Chronic myelogenous leukemia (CML) is a clonal myelo-proliferative disease characterized by the Philadelphia (Ph) chromosome genetic abnormality, which arises from the reciprocal chromosomal translocation t(9;22)(q34;q11) (1, 2). This translocation fuses the genes encoding BCR and ABL, resulting in expression of the constitutively active protein tyrosine kinase, BCR-ABL. Depending on the precise translocation breakpoints and differential mRNA splicing, various molecular weight isoforms of BCR-ABL are generated. These isoforms associate with distinct types of leukemia (2, 3). Most (>90%) of patients with CML and one third of patients with Ph+ acute lymphocytic leukemia (ALL) express the 210-kDa oncoprotein. Twenty percent to 30% of cases of Ph+ ALL and a few cases of CML are associated with 185-kDa BCR-ABL. Twenty percent to 30% of cases of Ph+ ALL and a few cases of CML are associated with 185-kDa BCR-ABL. A subset of patients with indolent CML express the 230-kDa oncoprotein. Differences in intrinsic BCR-ABL kinase activity, but the pathways downstream of BCR-ABL critical for oncogenesis and transformation are not well understood.

Based on its role in malignant transformation, BCR-ABL has served as a target for therapeutic intervention in CML. Imatinib is a relatively specific inhibitor of BCR-ABL, ABL, and ARG development of therapeutic agents to treat these BCR-ABL–driven malignancies (3).

### BCR-ABL Signaling

Unlike ABL, which is primarily localized in the nucleus and is expressed ubiquitously, BCR-ABL is found in the cytoplasm of Ph+ somatic cells (4). Constitutive BCR-ABL kinase activity is transforming in animal models (5–7), supporting the hypothesis that BCR-ABL is oncogenic in human leukemias.

BCR-ABL is considered necessary, but may not be sufficient, to cause malignant transformation in CML (8, 9). The causal insufficiency is supported by detection, using highly sensitive methods, of BCR-ABL expression in blood samples from normal humans, suggesting that other factors contribute to transformation (10, 11). BCR-ABL triggers intracellular signal transduction pathways that promote proliferation and genetic instability while suppressing apoptosis and weakening cellular adhesion (4, 12, 13). Biochemical signaling pathways known to be activated by BCR-ABL include RAS, mitogen-activated protein kinase, c-Jun NH2-terminal kinase/stress-activated protein kinase, phosphatidylinositol 3-kinase (PI3-K), nuclear factor-κB, CRK oncogene–like protein/focal adhesion kinase, and signal transducer and activator of transcription (STAT; Fig. 1). These and other pathways are activated by BCR-ABL kinase activity, but the pathways downstream of BCR-ABL critical for oncogenesis and transformation are not well understood.

## Abstract

**Purpose:** Review the state-of-the-art knowledge of the biology and therapy of chronic myelogenous leukemia (CML).

**Experimental Design:** A review of the literature was undertaken to summarize current information on the pathophysiology of CML and to update data of imatinib mesylate therapy, mechanisms of resistance, and in vitro and clinical data with the new tyrosine kinase inhibitors.

**Results:** Imatinib, which targets the ABL kinase activity of BCR-ABL, has prolonged survival in CML. Despite the efficacy of imatinib, some patients in chronic phase and more in advanced phases of CML develop resistance, frequently as a result of BCR-ABL tyrosine kinase domain mutations that impair imatinib binding but retain enzymatic activity. New tyrosine kinase inhibitors inhibit BCR-ABL more potently than imatinib and maintain activity against an array of imatinib-resistant BCR-ABL mutants. The IC₅₀ values of nilotinib and dasatinib are at least 10- to 100-fold lower for BCR-ABL compared with imatinib. Phase I-II trials of nilotinib and dasatinib showed high activity in imatinib-resistant CML and Philadelphia chromosome–positive ALL. Dasatinib also inhibits members of the Src family of kinases (SFKs); nilotinib does not. Whether SFKs have a critical role in imatinib resistance or BCR-ABL–mediated oncogenesis is unresolved. Agents that target signals downstream of BCR-ABL (e.g. Ras/Raf and phosphatidylinositol 3-kinase) are under investigation.

**Conclusions:** Understanding the pathophysiology of CML and mechanisms of resistance has produced effective targeted strategies for imatinib-resistant CML.

