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Development of an Effective Therapy for Chronic Myelogenous Leukemia

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Abstract: Targeted small-molecule drugs have revolutionized treatment of chronic myeloid leukemia (CML) during the last decade. These agents interrupt a constitutively active BCR-ABL, the causative agent for CML, by interfering with adenosine 5’ triphosphate–dependent ABL tyrosine kinase. Although the efficacy of tyrosine kinase inhibitors (TKIs) has resulted in overall survival of greater than 90%, TKIs are not curative. Moreover, no currently approved TKIs are effective against the T315I BCR-ABL variant. However, a new generation of TKIs with activity against T315I is on the horizon. We will highlight the clinical utility of historical CML therapeutics, those used today (first- and second-generation TKIs), and discuss treatment modalities that are under development. Recent advances have illuminated the complexity of CML, especially within the marrow microenvironment. We contend that the key to curing CML will involve strategies beyond targeting BCR-ABL because primitive human CML stem cells are not dependent on BCR-ABL. Ultimately, drug combinations or exploiting synthetic lethality may transform responses into definitive cures for CML.

Key Words: Chronic myeloid leukemia, BCR-ABL, tyrosine kinase inhibitors, drug resistance, synthetic lethality

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Use of TKI therapy has drastically improved patient survival, and projections indicate that CML prevalence will continue to increase as a result. In fact, it has been estimated that there may be up to 250,000 CML patients in the United States in 2040.30 Tyrosine kinase inhibitors are very effective inhibitors of BCR-ABL kinase activity; second-generation agents are more potent, and they have expanded inhibition against various BCR-ABL mutants resistant to the first-generation drug, imatinib.31 As we mark a decade of imatinib use, we have developed an understanding of disease response to these targeted agents, although many questions still remain. Will long-term BCR-ABL inhibition by TKIs eradicate all disease-causing cells, at least at some patients? If not, how can this be accomplished? Will it be possible for one compound to completely inhibit all BCR-ABL variants, including the T315I gatekeeper mutant? This review will discuss currently approved standard-of-care drugs and highlight promising novel agents. In addition, we will cover therapeutic roadblocks, such as targeting the bone marrow microenvironment and BCR-ABL–independent survival of CML stem cells.

**FOOD AND DRUG ADMINISTRATION-APPROVED FIRST-LINE TKIS**

Measuring Response  Disease stage is monitored using peripheral blood and marrow differentials, marrow cytogenetics, BCR-ABL detection by fluorescence in situ hybridization (FISH), and BCR-ABL copy number surveillance by quantitative real-time polymerase chain reaction (PCR). Normalization of blood counts and spleen size is termed complete hematologic remission (CHR) and is the earliest measure of response. Cytogenetic response is measured as the percentage of Ph+ karyotypes in 20 bone marrow metaphases. Zero Ph metaphases constitutes a complete cytogenetic remission (CCR); 1% to 35%, a partial response (PCR); 35% to 65%, a minor response; and 66% to 95%, a minimal response.42 Major cytogenetic response (MCyR) includes both CCyR and PCR. A major molecular response is defined as a 3-log reduction of BCR-ABL messenger RNA compared with a standardized baseline as measured by quantitative real-time PCR.43 For an excellent perspective on response to TKI therapy, please see the recent review by Radich.34

**Imatinib**

Imatinib mesylate (STI571/Gleevec; Novartis) is a competitive inhibitor of the adenosine 5′ triphosphate (ATP)–binding site of the BCR-ABL tyrosine kinase. Its development is regarded as a prototype for structure-based design of specifically targeted inhibitors.55 Preclinical efficacy was described first in patient-derived BCR–ABL–expressing cells and finally in a mouse model expressing BCR–ABL–positive cells.56 A phase I trial included an initial cohort of 83 patients. Despite dose escalation up to 1000 mg daily, the maximum tolerated dose was not achieved, and 400 mg/d was selected as an effective dose.7 Clinical efficacy (phase II) studies were conducted for each disease phase (CP, AP, and BC) enrolling more than 1000 patients. Impressively, these studies confirmed or surpassed the efficacy seen in phase I but also confirmed that responses in AP/BC are less frequent and less durable.37–39 The phase III International Randomized Study of Interferon and STI571 (IRIS) study demonstrated clear superiority of imatinib over IFN plus low-dose cytarabine for CP-CML. Specifically, at 18 months, freedom from progression to AP/BC was 96.7% in the imatinib group and 91.5% in the IFN group (P < 0.001), with a CCyR of 76.2% compared with 14.5%.40 On the basis of the efficacy seen in these studies, imatinib gained approval from the US Food and Drug Administration (FDA) for the treatment of patients who had failed IFN (2001) and for newly diagnosed patients in 2003. Subsequent updates of the IRIS study at 60 months confirmed these results.

