrombocytopenia (28%), and...
anemia (10%). QTcF prolongation greater than 500 milliseconds was rare, occurring in only 3 patients (<1%). Nilotinib was active in patients harboring different mutations, except T315I, but had limited activity in patients with Y253H, E255K/V, and F359C/V.

A phase II trial to characterize the efficacy and safety of nilotinib, 400 mg twice daily, in patients with imatinib-resistant advanced-phase CML was recently updated.39-40 This analysis included 138 patients, with 80% imatinib-resistant and 20% imatinib-intolerant. Of 134 patients with at least 6 months of follow-up, 56% experienced a hematologic response, including 30% who had a CHR. MCyR and CCyR occurred in 32% and 19% of patients, respectively. Cytogenetic responses were durable, with 69% of patients maintaining MCyR at 18 months. The median time to progression was 16 months, and the estimated overall survival at 1 year was 82%. The most frequently reported grade 3 to 4 laboratory abnormalities were thrombocytopenia (40%), neutropenia (40%), anemia (25%), elevated serum lipase (17%), and hypophosphatemia (12%). Grade 3 to 4 nonhematologic adverse events were uncommon (<1%) and included rash, nausea, fatigue, and diarrhea.

Data from a phase II trial evaluating nilotinib in patients with previously untreated chronic-phase CML were recently reported.41 Forty-nine patients were treated for a median of 13 months at a median dose of 400 mg twice daily. Of 48 patients, 46 (96%) experienced a CCyR, at a rate of 3, 6, and 12 months comparing favorably with imatinib. MMR was observed in 45% of patients at 6 months and 52% at 12 months. The 24-month event-free survival rate was 95%. The actual median dose was 800 mg daily. Toxicity was manageable, with myelosuppression being the main adverse event. A multicenter phase III trial is underway comparing nilotinib with standard-dose imatinib (400 mg/d) for treating patients with newly diagnosed chronic-phase CML.

### Dual Src Family/Abl TKIs: Dasatinib

Dasatinib (Sprycel, Bristol-Myers Squibb, New York, New York) is an orally available multi-targeted inhibitor of BCR-ABL, Src family, c-Kit, EPHA2, and PDGFRβ, among others.42-45 Dasatinib is approximately 325-fold more potent than imatinib against BCR-ABL. Unlike imatinib, dasatinib can bind to both the active and inactive conformations of the ABL kinase domain and has activity against many imatinib-resistant BCR-ABL mutations, including those within the P-loop, the activation loop, and other sites in the COOH-terminal loop, except for T315I.36,46,47

Based on its activity after imatinib failure in patients with CML (Table 1), the FDA approved dasatinib in 2006 for treating CML resistant to prior therapy. The START-C trial evaluated 387 patients (288 imatinib-resistant and 99 imatinib-intolerant)
with chronic-phase CML. The starting dose was dasatinib, 70 mg twice daily, and the primary end point was MCyR rate. Best response to prior imatinib therapy was CHR in 82%, MCyR in 37%, and CCyR in 19% of patients.

After a minimum follow-up of 24 months, CHR was noted in 91% of patients (95% CI, 88%–94%), MCyR in 62% (95% CI, 57%–67%), CCyR in 53%, and MMR in 47%. MCyR occurred in 55% of patients resistant to imatinib, and in 63% of those with baseline BCR-ABL mutations, except those carrying T315I. At 24 months, progression-free survival was 80% (75% in imatinib-resistant and 94% in -intolerant patients) and overall survival was 94% (92% in imatinib-resistant and 100% in -intolerant patients). The main grade 3 to 4 toxicities included thrombocytopenia (49%), neutropenia (50%), pleural effusion (9%); 35% all grades), dyspnea (6%), and bleeding (4%).

Another phase II study, the START-R trial, randomized (2:1) patients with imatinib-resistant chronic-phase CML to receive dasatinib, 70 mg twice daily (n = 101), or imatinib, 800 mg daily (n = 49). At a minimum follow-up of 2 years, dasatinib showed higher rates of CHR (93% vs. 82%; P = .034), MCyR (53% vs. 33%; P = .017), and CCyR (44% vs. 18%; P = .0025). At 18 months, MCyR was maintained in 90% of patients on the dasatinib arm and 74% on the high-dose imatinib arm. MMR rate was also higher in patients treated with dasatinib than with high-dose imatinib (29% vs. 12%; P = .028). The estimated progression-free survival also favored dasatinib. Grade 3 to 4 toxicities included thrombocytopenia (57%), neutropenia (63%), and pleural effusion (5%).