## Chronic myelogenous leukemia (CML)

CML is a clonal myeloproliferative disease characterized by the Philadelphia (Ph) chromosome genetic abnormality, which arises from the reciprocal chromosomal translocation t(9;22)(q34;q11) (1, 2). This translocation fuses the genes encoding BCR and ABL, resulting in expression of the constitutively active protein tyrosine kinase, BCR-ABL. Depending on the precise translocation breakpoints and differential mRNA splicing, various molecular weight isoforms of BCR-ABL are generated. These isoforms associate with distinct types of leukemia (2, 3). Most (>90%) of patients with CML and one third of patients with Ph+ acute lymphocytic leukemia (ALL) express the 210-kDa oncoprotein. Twenty percent to 30% of cases of Ph+ ALL and a few cases of CML are associated with 185-kDa BCR-ABL. A subset of patients with indolent CML express the 230-kDa BCR-ABL oncoprotein. Differences in intrinsic BCR-ABL kinase activity and cell context may influence the type of leukemia that arises with each BCR-ABL isoform and the development of therapeutic agents to treat these BCR-ABL–driven malignancies (3).

## BCR-ABL Signaling

Unlike ABL, which is primarily localized in the nucleus and is expressed ubiquitously, BCR-ABL is found in the cytoplasm of Ph+ somatic cells (4). Constitutive BCR-ABL kinase activity is transforming in animal models (5–7), supporting the hypothesis that BCR-ABL is oncogenic in human leukemias.

BCR-ABL is considered necessary, but may not be sufficient, to cause malignant transformation in CML (8, 9). The causal insufficiency is supported by detection, using highly sensitive methods, of BCR-ABL expression in blood samples from normal humans, suggesting that other factors contribute to transformation (10, 11). BCR-ABL triggers intracellular signal transduction pathways that promote proliferation and genetic instability while suppressing apoptosis and weakening cellular adhesion (4, 12, 13). Biochemical signaling pathways known to be activated by BCR-ABL include RAS, mitogen-activated protein kinase, c-Jun NH2-terminal kinase/stress-activated protein kinase, phosphatidylinositol 3-kinase (PI3-K), nuclear factor-κB, CRK oncogene–like protein/focal adhesion kinase, and signal transducer and activator of transcription (STAT; Fig. 1). These and other pathways are activated by BCR-ABL kinase activity, but the pathways downstream of BCR-ABL critical for oncogenesis and transformation are not well understood.

Based on its role in malignant transformation, BCR-ABL has served as a target for therapeutic intervention in CML. Imatinib is a relatively specific inhibitor of BCR-ABL, ABL, and ARG...
tyrosine kinase activity (14, 15). Imatinib also inhibits members of the class III receptor tyrosine kinases that include KIT, platelet-derived growth factor receptors, and c-FMS (16–18).

### Inhibition of BCR-ABL with Imatinib as Therapy for CML Prolongs Survival

Imatinib has been investigated in all CML phases. In a phase II trial of 229 patients with a confirmed diagnosis of blastic-phase CML, the cytogenetic response rates were 6% and 18% in patients treated with 400 or 600 mg/d, respectively (19). Follow-up indicated survival rates of 14% to 17% at 24 to 36 months (20, 21).

In accelerated phase, the phase II trial enrolled 235 patients treated with imatinib 400 or 600 mg/d in sequential cohorts (22). Hematologic responses were obtained in 65% of patients treated with 400 mg/d imatinib and in 71% of patients treated with 600 mg/d imatinib; 3-year survival rates were 44% and 66%, respectively (20). The 4-year estimated survival rate was 53% for similar patients in accelerated phase treated with imatinib in a single institution (Table 1; ref. 23).

In the phase II trial, including 454 patients in late chronic phase treated with imatinib after failure of, or intolerance to IFN-α, the complete hematologic response (CHR) rate was 95%, the cytogenetic complete response (CGCR) rate at 5 years was 57%, and the 5-year survival rate was 79% (24, 25). An analysis that included patients in this trial and patients treated in an Expanded Access Trial yielded an estimated 4-year survival rate of 86% (23, 26).

The phase III International Randomized Study of IFN versus STI571 (IRIS) trial compared imatinib with IFN-α plus cytarabine, the standard of care at that time, in 1,106 newly diagnosed chronic-phase patients with CML (27, 28). At 18 months, the estimated CGCR rate was 87% with imatinib compared with 35% with IFN-α plus cytarabine. The freedom from progression to accelerated or blastic phase were 97% and 92%, respectively ($P < 0.001$).

With a present follow-up of 5 years, the cumulative CGCR rate with imatinib is 87%, the estimated 5-year survival rate is 89%, and survival without transformation rate is 93% (Table 1; ref. 29). In two studies, comparison of survival with imatinib to historical experience with IFN-α regimens in newly diagnosed CML confirmed the survival advantage with imatinib (30, 31).