Overall survival in the patients treated with first-line imatinib was 89%, a revolutionary improvement over previous IFN-based regimens. No survival difference was demonstrated compared with the IFN/cytarabine arm because most IFN patients crossed over to imatinib for intolerance or lack of efficacy.41 Single-center studies had suggested that increasing imatinib from 400 to 800 mg/d could improve response rates. However, randomized comparisons failed to confirm these initial results.42 More recently, the German CML IV study showed a significant difference in the rate of mismatch repair (MMR) in favor of higher doses of imatinib. It has been suggested that the more flexible dosing regimen in this study led to an overall higher dose intensity and a superior result.43 At this point, the standard dose of imatinib for newly diagnosed patients remains 400 mg daily, and the drug remains a viable option for newly diagnosed patients in the chronic phase.42 Imatinib, however, falls short of effectively treating most patients in AP/BC.

**Dasatinib**

Inhibitors targeting Src kinases were the goal of Lombardo et al44 when they discovered a dual-Src/ABL kinase inhibitor initially referred to as BMS-354825 and now known as dasatinib (Sprycel; Bristol-Myers Squibb). Dasatinib binds with high affinity to both ABL and the SRC kinase in the ATP-binding site, translating to an ABL inhibition potency 300 times that of imatinib in biochemical and cell proliferation assays. In addition to SRC-family kinases, c-KIT, PDGFR-α/β, and the ephrin receptor kinases are also inhibited by dasatinib.45 Uniquely, this TKI binds ABL in both the active and inactive states, leading to a more complete inhibition regardless of protein confirmation.46 Dasatinib dose escalation studies were conducted in a cohort of 84 patients across all CML disease phases including a minority with Ph+ acute lymphoblastic leukemia (ALL). A maximum tolerated dose for dasatinib was not determined, but importantly, patients who enrolled after previous imatinib intolerance showed no similar toxicities.47 Efficacy of this phase I trial established 70 mg twice daily as the optimal dose for further studies. The phase II trials for Src/ABL Tyrosine kinase inhibition Activity Research Trials of dasatinib (START) were conducted separately for each disease phase. Dasatinib demonstrated a robust and durable response in CP (CHR, 87%; MyCr, 52%) and a progression-free survival of 92% at 8 months.48 Responsive responses were seen in AP (MCyR, 33%) and BC (MCyR, 31% myeloid and 50% lymphoid); however, these responses were much less durable than those in CB49,50. In 2006, the FDA granted approval of dasatinib at 70 mg twice daily for refractory CML patients. Further dose optimization studies led recommendations of 100 mg once daily for CP-CML51,52 whereas 70 mg twice daily remained the dose for advanced CML.53

**Nilotinib**

To overcome imatinib resistance, nilotinib (AMN107/Tasigna; Novartis) was rationally designed based on a thorough analysis of the ABL-imatinib complex to increase binding affinity. Nilotinib is more selective than imatinib, favoring ABL inhibition over the 2 other target kinases KIT and PDGFR.54 Nilotinib is 10 to 50 times more potent than imatinib and is an inhibitor of many BCR-ABL mutants that are resistant to imatinib.55,56 Phase I studies for nilotinib in imatinib-resistant CML or Ph+ ALL patients revealed significant activity in the chronic...
Resistance to Currently Approved TKIs