The efficacy of dasatinib in advanced- and blast-phase CML was also shown in the phase II START-A and -B trials, respectively. In the START-A trial, 174 patients with imatinib-resistant or -intolerant advanced-phase CML were treated with dasatinib. At a follow-up of 2 years, the reported CHR, MCyR, and CCyR rates were 50%, 45%, and 33%, respectively. The 2-year progression-free survival rate was 46%, with a median progression-free survival of 19.5 months. The 2-year overall survival rate was 72%; median overall survival had not been reached. Dasatinib was generally well tolerated; the most frequent nonhematologic severe treatment-related adverse event was diarrhea (52%; grade 3 to 4 in 8%).

Cytopenias were common, including grade 3 to 4 neutropenia (76%) and thrombocytopenia (82%). Pleural effusion occurred in 27% of patients (grade 3–4 in 5%).

The START-B trial evaluated 157 patients with imatinib-resistant or -intolerant CML in blast phase. This trial reported a CHR rate of 26% in patients with myeloid blast phase and 29% in those with lymphoid blast phase, and an MCyR rate of 34% and 52%, respectively. The CCyR rate was 27% and 46%, respectively. Overall survival was higher in those with myeloid blast crisis than lymphoid blast crisis, with a median overall survival of 11.8 versus 5.3 months.

The currently approved dose for chronic-phase CML is 100 mg daily based on results from the phase III dose optimization trial showing that this dose schedule was not inferior to twice-daily schedules and that it had an improved toxicity profile, particularly regarding rates of grade 3 to 4 neutropenia, thrombocytopenia, and pleural effusion. Dasatinib received full FDA approval in May 2009. The currently approved starting dosage for patients with advanced-phase CML, myeloid blast phase, and Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph+ ALL) resistant or intolerant to prior therapy is 140 mg daily.

Given the activity of dasatinib in patients for whom therapy with imatinib had failed, a phase II trial is exploring this TKI in treatment-naive patients with chronic-phase CML. Results evaluating 50 patients showed CCR rates of 78%, 93%, and 97% at 3, 6, and 12 months, respectively. A multicenter phase III trial comparing dasatinib with standard-dose imatinib (400 mg) for treating patients with newly diagnosed chronic-phase CML is underway.

Bosutinib: Bosutinib is an orally available potent dual Src/Abl kinase inhibitor. It has been shown to be up to 200-fold more potent than imatinib as an inhibitor of Bcr-Abl phosphorylation, but it has minimal inhibitory activity against PDGRF or c-Kit. In addition, bosutinib has shown activity against most imatinib-resistant mutants of Bcr-Abl, except for T315I.

Results were recently reported from a phase II study involving 283 patients with chronic-phase CML for whom treatment with imatinib or other second-generation TKIs failed (Table 1). In patients for whom only treatment with imatinib failed,
bosutinib rendered a CHR rate of 79% and a MCyR rate of 40% (29% CCyR). The most common adverse events were gastrointestinal (mostly diarrhea), but these were usually grade 1 to 2, manageable, and diminishing in frequency and severity after the first 3 to 4 weeks of treatment.

A separate phase II trial evaluating patients with advanced- or blast-phase CML for whom prior therapy with imatinib had failed showed a 73% MCyR rate and a 36% MMR rate. These data, although preliminary, indicate that bosutinib has activity in advanced-phase CML after imatinib failure. In addition, it seems to have a safer toxicity profile than other second-generation TKIs, perhaps because of its lack of activity against c-kit and PDGFR. An ongoing phase III trial is evaluating bosutinib versus imatinib.

**Compounds With Activity Against T315I:** None of the TKIs described have activity against the T315I mutation, which accounts for 10% to 20% of the BCR-ABL1 mutants detected after failure of TKI therapy. A series of compounds have shown important preclinical activity against BCR-ABL1 T315I kinase; some have entered clinical trials for evaluation in patients for whom treatment with 2 or more TKIs failed or who are carrying the T315I mutation. Many of these agents are multikinase inhibitors with activity against, among others, BCR-ABL1 and Aurora kinases. Aurora kinases are key regulators of cellular mitosis and have been shown to be overexpressed in multiple cancers.

