Multifaceted Approach to the Treatment of Bcr-Abl-Positive Leukemias

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Key Words. Arsenic trioxide · Bcr-Abl · Farnesyltransferase · Imatinib mesylate · Leukemia · Tyrosine kinase

ABSTRACT
Bcr-Abl-positive leukemias include chronic myelogenous leukemia (CML), both myeloid and lymphoid blast-phase CML, and some cases of acute lymphoblastic leukemia. The chimeric bcr-abl gene codes for a tyrosine kinase that is constitutively activated in the leukemic cells and plays the central role in leukemogenesis. Hematologic malignancies, including Bcr-Abl-positive leukemias, also frequently have overactivity of the Ras signaling pathway, leading to abnormal transduction of growth and survival signals. New and investigational therapeutic options that target these specific molecular defects of leukemic cells include the tyrosine kinase inhibitor imatinib mesylate (STI571) and farnesyltransferase inhibitors (R115777, SCH66336), which block localization of Ras proteins to the cell membrane. While single-agent therapy with these new agents may produce hematologic and cytogenetic remissions in patients with Bcr-Abl-positive leukemias, molecular remissions are less common, and resistance may develop. Therefore, the development of a multifaceted therapeutic approach to these leukemias is of great interest. Arsenic trioxide (ATO), which has significant activity in patients with relapsed and refractory acute promyelocytic leukemia, is a potential addition to the therapeutic arsenal. While some of the molecular activities of ATO are specific to acute promyelocytic leukemia, arsenicals also have a broad variety of antineoplastic properties that may be useful in combination therapy with agents that target specific molecular defects of Bcr-Abl-positive leukemias. The Oncologist 2002;7(suppl 1):30-38

INTRODUCTION
Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disease of transformed hematopoietic progenitor cells. With an incidence of one to two cases per 100,000 persons per annum, CML accounts for approximately 15% of all adult leukemias [1]. Although CML can affect people at any age, including children, it is predominantly a disease of the elderly. The median age at presentation is 65.8 years [2].

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Approximately 85% of patients are in the chronic phase of CML at the time of diagnosis [1]. After a period of 3 to 5 years, the disease progresses to a rapidly fatal blast phase, sometimes preceded by an interim accelerated phase [3]. Chemotherapy for patients in blast crisis is usually tailored to the predominant blast type (myeloid versus lymphoid). It is generally ineffective, however, with remissions occurring in only small numbers of patients for brief intervals.

During the last decade, the survival of patients with CML has increased because of earlier diagnosis from routine complete blood counts and treatment with allogeneic bone marrow transplantation or interferon-α (IFN-α). These treatments have important limitations, however. Transplantation is associated with significant mortality and potential post-transplantation morbidity from chronic graft-versus-host disease. Despite the fact that treatment with IFN-α is standard therapy for CML, it produces only a low level of complete cytogenetic remission (6%-20%) and its durability of response is unknown [3]; in addition, side effects cause a majority of patients to discontinue treatment [4].

Advances in cell biology have facilitated the development of therapies targeted to specific molecular events responsible for CML (Table 1). It is now clear that the chimeric Bcr-Abl fusion protein, which provides constitutively active tyrosine kinase activity, plays a central role in the pathophysiology of CML [3]. Accordingly, tyrosine kinase inhibition has been explored as a therapeutic strategy in Bcr-Abl-positive leukemias. Currently, the tyrosine kinase inhibitor, imatinib mesylate (formerly STI571) is successful in achieving complete hematologic responses in patients with chronic phase CML. Yet, while imatinib mesylate also has activity in advanced phases of the disease, the responses tend not to be durable and patients eventually relapse and become resistant to further treatment with imatinib mesylate. Similarly, farnesyltransferase inhibitors, modulating ras signaling, and arsenic trioxide (ATO), promoting apoptosis as well as other antileukemic effects, have also been investigated as therapeutic agents in CML. Suboptimal results with all of these agents as monotherapy have substantiated the need for multifaceted approaches in treating CML.