### Molecular Responses with Imatinib Associated with Improved Progression-Free Survival

Achievement of CGCR or molecular response has been associated with prolonged survival in CML with IFN-α therapy (32, 33). With most patients achieving cytogenetic responses with imatinib, the goal of therapy has shifted to achieving molecular responses as measured by the reduction or elimination of BCR-ABL transcripts.

**BCR-ABL** transcript levels can be quantitatively expressed as a log reduction from baseline levels standardized in individual laboratories or as a ratio of BCR-ABL to a control gene transcript. Major molecular responses (MMR) in the IRIS trial were defined as a $\geq 3$ log reduction in BCR-ABL/BCR transcripts from a standard baseline. After 12 months, 38% of patients taking imatinib, compared with <5% of patients taking IFN-α plus cytarabine, attained a MMR. A MMR was associated with significantly better long-term remission duration (34) and progression-free survival (28).

At 60 months of follow-up in the IRIS trial, achievement of CGCR and MMR at 12 months was associated with a progression-free survival rate of 97% compared with 89% for CGCR without MMR and 72% for less than CGCR (29, 35).

Achieving an early molecular response also predicted for better outcome. In studies of patient subsets from the IRIS trial, disease progression correlated with failure to achieve a 1 log reduction in BCR-ABL transcript levels by 3 months or a 2 log reduction by 6 months (36). The median best BCR-ABL/ABL ratio in patients achieving CCR on imatinib and then relapsing was 0.24% versus 0.029% in those maintaining CGCR (37).

### Table 1. Survival rates of patients with CML treated with imatinib

<table>
<thead>
<tr>
<th>Disease phase (reference)</th>
<th>Imatinib daily dose (mg)</th>
<th>Estimated survival rate (at X year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic, newly diagnosed 29)</td>
<td>400</td>
<td>89% (5)</td>
</tr>
<tr>
<td>Chronic, previously treated 24)</td>
<td>400</td>
<td>86% (4)</td>
</tr>
<tr>
<td>Accelerated phase 20)</td>
<td>400 vs 600</td>
<td>44% (3) vs 66% (3)</td>
</tr>
<tr>
<td>Blastic 21)</td>
<td>400-600</td>
<td>17% (2); 14-17% (2-3)</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Schematic BCR-ABL related downstream pathophysiologic events in CML.

**Table 1.** Survival rates of patients with CML treated with imatinib
Resistance to Imatinib in CML

Particular levels of response to imatinib in CML at defined time points have correlated with prognosis. Recent recommendations of definitions of resistance (38) versus suboptimal response to imatinib have been proposed (Table 2; ref. 38). Resistance to imatinib is defined as a failure to achieve CHR at 3 months, a cytogenic response at 6 months, or a major cytogenetic response at 12 months (38, 39). Whereas progression-free survival with MMR is better, CGCR without MMR at 12 months is still associated with a favorable progression-free survival (89% at 60 months); lack of MMR may be considered as suboptimal response rather than failure (Table 2). Resistance rate to imatinib averages 4% yearly in newly diagnosed CML, but has decreased to 1% to 1.5% in years 4 to 5 of study (29). In patients achieving CGCR, the resistance rate is 1% or less in years 3 to 4. In contrast, a substantial portion of patients in advanced-phase CML exhibit resistance to imatinib. The estimated 4-year resistance rates are 20% in later chronic phase and 70% to 90% in accelerated-blastic phases.

Resistance to imatinib is often attributed to the emergence of clones expressing mutant forms of BCR-ABL, in which amino acid substitutions in the ABL kinase domain impair imatinib binding but retain kinase activity (36, 39–42). Initial reports suggested these mutations to be present in up to 90% of patients failing imatinib therapy; recent studies suggested rates of 40%. Other proposed mechanisms of imatinib resistance include amplification of the fusion gene, overexpression of the BCR-ABL oncoprotein, overexpression of molecules downstream of BCR-ABL signaling, such as Lyn kinase, clonal evolution, expression of the multidrug resistance phenotype, and binding of α1 acid glycoprotein (39–51). In addition, leukemic stem cell quiescent and insensitive to imatinib may cause persistence of CML.