Despite the promise of TKIs in treating CML, drug resistance does occur. Resistance can be primary (failure of a newly diagnosed patient to achieve satisfactory response to drug) or secondary/acquired (failure of a patient on treatment who initially responded to maintain this response). Tyrosine kinase inhibitor failure has been linked to mutations in the ABL kinase domain that impair drug binding, increased BCR-ABL expression, and changes in drug efflux transporters that result in low intracellular drug concentrations, particularly with imatinib.60,61 These changes can occur during progression to advanced disease phases, but they do not, in and of themselves, cause progression. In vitro mutagenesis screens have been used to profile TKIs. These studies revealed the broadest activity for dasatinib, followed by nilotinib, whereas imatinib has extensive gaps in coverage, consistent with clinical data.62,63 On the basis of in vitro profiles, we and others have developed heat maps of predicted in vivo activity.64 However, it is important to note that the in vivo response is more complex, involving additional parameters such as plasma protein binding and plasma peak and trough drug concentrations.65 As a result, the correlation between in vitro predictions and clinical responses is relatively weak.66,67 with the notable exception of the T315I mutant, which is resistant to all currently approved TKIs. This poses a significant challenge to therapy because the T315I mutation is reported to represent 15% to 20% of all mutations.68 Tyrosine kinase inhibitors have transformed a previously fatal disease into a manageable chronic condition, but drug discontinuation usually results in disease recurrence, even in patients with profound responses such as MMR or “PCR-negative” CML, although rare exceptions may exist.69,70 Thus, drug treatment must continue indefinitely, a significant drawback to current TKI therapy. Consistent with these clinical observations, there is evidence that all 3 agents fail to eliminate primitive CML cells and that the bone marrow environment is a potential safe haven for these cells.71 Taken together, this suggests that minimal residual disease may be beyond the reach of our current TKI-based therapeutic arsenal. This is often referred to as disease persistence.

Second-Generation TKIs in First-Line Therapy

Treatment advantages of second-generation TKIs over imatinib were suggested during phase II studies; additional trials comparing these inhibitors were quickly planned and executed. The phase III trial, Evaluating Nilotinib Efficacy and Safety in Clinical Trials—Newly Diagnosed Patients, compared nilotinib 300 or 400 mg twice daily and imatinib (400 mg once daily). After 1 year, MMR for either nilotinib dose (43%–45%) was nearly double that of imatinib, and CCyR was significantly higher in the nilotinib cohorts (78%–80% vs 65%).72 In addition, nilotinib was superior in progression-free survival. As a result, the FDA granted accelerated approval of nilotinib in June 2010 for newly diagnosed CML patients.73

The Dasatinib versus Imatinib Study in Treatment-Naive CP-CML Patients (DASISION) trial tested dasatinib at 100 mg daily versus imatinib 400 mg daily in newly diagnosed chronic phase patients. This report indicated a comparable advantage as seen in the Evaluating Nilotinib Efficacy and Safety in Clinical Trials—Newly Diagnosed Patients trial regarding MMR for dasatinib over imatinib (46% vs 28%) and CCyR (77% vs 66%).74 Progression-free survival was also improved, although the difference failed to reach statistical significance. Regulatory approval of dasatinib for newly diagnosed CP-CML patients was granted in October 2010.

Adverse Effects of Currently Approved TKIs

A comprehensive appreciation of TKI-related toxicities is beyond the scope of this review. Hematologic toxicity is common and correlates with disease state, being more frequent in patients with advanced disease compared with newly diagnosed patients. It is generally believed that this reflects the more limited reserve of normal hematopoiesis in patients with long-standing or more aggressive CML. Nonhematologic toxicity is diverse and dependent on the specific TKI. The good news is that these toxicities are largely nonoverlapping, which implies that cross-tolerance to all 3 approved TKIs is rare. For a comprehensive and detailed review of toxicity, the reader is referred to a recent review.75 Importantly, annual updates of the IRIS study, as well as independent studies, confirmed the safety of long-term imatinib therapy in the sense that grades 3 to 4 toxicities are rare, and no new and unexpected adverse effects became apparent with longer follow-up.76 The body of data available for dasatinib and nilotinib is more limited, and it will be important to remain vigilant as therapeutic time increases for these drugs.