This article reviews the experience to date with single-agent and combination therapies involving tyrosine kinase inhibitors, IFN-α, farnesyltransferase inhibition, and ATO, toward the objective of improving remission rates (hematologic, cytogenetic, and molecular) and prolonging survival in refractory CML.

**Molecular Biology of CML**

The transforming event in CML is the appearance of a specific karyotypic abnormality, a shortened chromosome 22, known as the Philadelphia chromosome. Although the Philadelphia chromosome is found in monocytes and lymphocytes, the clinical and hematologic manifestations of CML are myeloid in origin and are marked by myeloid hyperplasia in the bone marrow [3, 5]. The Philadelphia chromosome is the result of a translocation—(9;22) (q34;q11)—that fuses the breakpoint cluster region (bcr) gene on chromosome 22 to the Abelson leukemia virus (abl) gene on chromosome 9 to produce a fusion bcr-abl transcript that codes for a chimeric Bcr-Abl protein with constitutive tyrosine kinase activity [1, 3, 6-8]. The Philadelphia chromosome is present in approximately 95% of patients with CML; its molecular equivalent is found in all patients with classic forms of the disease. The translocation is also identified in 15%-30% of patients with acute lymphoblastic leukemia (ALL) and approximately 2% of patients with acute myeloid leukemia [7].

The Bcr-Abl fusion protein can vary in size from 185 kDa to 230 kDa (p185bcr-abl to p230bcr-abl) [3]. Since the Abl tyrosine kinase portion remains the same size in all molecular species of the chimeric protein, the size of the fusion protein is a function of the length of the N-terminal Bcr segment [3]. In patients with typical chronic-phase CML, the protein is usually 210 kDa in size, and cells transfected with p210bcr-abl become dominant in cell culture systems and progress toward a fully malignant phenotype [9]. Patients with Philadelphia-positive (Ph+), ALL, on the other hand, express either a p210bcr-abl or a p190bcr-abl. The size of the protein appears to be one determinant of the biologic behavior of the disease, since the 190-kDa protein has enhanced tyrosine kinase activity relative to the 210-kDa version [3].

As a tyrosine kinase, the Bcr-Abl protein can phosphorylate a variety of proteins involved in cell growth, differentiation, adhesion, and apoptosis of CML cells. When the bone marrow of lethally irradiated mice is reconstituted with progenitor cells transfected with a bcr-abl gene, for example, 50% of them develop a myeloproliferative syndrome resembling CML [10]. Signaling pathways that are activated by Bcr-Abl activity include Ras, phosphatidylinositol 3-kinase/AKT, NF-kB, and Stat-5. These signaling pathways have important limitations, however. Transplantation is associated with significant mortality and potential post-transplantation morbidity from chronic graft-versus-host disease. Despite the fact that treatment with IFN-α is standard therapy for CML, it produces only a low level of complete cytogenetic remission (6%-20%) and its durability of response is unknown [3]; in addition, side effects cause a majority of patients to discontinue treatment [4].

Table 1. Targeted therapies for CML

<table>
<thead>
<tr>
<th>Therapy Type</th>
<th>Specifics</th>
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<tbody>
<tr>
<td>Inhibition of bcr-abl gene products</td>
<td>May include:</td>
</tr>
<tr>
<td>Inhibitors of the Bcr-Abl tyrosine kinase (e.g., imatinib mesylate [STI571])</td>
<td>- Antisense oligonucleotides</td>
</tr>
<tr>
<td>- Ribozyme inhibition of bcr-abl transcripts</td>
<td>- Inhibition of ras gene products</td>
</tr>
<tr>
<td>- Farnesyltransferase inhibitors (e.g., R115777, SCH66336)</td>
<td>- Multiple mechanisms of action (e.g., apoptosis induction, growth modulation, alteration of gene expression)</td>
</tr>
<tr>
<td>- Arsenic trioxide</td>
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</table>

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cascades govern physiologic hematopoiesis; however, the constitutively active state of the fusion tyrosine kinase results in deregulated activity contributing to malignant transformation. Activation of these pathways results in growth factor-independent proliferation and enhanced survival of CML cells. Bcr-Abl also affects integrin functioning, impairing cell-cell contact. This alters the ability of the transformed cells to respond to negative regulatory influences of bone marrow stromal cells and contributes to circulation of CML progenitor cells [11]. Consequently, the leukemic cells may be resistant to immune surveillance, chemotherapy, or radiation. As a result of these various alterations, CML cells undergo massive clonal expansion.