Table 2. Criteria for failure to imatinib

<table>
<thead>
<tr>
<th>Time on imatinib (mo)</th>
<th>Failure</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loss of CHR</td>
<td>No CHR</td>
</tr>
<tr>
<td>3</td>
<td>Ph+ ≤ 5%</td>
<td>Ph+ &gt; ≥ 35%</td>
</tr>
<tr>
<td>6</td>
<td>Ph+ ≤ 5%</td>
<td>Ph+ &gt; ≥ 35%</td>
</tr>
<tr>
<td>12</td>
<td>Loss of CHR</td>
<td>Ph+ &gt; ≥ 5%</td>
</tr>
<tr>
<td>18</td>
<td>Loss of cytogenetic response</td>
<td>No MMR (&lt;3-log reduction of BCR-ABL/ABL)</td>
</tr>
<tr>
<td>Any</td>
<td>Mutation</td>
<td>Clonal evolution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss of MMR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mutation</td>
</tr>
</tbody>
</table>

Abbreviation: HR, hematologic response.

Circumventing Imatinib Resistance in CML

Nilotinib. Nilotinib (Tasigna; AMN107) is a novel tyrosine kinase inhibitor designed to be highly selective and potent against ABL kinase. Structurally related to imatinib, nilotinib is 20- to 50-fold more potent than imatinib against BCR-ABL (Fig. 2; refs. 52–55). It maintains activity against most imatinib-resistant BCR-ABL point mutants. In proliferation assays, 32 of 33 imatinib-resistant mutant cell lines were inhibited by nilotinib, except T315I. Nilotinib prolonged survival in mice injected with leukemic cells expressing wild-type and imatinib-resistant mutants of BCR-ABL (52–54). At concentrations comparable with imatinib, nilotinib inhibits cells expressing activating mutants of ARG, KIT, platelet-derived growth factor receptor α, or platelet-derived growth factor receptor β (53) but has minimal effects on Src, fms-like tyrosine kinase 3, vascular endothelial growth factor receptor, or epidermal growth factor receptor kinases.

In a phase I trial of nilotinib in imatinib-resistant CML and Ph+ ALL (56), 119 patients were treated, including 17 in chronic phase, 56 in accelerated phase, 24 in myeloid blast–phase CML, 22 in lymphoid blast–phase CML, and Ph+ ALL. Nilotinib doses ranged from 50 mg once daily to 600 mg twice daily. Grade 3/4 thrombocytopenia was observed in 20%, and grade 3/4 neutropenia was observed in 13%. Nonhematologic grade 3/4 adverse events included elevated bilirubin in 7% and elevated serum lipase in 5%. Eleven of 12 (92%) patients in chronic phase with active disease attained CHR with nilotinib; a cytogenetic response was achieved in 9 of 17 (53%) patients in chronic phase, 6 of them achieving a CGCR. The overall hematologic and cytogenetic response rates to nilotinib in accelerated-phase CML were 72% and 48%, respectively. Patients with accelerated-phase CML only exhibiting clonal evolution (n = 10) had hematologic and cytogenetic response rates of 100% and 90%, respectively. Among patients with CML in myeloid blast phase (n = 24), the hematologic response rate was 42% and the cytogenetic response rate was 29%. Patients in lymphoid blastic phase disease (n = 9) had a hematologic response rate of 33% and a cytogenetic response rate of 22%. Response rates to nilotinib were similar in patients with or without mutations. The recommended dose for phase II studies was 400 mg orally twice daily (56).

Phase II pivotal studies of nilotinib in all CML phases after imatinib failure are ongoing and have confirmed the encouraging anti-CML activity post-imatinib failure in all CML phases (Table 3; refs. 57–60). In chronic-phase CML, 81 patients were evaluable: CHR was achieved in 69% and a cytogenetic response rate was achieved in 68%, this being major in 46% and complete in 32% (57). Severe neutropenia was noted in 16% and thrombocytopenia was noted in 19%. Similarly, a pilot study of nilotinib in newly diagnosed CML showed higher rates of CGCR compared with imatinib therapy (60).

Dasatinib. Dasatinib (Sprycel; BMS-354825) is a tyrosine kinase inhibitor originally designed as a Src family of kinases designed to be highly selective and potent against ABL kinase.
Dasatinib inhibits ABL, BCR-ABL, EPHA2, KIT, and platelet-derived growth factor receptor. The IC_{50} of dasatinib for BCR-ABL is <1 nmol/L compared with 25 nmol/L for nilotinib (53). Dasatinib also inhibits certain SFK members (i.e., Fyn, Yes, Src, and Lyk) with IC_{50} values in the range of 0.5 nmol/L (61–63). Dasatinib inhibits proliferation of cells expressing all imatinib-resistant BCR-ABL mutants tested to date, except the T315I (46, 55). Dasatinib prolongs survival of mice with wild-type or imatinib-resistant forms of BCR-ABL myeloproliferative disease.

