New Drug

Dasatinib: A Tyrosine Kinase Inhibitor for the Treatment of Chronic Myelogenous Leukemia and Philadelphia Chromosome–Positive Acute Lymphoblastic Leukemia

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ABSTRACT

Background: The Philadelphia chromosome is formed from a translocation of genetic material involving human chromosomes 9 and 22. The resulting gene product, BCR-ABL, encodes for an abnormal tyrosine kinase (TK) that is a factor in the pathology of chronic myelogenous leukemia (CML). Use of targeted therapy that inhibits BCR-ABL kinase activity may lead to hematologic and cytogenetic responses in affected individuals. The oral TK inhibitor dasatinib was approved in 2006 for use in patients with CML or Philadelphia chromosome–positive acute lymphoblastic leukemia (ALL) who are unable to tolerate or have not responded to other treatments.

Objective: This paper reviews the available data on dasatinib, including its pharmacokinetic and pharmacodynamic properties, findings of in vitro and in vivo studies, adverse effects, and potential place in therapy.

Methods: Pertinent information was identified through searches of MEDLINE (1966–May 2007), EMBASE (1980–first quarter 2007), and International Pharmaceutical Abstracts (1970–May 2007) using the terms dasatinib, BMS-354825, chronic myelogenous leukemia, Sprycel, Philadelphia chromosome, and acute lymphoblastic leukemia. All clinical studies and case reports published at the time of the search were included in this review.

Results: Observed mutations in the amino acid sequence of BCR-ABL cause the failure of treatment with existing TK inhibitors. Dasatinib has shown in vitro and in vivo activity against BCR-ABL, including mutations that are resistant to other available TK inhibitors. Preliminary results are available from several noncomparative studies of dasatinib in patients who were unable to tolerate or were resistant to previous therapeutics. The 5 phases of START (SRC/ABL Tyrosine kinase inhibition Activity Research Trials of dasatinib) represent the largest and most comprehensive evaluation of dasatinib in the treatment of patients in all stages of CML or Philadelphia chromosome–positive ALL who had undergone previous treatment for leukemia. Dasatanib had the greatest benefit in patients in the chronic phase of CML, with complete hematologic responses in 90% of patients, 52% of whom achieved a major hematologic response. Compared with those in the chronic phase, patients in the accelerated phase or blast crisis of CML, or with Philadelphia chromosome–positive ALL had lower responses. In the START-R trial, which compared the response to dasatinib and high-dose imatinib (800 mg/d), both regimens had comparable ability to induce a complete hematologic response (95% and 93%, respectively), although more patients achieved a major cytogenetic response with dasatinib (32% vs 7%). Adverse effects include significant myelosuppression. Dasatinib may have the potential for use in the management of nonleukemic malignancies.

Conclusions: Dasatinib has a wider spectrum of activity against a broader range of BCR-ABL forms than existing TK inhibitors. It has shown clinical benefit and tolerability in patients in all phases of CML, as well as in those with Philadelphia chromosome–positive ALL. Dasatinib illustrates the potential for targeted drug development based on an understanding of the genetic alterations leading to CML and the development of

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INTRODUCTION
The focus of research in many types of cancers is to gain molecular insights into the genetic aberrations that result in malignant transformation, with the goal of developing targeted treatments that are capable of maximizing destruction of malignant cells and minimizing damage to normal cells. The inherent plasticity of cancer-cell genetics complicates the development of targeted therapy. The application of new technologies allows better understanding of the interactions between therapeutic agents and their targets, leading to purposeful design of novel molecules rather than serendipitous discovery.