NOVEL AGENTS

ATP-Competitive ABL Inhibitors Without Activity Against T315I

Several TKIs have been developed that exhibit a target spectrum similar to the approved drugs, although they are distinct in off-target effects. The most advanced of these drugs is bosutinib (SKI-606; Wyeth), originally developed as a Src kinase inhibitor.75 Bosutinib has shown inhibitory activity in CML cell lines and primary cells and has demonstrated tumor regression in CML xenograft models. Unlike approved TKIs, bosutinib does not inhibit e-Kit or PDGFR.76 Phase I and II studies revealed drug activity in patients who failed imatinib. However, as expected, efficacy in patients who failed a second-generation TKI was lacking. A phase III study did not meet the primary end point (ie, superior rates of CCyR at 12 months in comparison with imatinib 400 mg daily). Current speculation attributes lack of efficacy to insufficient dose intensity triggered by dose interruptions due to diarrhea, a common, but transient adverse effect that should have been managed with supportive care. Bosutinib could possibly add to the therapeutic armamentarium as another drug with a unique adverse effect profile. However, it does not address the problems of the T315I mutant and BCR-ABL–independent resistance. Overall, the future of bosutinib is unclear.77

T315I Active Inhibitors

The most advanced third-generation inhibitor of BCR-ABL is ponatinib (AP24534; Ariad).78 Unlike all approved TKIs, ponatinib is effective against the T315I mutant as well as a large sample of other mutants previously detected in patients with clinical TKI resistance.68 In vitro screens revealed no mutational vulnerabilities in BCR-ABL, suggesting that ponatinib may be
the first true “pan-BCR-ABL” TKI. This drug also inhibits other kinases including FLT3, FGFR, vascular endothelial growth factor receptor (VEGFR), c-Kit, and PDGFR.\(^{79,80}\) Ponatinib showed significant activity in a phase I study of patients with Ph+ leukemia, mostly CML, who had failed other TKIs. Interestingly, responses were most impressive in patients with the T315I mutation, turning a poor prognostic factor into a favorable one.\(^{81}\) Ponatinib is currently in phase II clinical trials (PACE trial, Ponatinib Ph+ ALL and CML Evaluation). PACE is a global, single-arm clinical study including patients in all disease phases of CML and Ph+ ALL. Given its activity against the T315I mutant, ponatinib may well replace nilotinib and dasatinib in salvage therapy. A phase III study for ponatinib in first-line therapy is in the planning stage.

Aurora kinases are serine/threonine kinases known to regulate mitosis.\(^{82}\) Because of their role in cell cycle progression and the fact that they are overexpressed in leukemias and solid tumors,\(^{83}\) aurora kinases make attractive targets in CML therapeutic development. Several compounds with activity against ABL mutants, including T315I, were developed and have entered clinical trials. Among these, the most tested candidate is AT9283 (Astex Therapeutics) with activity against ABL, as well as Aurora A/B kinases, and Janus kinases 2/3 (JAK2 and JAK3).\(^{84}\) Preclinical efficacy was demonstrated in mouse models leading to initiation of clinical trials.\(^{84}\) Phase I and IIa clinical trials were completed in October 2010, and a recommended phase II dose was determined (NCT00522990). Danusertib, another Aurora kinase inhibitor, is currently in phase I studies in patients with refractory Ph+ leukemias.\(^{85}\) Results have not yet been published. Two other Aurora kinase inhibitors with activity against T315I mutant ABL, MK-0457 and XL228, failed in clinical trials (NCT00464113) for various reasons, including toxicity.\(^{86}\) The clinical efficacy of compounds inactive against T315I, but which inhibit other pathways (like the Src-family kinases) remains to be determined. Table 1 provides an overview of new compounds in development for Ph+ leukemias.

### Allosteric/Non-ATP Competitive Inhibitors

DCC-2036 (Deciphera) is an inhibitor of BCR-ABL that forces a conformational change of ABL on drug binding. ABL can exist in either an active (type I) or inactive (type II) conformation based on phosphorylation status. Structure-based design of DCC-2036 elucidated a “switch-pocket” in ABL, inducing a stable and inactive state.\(^{87}\) DCC-2036 inhibits ABL in a non-ATP competitive manner; it also inhibits Src, Lyn, Fgr, Hck, Flt3, and Tie2 but spares Kit. Based on efficacy in preclinical studies, a phase I trial has been initiated and is currently recruiting.