**CLINICAL PRESENTATION AND MONITORING**

CML patients may present symptoms of fatigue, weight loss, abdominal fullness, easy bruising, or bleeding. However, approximately 40% of patients are asymptomatic and are diagnosed by a routine complete blood count [3]. The peripheral blood smear reveals a granulocytic leukocytosis with a spectrum of myeloid cells in various stages of maturation; the complete blood count may also demonstrate anemia and/or thrombocytosis. Splenomegaly is present at the time of the initial examination in approximately one-half of patients.

At the onset of the blast phase, the peripheral blood and bone marrow contain 20% or more blasts. In most instances, the blasts possess myeloid markers, but in approximately one-third of cases the blast crisis is lymphoid in origin [1]. Some patients may have extramedullary blast crisis, in which the peripheral blood and bone marrow resemble chronic-phase disease but extramedullary tissue, such as the spleen or lymph nodes, is infiltrated by large numbers of blasts.

Several types of tests are used to monitor disease-state activity, before and after treatment, in CML (Table 2). These include hematologic tests (peripheral blood and bone marrow), cytogenetic tests (Ph chromosome status), and molecular tests (e.g., fluorescent in-situ hybridization [FISH] for detection of bcr-abl and reverse transcriptase-polymerase chain reaction [RT-PCR] for detection of bcr-abl mRNA transcripts) [7]. The relative sensitivities of each assay differ: FISH techniques, for example, can detect the fusion gene in 1 in 250 interphase nuclei, while RT-PCR can detect the fusion transcript in 1 in 10^6 cells. Use of these monitoring tests allows detection of minimal residual disease.

**THERAPEUTIC STRATEGIES IN CML**

**Tyrosine Kinase Inhibition**

In the early 1990s, investigators screened large compound libraries for substances with protein kinase inhibitory activity [12]. They subsequently synthesized a series of small phenylaminopyrimidine molecules that could potentially optimize kinase inhibitory activity. CGP57 1488 was identified as a potent molecule that occupies the kinase pocket of the Bcr-Abl protein, blocking access to ATP and thus preventing phosphorylation of any substrate. This molecule was a significant inhibitor of the tyrosine kinase activity of Abl, c-kit (stem cell factor receptor), and the platelet-derived growth factor receptor, without activity on other tyrosine kinases [6]. Since it blocked signal transduction, it was referred to initially as a signal transduction inhibitor (STI571). More recently, it was renamed imatinib mesylate. In preclinical studies, the compound inhibited proliferation of CML cell lines and clonogenic cells from patients with CML without affecting control cells [13].

Phase I studies indicate that tyrosine kinase inhibition with imatinib mesylate is a significant advancement in the treatment of patients with both chronic-phase and blast-phase CML. The drug has significant antileukemic activity and is well tolerated. In a dose-ranging study, imatinib mesylate was administered orally to 83 patients with chronic-phase CML who had failed treatment with IFN-α [14]. Patients were successively assigned to 1 of 14 doses ranging from 25 mg/day to 1,000 mg/day. Adverse effects were minimal, and a maximum-tolerated dose was not identified. Complete hematologic responses were observed in 53 of 54 patients treated with daily doses of ≥300 mg or greater, typically occurring during the first 4 weeks of therapy. Of the 54 patients treated with ≥300 mg, cytogenetic responses occurred in 54%. Complete cytogenetic responses (absence of Ph chromosome) occurred in 13% [14]. A phase II study was designed to further evaluate responses to imatinib mesylate in chronic-phase CML patients. Among 454 patients evaluated, 60% achieved a major cytogenetic response, but only 41% achieved a complete cytogenetic response [15].