Chronic myelogenous leukemia (CML) is a disorder of hematopoietic stem cells that results in uncontrolled myeloproliferation. The disease was first described in 1845 by 3 pathologists, Bennet, Craigie, and Virchow, working independently. In 1960, Peter Nowell and David Hungerford identified the molecular cause of the disease through discovery of the Philadelphia chromosome (Ph), named after the city in which it was identified. This was the first time that a chromosomal rearrangement was linked to development of a specific cancer. The Ph is created through translocation of a section of human chromosome 9 that contains the Abelson (ABL) kinase domain, with a specific breakpoint cluster region (BCR) on chromosome 22. This results in the gene product BCR-ABL, which is a constitutively active oncogenic tyrosine kinase (TK). This kinase activity gives the transformed stem cells the ability to hyperproliferate and eventually to be released into the periphery as differentiated leukemic white blood cells. Although not universally found in patients with CML, BCR-ABL is found in >90% of individuals with CML, as well as in 10% to 15% of patients with acute lymphoblastic leukemia (ALL). Besides the Ph, additional genetic defects have been observed in >80% of patients in the blast crisis (BC) phase of CML, which is the most aggressive phase of the disease.

CML is a disease of the elderly; the median age at diagnosis is 65 years. The diagnosis is often made when the finding of leucocytosis leads to additional evaluation and subsequent identification of immature blasts and promyelocytes. The disease is classified into 3 phases according to the level of clonal expansion of blast cells and promyelocytes in the peripheral blood and bone marrow. The chronic phase (CP) is the early stage of the disease and involves a lower level of myeloproliferation than in the advanced stages. Of the ~4600 patients diagnosed with CML in the United States each year, >90% are in the CP. Although patients are generally asymptomatic, expansion of the CML clone may lead to development of malaise, weight loss, and an enlarged spleen. Patients may remain in the CP for 3 to 5 years. The accelerated phase (AP) involves a more aggressive form of CML marked by genetic instability of the clone. The defining criteria of the AP may include the presence of >10% to 15% blasts, 20% to >30% blasts plus promyelocytes, >20% basophils, or a non-treatment-related platelet count of <100 x 10^9/L. The absence of physical symptoms denoting the progression to the AP makes this step difficult for patients to identify. The AP generally lasts between 4 and 6 months before progression to BC, which is the most aggressive form of CML. This phase is characterized by >30% blasts in the bone marrow or peripheral blood. Extramedullary sites of blast-cell proliferation also may be present. Patients in BC may have fever, night sweats, weight loss, anorexia, and fatigue. Splenomegaly is often present, and patients may have bone pain and show signs of infection. The median survival of patients in BC is 3 to 6 months. Criteria from the International Bone Marrow Transplant Registry and the World Health Organization used to diagnose CML progressing to AP and BC are summarized in Table I.

ALL generally occurs in children, but ~20% of cases are in adults. Although several genetic abnormalities are observed in patients with ALL, the Ph is present in 15% to 25% of adults and 2% to 6% of children with ALL and indicates a poor prognosis. Morphologic criteria and immunophenotyping are used to differentiate the types of ALL. As in CML, symptoms are nonspecific and are related to the inability to produce adequate quantities of platelets, red blood cells, and white blood cells. Multiple chemotherapy drugs are typically used in the treatment of ALL to restore normal hematopoiesis, prevent emergence of resistant clones, prevent distribution of disease to sanctuary sites such as the central nervous system and testicles, and eliminate minimal residual disease through prolonged treatment. Conventional
Table I. Criteria for the diagnosis of accelerated and blast crisis chronic myelogenous leukemia (CML).

International Bone Marrow Transplant Registry criteria

Chronic
- Morphologic and cytogenetic findings consistent with CML
- No features of accelerated phase or blast crisis

Accelerated (requires ≥1 of the following)
- >10% Blasts in peripheral blood or bone marrow
- >20% Blasts plus promyelocytes in blood or bone marrow
- >20% Basophils plus eosinophils in peripheral blood
- Thrombocytopenia unresponsive to therapy
- Persistent thrombocytosis
- Increasing spleen size
- Rapid doubling of white blood cell count in <5 days
- Appearance of additional cytogenetic abnormalities
- Development of chloromas or myelofibrosis