**Switch Pocket Inhibitors:** An alternate approach to tackling the T315I mutation is to develop compounds that target BCR-ABL1 kinase by binding to regulatory sites distant from the catalytic domain of the enzyme. A novel chemical class of TKIs circumvents the gatekeeper T315I mutation by binding to several pockets involved in the “switch” mechanism the ABL kinase uses to control its transition from the inactive to the active state. DCC-2036, the first agent of this class, inhibits purified ABL kinase that is either unphosphorylated (switch-off) or phosphorylated (switch-on) with high specificity for Bcr-Abl tyrosine kinases.
became undetectable in 19 (48%) of 40 evaluable patients. Among patients in chronic phase, the most frequent grade 3 to 4 toxicities were thrombocytopenia (70%), anemia (55%), and neutropenia (43%). If maintained, the results of this trial may lead to the approval of omacetaxine for treating patients with BCR-ABL1 T315I-positive CML.

Another approach to the management of patients with BCR-ABL1 T315I-positive CML is the use of epigenetic modifiers. Histone deacetylases (HDAC) catalyze the deacetylation of lysine residues at the amino termini of core nucleosomal histones. Inhibition of HDACs with HDAC inhibitors cause hyperacetylation of histones, leading to transcriptional upregulation of cyclin-dependent kinase inhibitor, p21, cell-cycle arrest, and apoptosis in tumor cells. HDAC inhibitor treatment results in depletion of BCR-ABL1 protein and sensitization to imatinib-induced apoptosis. In preclinical studies of cells obtained from patients with CML expressing the T315I mutation, treatment with the HDAC inhibitor LBH589 in combination with nilotinib resulted in synergistic reduction of STAT5 and ERK1/2 phosphorylation, and enhanced apoptosis.

Table 2: Agents with Inhibitory Activity Against BCR-ABL1 and Aurora Kinases in Clinical Development for Imatinib-Resistant CML

<table>
<thead>
<tr>
<th>Drug</th>
<th>Chemical Class</th>
<th>Aurora Selectivity</th>
<th>Other Targets</th>
<th>Route</th>
<th>Stage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK-0457</td>
<td>Pyrazolo-quinazoline</td>
<td>Aurora A, B, and C</td>
<td>ABL1, FLT3, JAK2</td>
<td>IV</td>
<td>Phase II</td>
<td>Development stopped due to cardiac toxicity</td>
</tr>
<tr>
<td>XL228</td>
<td>Not available</td>
<td>Aurora A</td>
<td>ABL1, IGF1R, SRC</td>
<td>IV</td>
<td>Phase I</td>
<td>Tested in CML and Ph+ ALL after failure of imatinib or dasatinib</td>
</tr>
<tr>
<td>PHA-739358</td>
<td>Pyrrolo-pyrazole</td>
<td>Aurora A, B, and C</td>
<td>ABL1, FGFR1, RET, TRK-A</td>
<td>IV</td>
<td>Phase II</td>
<td>Tested in CML after failure of imatinib or other anti-ABL1 treatments</td>
</tr>
<tr>
<td>AT-9283</td>
<td>Not available</td>
<td>Aurora A, B, and C</td>
<td>ABL1, FLT3, JAK2, JAK3</td>
<td>IV/Oral</td>
<td>Phase I/II</td>
<td>Being tested in patients with refractory hematologic malignancies including CML</td>
</tr>
<tr>
<td>KW-2449</td>
<td>Not available</td>
<td>Aurora A</td>
<td>ABL1, FLT3, FGFR1, VEGFR</td>
<td>Oral</td>
<td>Phase I</td>
<td>Undergoing clinical testing in CML, acute leukemia, and high-risk MDS</td>
</tr>
</tbody>
</table>

Abbreviations: ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; FGFR1, fibroblast growth factor receptor-1; FLT3, FMS-like tyrosine kinase 3; IGF1R, insulin-like growth factor type-1 receptor; IV, intravenous; MDS, myelodysplastic syndrome; Ph+, Philadelphia chromosome positive.
combined with the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA). Ongoing clinical trials are evaluating the activity of HDAC inhibitors in patients with CML in all phases after TKI failure and in those carrying BCR-ABL1 T315I.

Bcr-Abl activates PI-3 kinase through a direct association with its 85 kd regulatory subunit; signaling by way of the PI-3 kinase is essential for the growth of CML progenitors. The mammalian target of rapamycin (mTOR) is a serine-threonine kinase downstream of PI-3 kinase that is activated on phosphorylation by Akt. Recently, RAD001 (Everolimus, Novartis International AG, Basel, Switzerland), a derivative of the mTOR rapamycin, has been shown to inhibit the growth of Ba/F3-Bcr-Abl, and prolong the survival of mice transplanted with bone marrow retrovirally transduced with Bcr-Abl. The combination of imatinib and rapamycin suppressed the growth of imatinib-resistant cell lines overexpressing Bcr-Abl. Studies investigating the safety and efficacy of combining imatinib and RAD001 are ongoing in patients with CML.