Imatinib mesylate has also been found to be active in patients with blast-phase disease, either myeloid blast-phase CML or Ph + ALL. In a dose-escalation pilot study of 58

Table 2. Monitoring treatment efficacy in CML

<table>
<thead>
<tr>
<th>Response</th>
<th>Definition</th>
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<tr>
<td><strong>Hematologic</strong></td>
<td>Reduction in WBC and platelet counts; no evidence of extramedullary disease</td>
</tr>
<tr>
<td><strong>Cytogenetic</strong></td>
<td></td>
</tr>
<tr>
<td>Major</td>
<td>0%-34% Ph^+ cells</td>
</tr>
<tr>
<td>Complete</td>
<td>0</td>
</tr>
<tr>
<td>Partial</td>
<td>1%-34% Ph^+ cells</td>
</tr>
<tr>
<td>Minor</td>
<td>35%-94% Ph^+ cells</td>
</tr>
<tr>
<td><strong>Molecular</strong></td>
<td></td>
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<tr>
<td>RT-PCR-negative</td>
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patients treated with oral imatinib mesylate at doses of 300 mg/day to 1,000 mg/day, responses occurred in 21 of 38 (55%) patients with myeloid blast crisis (CML) and in 14 of 20 (70%) with lymphoid blast crisis (ALL) [5]. Complete hematologic responses occurred in 19% and 20% of these patients, respectively. Among the myeloid blast crisis patients, who responded, 33% continued to receive treatment and remained in remission for durations ranging from 101 to 349 days after starting therapy. All but one patient with Ph+ ALL relapsed. The Food and Drug Administration (FDA) approved imatinib mesylate (Gleevec™; Novartis Pharmaceuticals; Basel, Switzerland) for the treatment of patients with CML in May 2001.

Rationale for Combination Therapy With Imatinib Mesylate

The hematologic remission rate for patients with chronic-phase CML treated with imatinib mesylate is almost 100%, although the complete cytogenetic remission rate is only 41% [14, 15]. This suggests that residual disease remains in many patients. In addition, as with other antineoplastic therapies, resistant clones can emerge in patients treated with imatinib mesylate [16]. Resistance may occur through several mechanisms, including bcr-abl gene amplification or increased drug efflux via a p-glycoprotein-mediated process. The development of point mutations that result in an amino acid change in a critical site of the Abl kinase domain may also contribute to resistance by altering the binding of imatinib mesylate to the tyrosine kinase pocket [16-19]. Consequently, it is reasonable to administer imatinib mesylate in combination with other agents [20].

The antitumor activity of imatinib mesylate has been evaluated in combination with IFN-α, hydroxyurea, daunorubicin, and cytarabine in colony-forming assays of bcr-abl-expressing myeloid cell lines [21]. In these in vitro systems, combinations of imatinib mesylate with another agent were more effective than imatinib mesylate alone. The role of combination therapy with imatinib mesylate will be evaluated in a three-arm multicenter study comparing imatinib mesylate monotherapy with imatinib mesylate plus IFN-α or cytarabine [20]. Additional studies will also evaluate the role of tyrosine kinase inhibition prior to allogeneic bone marrow transplantation.

IFN-α

Treatment with IFN-α is currently the standard, first-line therapy for patients with CML. Several studies involving patients in chronic-phase CML have documented that survival rates are better after treatment with IFN-α than after standard chemotherapy regimens consisting of busulfan or hydroxyurea [3, 22]. IFN-α induces hematologic remission in 70% to 80% of patients and at least partial cytogenetic responses in 20% to 30% [3]. However, only 6% to 20% of patients experience a sustained and complete disappearance of Ph+ cells, a marker that correlates with substantially longer survival periods [23]. Little is known about the mechanism by which IFN-α functions to diminish the percentage of Ph+ cells.

Combination Therapy with IFN-α

Combination of IFN-α, both natural and recombinant, with imatinib mesylate was shown to produce an additive-to-synergistic effect in three human Ph+ leukemia cell lines [21, 23]. These findings suggest that administering imatinib mesylate in conjunction with IFN-α may be a highly effective alternative treatment for CML and may help overcome imatinib mesylate resistance.