An allosteric, non-ATP competitive inhibitor of BCR-ABL is GNF-2 (Genomics Novartis Foundation), which was discovered during kinase activity screening.\(^{88}\) GNF-2 is hypothesized to bind at the myristoyl binding cleft of BCR-ABL, distant from the T315I mutation.

### Table 1. Drugs Developed for CML Therapy With Activity Against ABL-Kinase and Other Kinases Listed

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Non-ABL Kinase Target(s)</th>
<th>T315I Status</th>
<th>Status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCC-2036</td>
<td>Src, Lyn, Fgr, Hck, Flt3, Tie2</td>
<td>Active</td>
<td>Phase I/II</td>
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</tr>
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<td>GNF compounds</td>
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<td>Active</td>
<td>Preclinical</td>
<td></td>
</tr>
<tr>
<td>ON012380</td>
<td>ABL only</td>
<td>Active</td>
<td>Preclinical</td>
<td>†</td>
</tr>
<tr>
<td>PPF-A</td>
<td>ABL only</td>
<td>Active</td>
<td>Preclinical</td>
<td>‡§</td>
</tr>
<tr>
<td>SGX393</td>
<td>ABL only</td>
<td>Active</td>
<td>Preclinical</td>
<td></td>
</tr>
<tr>
<td>XL228</td>
<td>Aurora A/B, FAK, Src</td>
<td>Active</td>
<td>Phase I—terminated</td>
<td>NCT00464113</td>
</tr>
<tr>
<td>MK-0457</td>
<td>Aurora A-C, Flt3</td>
<td>Active</td>
<td>Phase II—terminated</td>
<td>NCT00405054</td>
</tr>
<tr>
<td>AT9283</td>
<td>Aurora A/B, JAK2/3</td>
<td>Active</td>
<td>Phase I/II</td>
<td>NCT00522990</td>
</tr>
<tr>
<td>Danusertib</td>
<td>Aurora A-C, Ret, Trk-A, FGFR-1</td>
<td>Active</td>
<td>Phase II</td>
<td>NCT0035868</td>
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<td>Ponatinib</td>
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<td>Bafetinib</td>
<td>Lyn</td>
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<tr>
<td>AP23464</td>
<td>Src family</td>
<td>Active</td>
<td>Preclinical</td>
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<td>Bosutinib</td>
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<td>Phase I/II/III</td>
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<td>DSA compounds</td>
<td>Src</td>
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<td>PD166326</td>
<td>Src</td>
<td>NA</td>
<td>No trials or recent reports</td>
<td>††</td>
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<tr>
<td>Saracatinib</td>
<td>Src family</td>
<td>Not in trials for CML</td>
<td>†‡</td>
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<tr>
<td>HG-7-85-01</td>
<td>Src, PDGFR, VEGFR, Flt3, Ret, Tie2, Kit, DDR1, b-raf</td>
<td>Active</td>
<td>Preclinical</td>
<td>§§</td>
</tr>
</tbody>
</table>

\(^*PloS One. 2011;6:e15929.\)
\(^‡Chem Biol Drug Des. 2007;70:171–181.\)
\(^§Med Res Rev. 2011;31:1–41.\)
\(^**Cancer Res. 2009;69:2384–2392.\)
\(†‡Expert Opin Investig Drugs. 2010;19:931–945.\)
\(§§Blood. 2010;115:4206–4216.\)

NA indicates not active.
the active site of BCR-ABL. GNF-2 has exceptional specificity for BCR-ABL, does not inhibit c-Kit, PDGFR, or other kinases (63 tested), and is nontoxic toward non–BCR-ABL–expressing cells. GNF-2 has been found to enhance imatinib activity against BCR-ABL, whereas a GNF-2 analog (21a-I) was found to synergize with dasatinib against the T315I mutant. Other GNF analogues are in development, but none are currently in clinical trials.