Blast crisis
- >30% Blasts plus promyelocytes in blood or bone marrow

World Health Organization criteria

Chronic
- Morphologic and cytogenetic findings consistent with CML

Accelerated (requires ≥1 of the following)
- 10%-19% Blasts in peripheral blood or bone marrow
- >20% Basophils in peripheral blood
- Platelet count <100 x 10^9/L unrelated to therapy or >1000 x 10^9/L unresponsive to therapy
- Increasing spleen size
- Increasing white blood cell count unresponsive to therapy
- Appearance of additional genetic abnormalities not present at diagnosis
- Megakaryotic proliferation and/or severe granulocytic dysplasia

Blast crisis (requires ≥1 of the following)
- >20% Blasts in peripheral blood or bone marrow
- Extramedullary blast proliferation
- Large clusters of blasts in bone marrow

Chemotherapy is associated with long-term survival of <10% of patients with Ph-positive ALL. In recent years, imatinib* has been used with some success to treat this form of leukemia.8,9 However, given the therapeutic limitations of imatinib, there is a need for new drugs with similar mechanisms of action but more potent inhibition of the TK activity of BCR-ABL.

This paper reviews the available information on the oral TK inhibitor dasatinib,† including its pharmacokinetic and pharmacodynamic properties, findings of in vitro and in vivo studies, adverse effects, and potential place in therapy.

METHODS
Pertinent information was identified through searches of MEDLINE (1966–May 2007), EMBASE (1980–first quarter 2007), and International Pharmaceutical Abstracts (1970–May 2007) using the terms dasatinib, BMS-354825, chronic myelogenous leukemia, Sprycel, Philadelphia chromosome, and acute lymphoblastic leukemia. All clinical studies and case reports published at the time of the search were included in this review. The reference lists of identified articles were...
scanned for additional publications. Abstracts from annual meetings of the American Society of Hematology were also reviewed.

TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA

Nonspecific treatments for CML have involved drug regimens that include interferon, as well as procedures such as allogeneic stem-cell transplantation. The latter has been the only curative treatment, although donor availability and iatrogenic complications of this method limit its applicability.

Imatinib

Imatinib has been found to have antileukemic activity in patients with CML and Ph-positive ALL, and was the first agent found to be clinically effective in specifically inhibiting a genetic abnormality that plays a role in malignant transformation. It acts as a competitive inhibitor at the adenosine triphosphate (ATP) binding site of BCR-ABL, preventing tyrosine phosphorylation and downstream signaling, leading to growth arrest and apoptosis. Imatinib is currently considered a first-line agent for newly diagnosed CML. Its availability in an oral formulation avoids the need for intravenous access and allows in-home administration.

Despite the durable cytogenetic responses obtained in patients with CML, limitations in imatinib’s spectrum of activity have become apparent. Disease relapse has been observed after an initial response to imatinib, particularly in patients whose treatment was initiated during the AP or BC. Relapse during imatinib treatment is most often related to mutations occurring within the kinase domain of BCR-ABL. These point mutations, occurring at >40 different amino acid positions, have been found to account for acquired resistance in 50% to 90% of patients. When imatinib is started in the CP, resistance has been predicted to occur at a rate of 4%, higher than in the advanced stages. The point mutations may either disrupt critical interactions between imatinib and BCR-ABL or prevent the kinase from adopting an inactive conformation. Formation of the inactive conformation can be accomplished by stabilizing the activation loop of the open conformation or destabilizing the molecule while in the closed conformation. Either way, the result is reactivation of BCR-ABL, which leads to downstream signaling of malignant processes and proliferation of clones containing the abnormal kinase. Other proposed mechanisms of resistance to imatinib include amplification of the BCR-ABL gene, overexpression of BCR-ABL mRNA, increased efflux of imatinib via P-glycoprotein-mediated actions, and activation of additional proteins, such as those belonging to the sarcoma (SRC) family of kinases.