The safety, tolerability, and efficacy of low-dose IFN-α administered in combination with imatinib mesylate is being evaluated in a phase I/II clinical study involving patients with chronic-phase CML [24]. Although it is too early for assessment of the cytogenetic response rates, the majority of the patients enrolled in the study have achieved complete hematologic responses. Preliminary safety data from this trial indicate that IFN-α in combination with imatinib mesylate appears to produce a greater myelosuppressive response than therapy with imatinib mesylate alone; several patients required a dose reduction because of hematologic toxicity [24].

Another phase I/II study is being conducted to assess the combination of pegylated IFN-α2b with imatinib mesylate in newly diagnosed patients with chronic-phase CML. Preliminary results are available from 46 patients. The hematologic response rate was 92.7% (86.8% complete response), and there was a high rate of complete cytogenetic response following 6 months of treatment [25]. A majority of patients experienced grade 3/4 hematologic toxicities (58.7%) requiring dose reductions of both the imatinib mesylate and pegylated IFN-α2b components [25].

Farnesyltransferase Inhibition

Increased signal transduction through the Ras family of proteins is particularly common in hematologic malignancies, particularly in the myeloid lineage. This can be as a result of mutations of the ras gene or through constitutive activation, as is the case in CML. The ras gene family is composed of H-, K-, and N-ras [26]. Ras gene products are low-molecular-weight G proteins that are anchored to the plasma membrane and act to switch on a diverse set of cellular activities, including cell cycling, differentiation, and apoptosis. Following synthesis, Ras proteins must undergo a series of posttranslational modifications before they can exhibit their full biologic activity [26]. Among these modifications is the addition of a farnesyl group to the Ras C-terminal cysteine. This modification, catalyzed by farnesyltransferase, is required for localization of the protein to the membrane [26, 27]. Thus, farnesyltransferase
inhibitors have significant therapeutic potential in Bcr-Abl-positive CML [26]. Several newly developed farnesyltransferase inhibitors have shown antitumor activity in preclinical studies, without significant toxicity to normal cells.

Clinical studies have been done with R115777, a selective farnesyltransferase inhibitor that inhibits the farnesylation of both lamin A and B and H- and K-Ras [26]. A phase I dose-escalation study has been completed in 35 adult patients with relapsed, refractory, or secondary acute myeloid or lymphoid leukemias or blast-phase CML. Patients were treated with doses of R115777 ranging from 100 mg to 1,200 mg twice daily for up to 21 days [28]. Dose-limiting central neurotoxicity occurred in patients treated with the maximum dose. Nondose-limiting toxicities included renal insufficiency, paresthesias, and myelosuppression [28]. Clinical responses were associated with a significant reduction in enzymatic activity and occurred in 10 of 34 (29%) patients for whom data could be evaluated, including patients with t(9;22).

SCH66336 is another potent, nonpeptidic, small-molecule inhibitor of farnesyltransferase with demonstrated activity in a murine model of Bcr-Abl leukemia and in vitro activity against CML cells [29]. When murine Bcr-Abl-positive cells are incubated with SCH6636, colony formation is inhibited, proliferation is decreased, the cells become sensitized to apoptotic stimuli, and a G2/M blockade occurs. The latter suggests that centromeric proteins that regulate the G2/M checkpoint must be farnesylated for biologic activity [29]. Experimentally, mice injected with BaF3 cells, a Bcr-Abl-positive cell line, developed acute leukemia with splenomegaly and died within 4 weeks. When treated with SCH6636, however, all animals survived and remained free of disease for more than 1 year [29]. In a murine model of p210 Bcr-Abl-positive ALL, SCH66336 was able to revert early signs of leukemia and significantly prolong survival in treated animals, while all control animals died [30].