A phase I trial of dasatinib has been completed in 88 patients with imatinib-resistant or imatinib-intolerant CML in chronic (n = 40) and advanced phases (n = 44; ref. 64). The maximum tolerated dose of dasatinib was 70 mg orally twice daily. Adverse events with dasatinib were mainly hematologic, including grade 3/4 neutropenia and thrombocytopenia in 50% to 60%. Non-hematologic adverse events included pleural effusions in 5% to 10% (64). The CHR rate in chronic-phase CML post-imatinib failure was 88%; the CGCR rate was 33%. The CHR rate was 50% in accelerated-phase CML, 18% in myeloid blastic phase, and 50% in lymphoid blastic phase. Overall, the CGCR rate in advanced disease was 21%, and the MMR rate was 24% (65).

Phase II trials of dasatinib in CML and Ph^{+} ALL have been completed and led to the approval of dasatinib for patients resistant to intolerant of imatinib (Table 4; 66–70). In these studies, dasatinib was administered at a dose of 70 mg twice daily. In the phase II study in patients with CML in chronic phase, 387 patients resistant (75%) or intolerant (25%) to imatinib were treated. A CHR was observed in 90% and major cytogenetic response in 78% of imatinib-intolerant (68% CGCR and 10% CGPR) and in 42% of imatinib-resistant (30% CGCR and 12% CGPR) patients. BCR-ABL mutations were detected in 160 of 363 (44%) assessable patients, G250E (n = 23) and T315I (n = 3) being the most and least frequently encountered mutations. Significant molecular responses were observed with a median BCR-ABL/ABL ratio of 0.3% at 9 months (66). The 10-month progression-free survival was 88%.

In accelerated-phase CML, 174 patients were treated. A major hematologic response was reported in 102 (59%) patients, including 59 (34%) who achieved CHR and 43 (25%) with no evidence of leukemia. A major cytogenetic response was obtained by 60 (34%) patients, including 43 (25%) CGCRs and 17 (10%) CGPRs.

One hundred and nine patients with CML myeloid blastic phase and 94 patients with either CML lymphoid blastic phase (n = 48) or Ph^{+} ALL (n = 46; ref. 69) were treated. The overall hematologic and cytogenetic response rates in myeloid blastic phase were 48% and 44% (25% CGCR and 6% CGPR), respectively. Major hematologic responses were observed in 33% (29% CHR) of patients with lymphoid blastic phase and in 39% (33% CHR) of those with Ph^{+} ALL. Major cytogenetic responses were reported in 44% (38% CGCR) of patients with lymphoid blastic phase and in 46% (44% CGCR) of those with Ph^{+} ALL.

The most common nonhematologic toxicities in these phase II studies were as follows: diarrhea (30-60%), headache (30%), rash (22-27%), superficial edema (20%), and pleural effusion.

### Table 3. Results of phase II nilotinib studies in CML post-imatinib failure

<table>
<thead>
<tr>
<th></th>
<th>Chronic</th>
<th>Accelerated</th>
<th>Blastic</th>
<th>Ph^{+} ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. treated</td>
<td>81</td>
<td>25</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>% CHR/HR</td>
<td>69/—</td>
<td>16/40</td>
<td>4/13</td>
<td>27/33</td>
</tr>
<tr>
<td>% Cytogenetic response</td>
<td>68</td>
<td>56</td>
<td>29</td>
<td>—</td>
</tr>
<tr>
<td>Complete</td>
<td>34</td>
<td>16</td>
<td>21</td>
<td>—</td>
</tr>
<tr>
<td>Partial</td>
<td>14</td>
<td>12</td>
<td>8</td>
<td>—</td>
</tr>
</tbody>
</table>

NOTE: Update of presentations at the American Society of Clinical Oncology, 2006 (57–60).
and associated with pleural effusions (11%). 21% versus 8%). Dasatinib was also more myelosuppressive

dasatinib versus high-dose imatinib (CGCR rates at 3 months, better rates of CGCR and progression-free survival with

imatinib (2:1 randomization) have been reported, post-failure

II study comparing the activity of dasatinib and high-dose

or thrombocytopenia was reported in 47%.

(15-25%). These were severe in <5%. Grade 3 to 4 neutropenia

Results from a randomized, multinational, open-label phase

II study comparing the activity of dasatinib and high-dose

imatinib at 400 to 600 mg daily (70), suggesting

better rates of CGCR and progression-free survival with dasatinib versus high-dose imatinib (CGCR rates at 3 months,

21% versus 8%). Dasatinib was also more myelosuppressive

and associated with pleural effusions (11%).

Therapeutic Interventions for CML Downstream or Parallel to BCR-ABL

BCR-ABL–dependent resistance to imatinib has raised

interest in agents that intervene downstream of BCR-ABL.