A novel approach to treating T315I-positive CML is the use of protein phosphatase 2A (PP2A) activators. BCR-ABL1 inhibits PP2A through up-regulating the phosphoprotein SET, a PP2A inhibitor. PP2A activates the phosphatase SHP1, which then catalyzes BCR-ABL1 dephosphorylation, resulting in proteosomal degradation.

FTY720 is a PP2A activator that is structurally similar to sphingosine and is being investigated as an immunomodulator in clinical trials for patients with multiple sclerosis or undergoing renal transplantation. FTY720 suppressed the growth, abolished Bcr-Abl phosphorylation, and induced Bcr-Abl down-regulation through activating PP2A in imatinib-sensitive and T315I-expressing cell lines and in primary CML cells. FTY720 also suppressed in vivo wild-type and T315I Bcr-Abl-driven leukemogenesis without causing side effects.

Recent preclinical findings suggest that targeting the Hedgehog signaling pathway or the promyelocytic leukemia protein, which are both critical elements in hematopoietic stem cell maintenance, may represent a promising therapeutic strategy for eradicating BCR-ABL1–positive leukemia stem cells, which are believed to be responsible for relapse after TKI therapy discontinuation in patients with CML.

**Identifying More Adequate Second-Generation TKIs for Patients Refractory and Intolerant to Imatinib**

Nilotinib and dasatinib are currently approved as salvage therapy for patients with Ph+ leukemia. Interestingly, dasatinib was initially developed as an immunosuppressant drug and later recognized as a potent bcr-abl inhibitor. Nilotinib was developed as a new-generation imatinib-like molecule with the goal of treating already known mechanisms of resistance, such as Abl kinase domain mutations. Although dasatinib is approved by the FDA for all phases of CML (chronic, accelerated, myeloid, and lymphoid blast crisis), nilotinib is approved for chronic- and accelerated-phase CML.

In the absence of randomized trials to clarify the differences in efficacy and toxicity between the agents, definite recommendations are difficult to make. Nevertheless, in most high-risk patients in chronic-phase CML, some suggestions can be made based on different toxicity profile and emerging data regarding kinase mutations clinical sensitivity.

A review of data from all clinical trials, and considering that the populations studied with both drugs were similar but not equal, suggests that both drugs have comparable response rates. Kinase domain mutations are responsible for 50% of imatinib-resistant cases; however, the drugs were equally effective independent of the presence of a kinase domain mutation. In the absence of a kinase domain mutation, deciding between the drugs will depend on a review of the potential toxicities, existing comorbid conditions, and patient preferences.

When the presence of a mutation causes clinical resistance, the presence of a T315I mutation must first be assessed (this is found in approximately 10% of cases). This mutation is not sensitive to any of the currently approved TKIs, and therefore an allogenic stem cell transplant or participation in a clinical trial is advised. In the presence of a P-loop mutation (residues 248–256) and other mutations outside this region (residues 299, 317, and 359), in vitro sensitivity to nilotinib or dasatinib could be a useful guide when choosing between these drugs. For example, dasatinib would be recommended in patients with Y253H and F359C/V, whereas nilotinib would be a better choice in those with F317V and V299L. However, most of these mutations represent only a small proportion and most are not associated with a different clinical response to either drug.
New Agents for CML Treatment

Conclusions
Imatinib mesylate represents the standard of care for patients with newly diagnosed CML. However, results from the IRIS trial show that an important fraction of patients must discontinue imatinib therapy because of intolerance or resistance. For those patients, alternative forms of therapy are required. Two agents have received approval by regulatory agencies for managing these patients, nilotinib and dasatinib. However, these second-generation TKIs can only rescue approximately half of the patients for whom imatinib therapy fails. Therefore, in light of the growing prevalence of CML, more patients will clearly require further forms of treatment over time. A wide array of promising compounds are being developed in clinical trials. Of particular importance is the development of agents with activity against the T315I mutation, which has proven resistant to imatinib, nilotinib, dasatinib, and bosutinib. Patients with this mutation should be enrolled in clinical trials. These studies will undoubtedly provide novel strategies to better treat patients resistant to conventional TKI therapy or carrying highly resistant BCR-ABL1 mutant isoforms, such as T315I.

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