Combination Therapy with Farnesyltransferase Inhibitors

Since the farnesyltransferase inhibitors and imatinib mesylate work through different molecular mechanisms, combination therapy might be effective in treating Ph+ leukemias. A recent study showed that SCH6636 inhibits the proliferation of imatinib mesylate-resistant Bcr-Abl-positive cells and inhibits hematopoietic colony formation in cells derived from CML patients who were unresponsive to imatinib mesylate [31]. SCH66336 also sensitized imatinib mesylate-resistant cells to imatinib mesylate-induced apoptosis. Clinical trials evaluating the safety and efficacy of combination therapy with a farnesyltransferase inhibitor and a tyrosine kinase inhibitor are ongoing.

Arsenic Trioxide

Historically, ATO therapy was the first chemotherapeutic intervention for CML. Advancements in molecular biology have clarified the scientific basis for its effectiveness, and, as a result, there is a resurgence of interest in utilizing this agent as therapy for patients with a variety of refractory and relapsed hematologic and solid tumors [32].

The antileukemic activity of arsenic was first reported in the late 1800s. Fowler’s solution (potassium arsenite) was noted to reduce white blood cell counts in normal individuals and in a patient with “leucocythemia” [33]. In the 1930s, the efficacy of arsenic in the treatment of CML established it as a primary therapeutic agent for this disease [34]. Until the advent of modern chemotherapy, arsenic and radiation were the mainstays of treatment for patients with CML. In the early- to mid-1990s, reports from China described the ability of ATO to induce dramatic clinical and hematologic responses in patients with de novo and relapsed acute promyelocytic leukemia (APL) [35-37]. A complete clinical response rate of 90% was reported from one study of 10 patients with relapsed APL [35]. Since approximately 20% to 30% of patients with APL relapse despite treatment with all-trans retinoic acid and combination chemotherapy, this represented a significant advance in the chemotherapy of this disease [38]. Arsenic therapy was not associated with bone marrow suppression and produced only limited side effects. Clinical trials conducted in the U.S. confirmed the results of these observational studies and established the arsenic-induced remission as both clinical and molecular [38, 39]. ATO (Trisenox®; Cell Therapeutics; Seattle, Washington; http://www.ctisecondle.com) was approved by the FDA for the treatment of relapsed/refractory APL in September 2000.

ATO has a number of molecular effects specific for APL. The multiple mechanisms of action of ATO have been shown to induce the loss of the PML/RARα fusion protein, growth cessation, differentiation, and apoptosis with caspase activation of neoplastic cells [38, 40, 41]. However, ATO also inhibits growth and promotes apoptosis of non-APL cells, suggesting that it may be efficacious in Bcr-Abl-positive leukemias as well as a variety of other hematologic neoplasms and solid tumors (Table 3).

Arsenic compounds generate reactive oxygen species, which damage mitochondrial membranes and allow leakage of cytochrome c into the cytosol [42, 43]. As a result, caspases are activated, which degrade cytoplasmic proteins, including the DNA repair enzyme poly-(ADP-ribose) polymerase. These events induce tumor cell apoptosis. Arsenic-induced upregulation of c-Jun NH2-terminal kinase activity may also contribute to apoptosis [44, 45]. Intracellular stores of reduced glutathione protect cells...
from arsenic-induced oxygen toxicity. Modulation of the glutathione redox system can be employed to increase the sensitivity of tumor cells to this novel agent [43, 46]. Other mechanisms that may also contribute to the antitumor activity of ATO in Bcr-Abl-positive leukemias include inhibition of NF-κB activation when ATO binds to the activation loop of 1kB [47].

Preclinical and clinical data support the role of ATO as therapy for Bcr-Abl-positive leukemias. Experimentally, ATO induces apoptosis in Ph⁺ lymphoblasts but not in Ph⁻ lymphoblast cell lines [48]. This activity is independent of the Bcr-Abl tyrosine kinase activity. In ex vivo studies of samples from patients with CML and newly diagnosed myeloid or lymphoid blast crisis, ATO inhibited growth and induced apoptosis of the blasts without influencing colony formation by CD34⁺ hematopoietic progenitor cells. The antitumor effect of ATO is also exhibited in myeloid cell lines resistant to a variety of apoptotic stimuli [49]. In Bcr-Abl-positive myeloid blast cell lines, exposure to clinically achievable concentrations of ATO generated a series of apoptotic events. These included increased caspase activity along with a decline in Bcr-Abl protein levels (via translational inhibition) and without alteration in the levels of other apoptotic proteins [50]. Hyperacetylation of histones H3 and H4 occurred in ATO-exposed cells; this biochemical observation indicates that arsenic has the potential to induce differentiation of primitive CML populations, analogous to the effect of ATO in patients with APL.