Src as a target for CML therapy. Src kinases are a family

of nine structurally homologous nonreceptor intracellular
tyrosine kinases (Src, Fyn, Yes, Blk, York, Fgr, Hck, Lck, and

Lyn). They regulate signal transduction pathways involved in
cell growth, differentiation, and survival (71). The expression

of some Src kinases is ubiquitous; others display different tissue-specific expression patterns. Hck, Lyn, Fgr, Lck, and Blk are

strictly restricted to hematopoietic cells (71). Hck expression is

restricted to myeloid cells and B-lymphocytes, whereas Lyn

is expressed in myeloid cells, B-lymphocytes, and natural killer

cells (71). Multiple domains of BCR-ABL interact with Hck and

Lyn leading to their activation (72). Experiments with Src

dominant-negative mutants suggest that Src kinases play a role

in proliferation of BCR-ABL–expressing cell lines (71–73).

Formation of the Hck–BCR-ABL complex and BCR-ABL–

mediated activation of Hck are not dependent on the ABL

kinase activity (74). Overexpression of Src kinases is implicated

in BCR-ABL–mediated leukemogenesis and in some cases of

imatinib resistance (45, 72, 75–77). Overexpression and/or

activation of Hck and Lyn occur during CML progression,
suggesting that acquired imatinib resistance may be BCR-ABL

independent and mediated by overexpression of Src kinases

(75). Activation of kinases may promote phosphorylation of

BCR-ABL and interaction with growth factor receptor binding

protein 2 (72, 74). ABL has significant sequence homology with

Src and, in its active configuration, bears remarkable structural

resemblance with Src family kinases. ATP-competitive com-

pounds originally developed as Src inhibitors frequently exert

potent inhibition of ABL kinase due to the striking resemblance

between the catalytically active state of both protein kinases

(78, 79). Based on the structural similarity between ABL and Src

and their critical role in the pathogenesis of CML, small-
molecule inhibitors with overlapping activity against both ABL

and Src may result in enhanced activity against CML compared

with imatinib.

When BCR-ABL interacts with and activates HCK, it

phosphorylates and activates STAT5 leading to myeloid cell

transformation in vitro. This suggests that the BCR-ABL-HCK-

STAT5 pathway may have an important role in Ph+ leukemias

(79). Cell context contributes to BCR-ABL–mediated transfor-
mation and differences may exist in the role of SFKs within
different hematopoietic cell lineages. BCR-ABL retrovirus-

transduced bone marrow cells from mice lacking expression

of Lyn, Hck, and Fgr SFKs induced CML but not Ph+ ALL (80).
This suggested that Lyn, Hck, and Fgr may have a role in

lymphoid leukemogenesis and that BCR-ABL uses different

signaling pathways to induce CML and Ph+ ALL. Exposing

lymphoid blasts from patients with blast phase CML in vitro

to small interfering RNA that specifically diminishes Lyn kinase

expression induced cell apoptosis, suggesting that Lyn kinase

activity contributed to the blast phase phenotype. Cells from

patients with myeloid blast phase CML were less sensitive to

LYN small interfering RNA (81).

Other Dual ABL/SFK Inhibitors in Development for CML

Bosutinib. Bosutinib (SKI-606) is a potent inhibitor of

SFKs and ABL tyrosine kinase activity (82). In three different

BCR-ABL–positive CML cell lines, SKI-606 showed in vitro

antiproliferative effects. SKI-606 caused regression of large

K562 xenografts in nude mice (82). Phase I–II studies are

ongoing to evaluate the safety and tolerability of bosutinib.

NS-187/INNO406. This dual-specificity ABL and Lyn kinase

inhibitor is 25 to 55 times more potent than imatinib in vitro

(83). Its inhibitory activity is less affected by BCR-ABL mutants

compared with imatinib, but it does not inhibit T315I. It also

suppressed the growth of BCR-ABL–expressing leukemic cell

lines and prolonged survival of mice bearing BCR-ABL–

expressing leukemia. The efficacy and safety of INNO406 is

now being tested in a phase 1 study.

Others. AZD0530 is a highly selective inhibitor of Src kinase

and BCR-ABL (84). Phase I dose-finding studies in normal

volunteers showed the drug to be tolerated at doses up to

500 mg daily. Dose-limiting toxicities were diarrhea and

vomiting. Efficacy of AZD0530 in imatinib-resistant CML

remains to be tested.