The Essential BCR Coiled Coil

Oligomerization of BCR-ABL through the coiled-coil domain (Fig. 1) is essential for oncogenicity, making this region an attractive target for therapeutic development. Non-small-molecule inhibitors targeting the BCR coiled-coil are exciting alternatives that disrupt BCR-ABL oligomerization and activation. We have recently reported the disruption of BCR-ABL via a rationally designed mutant coiled-coil peptide. Such peptides may reduce the risk of acquired resistance due to the numerous contact points between the coiled-coil and the protein or because peptides are not typical substrates for drug efflux transporters whose overexpression may lead to resistance. Delivery strategies for peptide therapeutics to the CML cell are a current focus of our laboratory.

Degrading BCR-ABL

A natural compound in vegetables, PEITC, was found to kill T315I harboring cells in culture and from patient samples. PEITC induces oxidative stress in CML cells leading to degradation of BCR-ABL. Another degradation strategy involves a novel ubiquitin cycle inhibitor, WP1130, reported to rapidly induce ubiquitination of BCR-ABL resulting in protein relocation into aggresomes, rendering it inactive. Both imatinib-sensitive and -resistant CML cells initiated apoptosis in response to WP1130.

Hsp90 (heat shock protein 90) inhibitors geldanamycin and 17-AAG were shown to induce degradation of BCR-ABL protein in vitro. Mechanistically, after dissociation of Hsp-90 from client proteins, Bag1 (B-cell lymphoma-2 [Bcl-2]-associated athanogene-1) mediates BCR-ABL localization to the proteasome and stimulates its degradation via an E3-ligase–dependent mechanism. However, clinical trials in CML were disappointing.

Immunotherapy

In addition to small molecules, immunologic targeting of BCR-ABL, rather than kinase inhibition, may be effective. Interferon may function by inducing cytotoxic T-cell responses against myeloid antigens. A more specific approach is vaccines targeting the BCR-ABL junction. Despite some encouraging results, the efficacy of this approach remains unproven in the absence of a prospective randomized trial. Antibodies to the BCR-ABL junction have also been produced. Updates to these are smaller fragments of antibodies such as iDabs, including those specific to BCR-ABL, and small

FIGURE 1. p210 BCR-ABL functional domains and effects of downstream signaling. BCR-ABL signaling leads to enhanced proliferation, reduced apoptotic potential, and altered cell adhesion. Contributions from both BCR and ABL domains on downstream signaling are illustrated. Dashed lines indicate additional intermediate signaling steps not detailed in this figure. ABD indicates actin binding domain; CC, coiled-coil; DBD, DNA binding domain; DH, Dbl homology; DH, Pleckstrin homology; S/T kinase, serine/threonine kinase; SH2 or SH3, Src homology 2/3; Y kinase, tyrosine kinase. Figure courtesy of Andrew Dixon.
antibody mimics or monobodies. The clinical utility of these antibodies is unclear.

TARGETING CML STEM CELLS AND THEIR MICROENVIRONMENT

Stem Cell Niche

In vitro, TKIs are known to have antiproliferative effects on primitive CML cells, but they do not induce apoptosis. This may explain why TKIs fail to eliminate CML stem cells in vivo, evident by disease persistence and the inability to discontinue therapy. We have reported that primitive human CML stem cells are not dependent on BCR-ABL, suggesting that, on TKI challenge, CML stem cells rely on survival signals other than BCR-ABL. It is likely that these signals are provided by the microenvironment. It follows that therapies that only biochemically target BCR-ABL will be unable to eliminate CML stem cells. Cytokines, chemokines, and the extracellular matrix, collectively referred to as the microenvironment, may activate signaling pathways involved in survival. Therapeutic strategies that target stem cells within this context hold promise to eliminate residual leukemia, including cytokine antagonists, adhesion molecule antagonists, and inhibitors of survival and self-renewal.