Clinical studies in China demonstrated that 2 weeks of treatment with ATO was effective in inducing complete remission in 25 of 34 (74%) patients with CML and partial remission in 7 of 34 (21%) patients [51]. Duration of the remissions ranged from 30 to 60 days (median 58 days). In this population, ATO therapy did not cause myelosuppression or significant cardiac, hepatic, or renal dysfunction.

\textbf{Combination Therapy With ATO}

The fact that ATO-induced apoptosis appears to be independent of the activity of the Bcr-Abl tyrosine kinase suggests that combined therapy with ATO plus imatinib mesylate might have synergistic activity in patients with Bcr-Abl-positive leukemias. In cell culture studies, the combination of the two agents induced an additive apoptotic response in a Bcr-Abl-positive human myeloid leukemia cell line, HL60/bcr-abl, which was greater than the response with either agent alone (p < 0.05) [52]. Furthermore, cotreatment of K562 and MO7p210 cells with approximately equipotent doses of ATO and imatinib mesylate inhibited cell proliferation by up to 80%; the combination was also significantly more potent than imatinib mesylate alone in inhibiting colony formation, thus suggesting synergy between these two agents [53]. Combination therapy also resulted in greater reductions of Bcl-X\textsubscript{L}, XIAP, and AKT expression, a decrease in AKT tyrosine kinase activity, and degradation of GATA-1 (Fig. 1). These proapoptotic properties support clinical studies of combination therapy with these agents in patients with Bcr-Abl-positive leukemias. ATO has recently received orphan drug designation from the FDA for the treatment of CML, and several clinical studies are planned.

\textbf{Conclusion}

Molecular targeting of signal transduction molecules in patients with Bcr-Abl-positive tumors has led to a revival in the use of chemotherapy for chronic-phase and blast-crisis CML. Inhibition of Bcr-Abl tyrosine kinase-induced signal transduction by imatinib mesylate can lead to hematologic remissions in patients with Bcr-Abl-positive leukemias, but many patients do not achieve a cytogenetic remission, and molecular remissions are rare. Furthermore, relapse eventually occurs in large numbers of patients with advanced disease. Among the promising treatment options under exploration are the farnesyltransferase inhibitors and ATO. The antitumor activity of ATO has been evaluated in patients with relapsed and refractory APL and in vivo and in vitro studies. These studies suggest that ATO affects metabolic pathways different from those influenced by tyrosine kinase and farnesyltransferase inhibitors. An important observation is that the reduction of endogenous Bcr-Abl tyrosine kinase activity in response to ATO appears to sensitize Ph⁺ cells to the activity of imatinib mesylate.

As with many other human tumors, combination therapy directed at alternate pathways and at both cycling and noncycling cells has a rational basis in CML. Both basic science and clinical studies support the clinical evaluation of combinations of imatinib mesylate with farnesyltransferase inhibitors and with ATO for the treatment of patients with Bcr-Abl-positive leukemias. Combination therapy is anticipated to increase cytogenetic and molecular remission rates for patients with chronic-phase CML and to improve response rates for patients with accelerated or blast-phase CML.
Figure 1. Mechanisms of action of ATO and imatinib mesylate in CML. Combined treatment of Bcr-Abl-positive leukemia cells with imatinib mesylate and ATO leads to cytochrome c release and caspase-mediated apoptosis by A) lowering both the levels and activity of Bcr-Abl; B) enhancing ATO-induced downregulation of antiapoptotic factors, Bcl-xL, and XIAP, and C) downregulation of inhibitors of proapoptotic factors, such as AKT.

Acknowledgment

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