Table 4. Summary of dasatinib results in CML post-imatinib failure

<table>
<thead>
<tr>
<th>Disease</th>
<th>No.</th>
<th>% Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic</td>
<td>387</td>
<td>90</td>
</tr>
<tr>
<td>Accelerated</td>
<td>174</td>
<td>34</td>
</tr>
<tr>
<td>Blastic myeloid</td>
<td>109</td>
<td>25</td>
</tr>
<tr>
<td>Blastic lymphoid</td>
<td>48</td>
<td>29</td>
</tr>
<tr>
<td>Ph+ ALL</td>
<td>46</td>
<td>33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHR</th>
<th>Hematologic response</th>
<th>Cytogenetic response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Major</td>
<td>Complete</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic</td>
<td>51</td>
<td>40</td>
</tr>
<tr>
<td>Accelerated</td>
<td>34</td>
<td>25</td>
</tr>
<tr>
<td>Blastic myeloid</td>
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<td>25</td>
</tr>
<tr>
<td>Blastic lymphoid</td>
<td>44</td>
<td>38</td>
</tr>
<tr>
<td>Ph+ ALL</td>
<td>46</td>
<td>44</td>
</tr>
</tbody>
</table>
AP23464 is also a potent SFK and ABL kinase inhibitor with an IC$_{50}$ in the nanomolar range (85). AP23464 has shown anti-proliferative activity and promoted apoptosis in CML cell lines. In addition, proliferation of cell lines expressing a different imatinib-sensitive BCR-ABL mutant (Q252H, Y253F, E255K, M351I, or H396P) was inhibited by AP23464. AP23464 was unable to inhibit cells expressing the BCR-ABL T315I mutation.

Preclinical studies have also investigated the two SFK inhibitors, PP1 and CGP76030 (85), with dual specificity for ABL and SFKs. Like imatinib, PP1 and CGP76030 binding to BCR-ABL depends on T315, the gatekeeper residue that controls access to the enzymatic active site. PP1 and CGP76030 induced growth arrest and apoptosis in BCR-ABL–expressing cells.

**Potential Consequences of SFK Inhibition**

Mice lacking certain single or combined SFKs have exhibited defective learning and memory (Fyn /), autoimmunity (Fyn / Yes and Lyn /), impaired immune cell function (Lyk and Lyn /), or osteopetrosis (Src /). However, these experiments primarily address issues of SFK ablation on development rather than effects on fully developed organs. The potential long-term consequences of SFK inhibition in CML are unknown. Clinical trials to date with SFK inhibitors do not suggest any significant adverse consequences.

**Novel Approaches to BCR-ABL Inhibition**

One approach to BCR-ABL inhibition involves the substrate recognition domain of the kinase rather than the ATP binding site (86). ON012380 is such an agent. It induces apoptosis of cells expressing imatinib-resistant mutants at a concentration of <10 nmol/L and causes regression of leukemias in mice induced by injection of cells expressing the imatinib-resistant T315I mutant.

Another approach to BCR-ABL inhibition is the use of agents that target mutants of BCR-ABL resistant to all available tyrosine kinase inhibitors, such as the T315I mutant. Aurora kinases are a family of serine/threonine kinases that play an important role in protein phosphorylation events regulating the mitotic phase of the cell cycle (87). Aurora kinases are overexpressed in several human cancers. This prompted the investigation of inhibitors of these kinases as therapeutic agents in cancer. One such Aurora kinase inhibitor, MK0457 (formerly VX680), inhibited the T315I mutant of BCR-ABL. It also inhibited proliferation of CML cells derived from a patient harboring a BCR-ABL T315I mutant. Trials with MK0457 are ongoing (88, 89).

**Non–BCR-ABL kinase inhibitors**. The kinase inhibitor sorafenib (formerly BAY 43-9006) is multitargeted and has shown disease-stabilizing activity in phase 2 trials for solid tumors (90). The mechanism of action of sorafenib may involve inhibiting tumor vasculature cell proliferation. Sorafenib is undergoing evaluation in CML.

**Other Potential Targets in CML Therapy**

**Farnesyl transferase inhibitors**. The RAS proteins are members of a family of G-proteins that bind guanine nucleotides and have intrinsic GTPase activity. These proteins have a central role in signal transduction pathways involved in cell growth and proliferation. RAS proteins are farnesylated, which entails covalent coupling with a 15-carbon isoprenyl group. Inhibition of the farnesyl transferase enzyme can block downstream signaling by RAS proteins. The efficacy of farnesyl transferase inhibitors in CML has been shown in preliminary trials. In a study of 22 patients with CML treated with tipifarnib, hematologic responses were noted in 7, including CHR in 6 patients in chronic-phase CML and cytogenetic responses in 3 of them (91). Tipifarnib and imatinib were given in combination to 23 patients with imatinib failure: hematologic responses were observed in 8 of 10 patients with active disease and cytogenetic responses in 9 of 22 evaluable patients (40%; 6 complete response, 3 partial response, and 4 minor; ref. 92).