The Hedgehog (Hh) signaling pathway has been implicated in hematopoietic stem cell renewal. Consistent with a critical role of Hh for CML pathogenesis, lack of Smoothened, an essential component of the pathway, was shown to attenuate CML in murine models. Similarly, the Hedgehog inhibitor LDE225 in combination with nilotinib resulted in elimination of CML stem and progenitor cells. Several Hedgehog inhibitors, including PF-04449913, for hematologic malignancies are also in clinical development. Akt, a well-established downstream target of BCR-ABL, phosphorylates the Foxo3a transcription factor, leading to its exclusion from the nucleus and suppression of transcription. Despite this, Foxo3a is nuclear in primitive CML cells. Recent data have suggested that TGF-β signaling may be responsible for this unexpected finding, and it has been inferred that this may allow CML stem cells to remain in a quiescent state, despite BCR-ABL activity. If so, this would suggest that inhibiting TGF-β may push the critical cells into cycle, thereby rendering them susceptible to BCR-ABL inhibition. Efficient depletion of CML in vivo was found with a combination treatment using imatinib, a TGF-β inhibitor, and Foxo3a depletion.

Yet another strategy is to interfere with stem cell homing. For example, CXCR4 is a receptor for the chemokine SDF-1 (stromal-derived factor 1), and plays a role in homing...
of CD34+ stem cells to the bone marrow microenvironment. Imatinib inhibition of BCR-ABL restores the CXCR4 interaction with SDF-1, leading to the migration and attachment of CML cells to the bone marrow microenvironment. However, a CXCR4 antagonist, AMD3465, partially inhibited cell migration to mesenchymal cells in coculture conditions. Similar results were seen with QLT0267, an integrin signaling inhibitor.115

**Drug Combinations and Synthetic Lethality**

Although stem cells express, but are not addicted to, BCR-ABL it may still be possible to manipulate other pathways which assume an essential role in response to ABL inhibition. This idea of synthetic lethality for cancer therapy is not new but has recently received more attention in the CML field propelled by emerging data demonstrating BCR-ABL–independent disease persistence on TKI therapy. In an RNAi-based screen for dysregulated genes in response to imatinib therapy, the Wnt pathway emerged as the viable target for a second hit.116 Other critical pathways involved in disease progression or leukemic cell function have become attractive targets to augment BCR-ABL inhibition. For example, inhibition of ATM,117 MUC1,118 Alox5,119 and mTOR120 have all been investigated in preclinical studies because they do not cause loss of hematopoietic stem cell function but instead target the leukemic clone in combination with TKIs. A list of recent clinical trials for combination therapies can be found in Table 2.

Finally, transcription factors such as STAT5 can mediate resistance to TKIs.121 Some patients in BC-CML have significant down-regulation of signal transducers and activators of transcription (STAT) inhibitor proteins, potentiating cell survival and residual disease.122 A new STAT5 inhibitor, pimozone, is able to decrease STAT5 and its target genes, resulting in growth inhibition of Ph+ patient samples independently of ABL mutations.123 The precise mechanism of action of this compound is not known. For a comprehensive discussion on other signal transduction pathways in CML, the reader is referred to the referenced chapter.124

**CONCLUSIONS**

The rational design of drugs targeting BCR-ABL has made CML a manageable disease, resulting in prolonged survival for most patients. Mutations resulting in resistance to imatinib have driven development of the second-generation TKIs nilotinib and dasatinib. These inhibitors are active against a broad spectrum of BCR-ABL mutants, with the notable exception of the T315I “gatekeeper” mutant, which, in turn, has led to third-generation inhibitors. The most advanced of these is ponatinib, which has been termed a pan-BCR-ABL inhibitor, as it does not have identifiable gaps in BCR-ABL coverage. As complete ablation of BCR-ABL activity becomes a reality, the question arises whether we will see BCR-ABL–independent resistance emerge as a unifying feature of TKI failure. As the field has focused on the role of kinase domain mutations, relatively little is known about these mechanisms.

On the other side of the response spectrum is minimal residual leukemia despite prolonged TKI therapy. Although the relapse rate in this population of patients is very low, the need for continued treatment has major health and economic implications, and it remains possible that we will see unexpected late adverse effects in patients after decades of TKI therapy. Recent evidence suggests that primitive CML cells survive despite inhibition of BCR-ABL, suggesting a biologic barrier to disease eradication by TKIs.71 We contend that eradicating CML will require targeting the stem cell niche. Several pathways have emerged as potential targets, and a clear winner has not yet been identified. In many respects, CML has served as a paradigm for cancer therapy, and it is likely that this will continue to be the case as we start to transform profound responses into definitive “cures.”

**REFERENCES**


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