Lonafarnib, another farnesyl transferase inhibitor, showed efficacy in CML (93). Two responses were noted among 13 patients with CML in chronic or accelerated phase resistant to imatinib treated with lonafarnib (93).

**Rationale for inhibition of the PI3-K signaling pathway**. The lipid kinase PI3-K is activated by growth factors to stimulate processes associated with cell cycle control and proliferation. BCR-ABL stimulates PI3-K activity, which participates in BCR-ABL–dependent cell growth and survival pathways. The lipid substrate of PI3-K, when phosphorylated, serves as a binding site for proteins containing the pleckstrin homology domain. An example of such a protein is AKT, a serine/threonine kinase recruited to the plasma membrane via a pleckstrin homology domain (94–96). After binding to phosphorylated lipids, AKT itself becomes phosphorylated and subsequently interacts with mammalian target of rapamycin, also a serine and threonine kinase, to further propagate the signal. Inhibition of mammalian target of rapamycin with rapamycin inhibits growth of primary CML cells and imatinib-resistant leukemic cells. Rapamycin and imatinib synergistically inhibited proliferation of leukemic cells in culture and in mice injected with BCR-ABL–expressing cells (97, 98). Rapamycin or similar inhibitors of mammalian target of rapamycin activity, either alone or in combination with imatinib, may be useful in imatinib-resistant CML.

A second approach to intercept the PI3-K signaling pathway via AKT kinase activity is to inhibit the heat shock protein-90, a member of the chaperone family of proteins, which forms a complex with AKT (99). Heat shock protein-90 inhibitors cause AKT degradation; loss of AKT activity is linked to enhanced apoptosis. Trials are under way to investigate the heat shock protein-90 inhibitor 17-allylamino-17-demethoxy-geldanamycin in CML.

**Hypomethylating Agents**

Methylation is a prevalent process in cancer progression and resistance (100). Decitabine, a hypomethylating agent, has activity in CML. In a study of 130 patients with CML, treatment with decitabine 50 to 100 mg/m$^2$ twice daily for 5 days (500-1,000 mg/m$^2$ per course) resulted in a response rate of 28% in blast phase and of 55% in accelerated phase (101).

A phase II study of low-dose decitabine (15 mg/m$^2$ daily × 10; 150 mg/m$^2$ per course) in 35 patients with CML resistant to imatinib resulted in an overall hematologic response rate of 54%; chronic phase, 83%; accelerated phase, 41%; and blast phase, 34% (102). The cytogenetic response rate was 46% and major cytogenetic responses were observed in 6 (17%) patients. Median duration of response was 3.5 months.
Homoharringtonine

The plant-derived alkaloid homoharringtonine was used in treatment of CML before the advent of imatinib. The antileukemic effects of homoharringtonine in CML were shown in several studies in CML (103). More recently, homoharringtonine was investigated in patients with CML achieving suboptimal responses to imatinib (104). Homoharringtonine was given subcutaneously at a dose of 1.25 mg/m² twice daily for 1 day every 28 days. Seven of 10 evaluable patients had significant declines in BCR-ABL transcript levels; 5 had reductions >1 log.

Conclusions

BCR-ABL signaling has a causative role in Ph+ CML. This is substantiated by clinical evidence that BCR-ABL inhibition is the most effective therapy for CML. Resistance to imatinib therapy often stems from BCR-ABL mutations that maintain kinase activity but compromise imatinib binding. This further highlights the key role of BCR-ABL signaling in onco genesis and affirms the position of BCR-ABL as the central therapeutic target in Ph+ CML. The efficacy of novel BCR-ABL inhibitors, such as nilotinib or dasatinib, in imatinib-resistant CML (higher affinities and inhibitory potency for BCR-ABL; inhibition of BCR-ABL mutants), reinforces these concepts. Nilotinib and dasatinib have shown efficacy in phase II trials in imatinib-resistant CML phases as well as in Ph+ ALL. The downstream pathways of malignant transformation induced by BCR-ABL are becoming better understood and are targets of interest for additional treatment strategies. With the rapid advances in molecular biology and pharmacology, the treatment of CML has evolved significantly, offering the potential for durable responses in most patients. The evolution of therapy from imatinib to the second generation of kinase inhibitors is a model that will undoubtedly be translated to other cancers.

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