A description of the structure of BCR-ABL may be helpful in understanding the activity of agents that inhibit this gene. Tokarski et al described ABL as typical of other kinases in having a protein fold separated by 2 subdomains, or lobes. The NH2 terminal lobe contains a 5-stranded β-sheet with an α-helix called α-C. The carboxyl terminal lobe is the larger of the 2 lobes and is predominantly helical in form. A polypeptide strand connects the 2 lobes and acts as a hinge that allows the lobes to rotate relative to each other on binding a substrate such as ATP, which would trigger activation of the kinase. The ATP binding site is actually a deep cleft located behind a highly flexible loop, referred to as the activation P-loop. Crystallographic analysis of imatinib bound to the ABL kinase has indicated that imatinib is able to bind to BCR-ABL only when the P-loop is in the closed, or inactive, conformation. As mentioned previously, resistance to imatinib has been found to occur due to variations in amino acids within the kinase domain that impair the ability of the kinase to adopt the closed form, preventing imatinib from imparting its inhibitory action. Although these amino acid variations reduce sensitivity to imatinib by 3- to >100-fold, BCR-ABL is still able to bind ATP and perform its aberrant activities.

The nomenclature used to identify these variations is based on peptide analysis. Letters are assigned to each of the 20 amino acids used in protein structures. The wild-type, or nonmutated, amino acid letter designation is followed by the residue number of the amino acid in the protein structure. The letter of the substituted amino acid acts as a suffix. For example, in the case of the T315I mutant of BCR-ABL, threonine (T) in position 315 is substituted for isoleucine (I). These amino acid variations impart different characteristics to the protein in relation to chemical interactions between neighboring atoms. These alterations can affect different actions, such as enzymatic activity or the ability to bind substrate.

Branford et al found evidence that individual point variations are responsible for the development of imatinib-resistant CML and Ph-positive ALL. Of 18 patients with imatinib-resistant disease, 12 had...
amino acid variations in the ATP binding site of BCR-ABL. These variations were not detected before the initiation of imatinib therapy in 25% of the 12 patients, indicating that the variations developed during treatment. Furthermore, these variations occurred in, or adjacent to, the ATP binding site, leading to disruption between imatinib and the residues to which it binds. The plasticity of the amino acid structure of BCR-ABL forms the basis for the evaluation of patients with Ph-positive disease who may be considered for treatment with an agent that inhibits TK activity. This instability also illustrates the need for a variety of agents that target BCR-ABL and its potential mutant forms.

**Dasatinib**

Dasatinib is an SRC-family TK inhibitor that is structurally distinct from imatinib. It has activity against many of the mutant forms of BCR-ABL that are resistant to imatinib, suggesting promise in the treatment of CML and Ph-positive ALL. Dasatinib's broader spectrum of activity derives from its ability to bind to both the inactive form of BCR-ABL (as does imatinib) and the active form. This expanded activity results from dasatinib's requiring less sensitive molecular interactions and thus fewer restrictions for binding.

**CHEMICAL STRUCTURE OF DASATINIB**

Lombardo et al performed the chemical manipulations that led to the creation of the dasatinib molecule, whose chemical structure is illustrated in Figure 1. The SRC proto-oncogene is believed to play a role in the development of several human cancers, contributing to cellular proliferation, adhesion, invasion, and motility. Dasatinib was selected from several compounds tested based on x-ray crystallographic analysis indicating a high potential for interactions consistent with those of other compounds having favorable kinase-inhibitory activity. The chemical name of dasatinib is N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl] amino]-5-thiazolecarboxamide, and its molecular formula is C_{22}H_{26}ClN_{7}O_{2}S·H_{2}O. Dasatinib is a piperazinyl ethanol with a molecular weight of 506.02 in the monohydrated form.

**MECHANISM OF ACTION**

SRC-family kinases, such as SRC and LYN, constitute a group of enzymes that, like BCR-ABL, modulate signal transduction by phosphorylating tyrosyl residues of molecules such as endothelial growth factor receptor (EGFR), human epidermal receptor type 2, platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), and vascular endothelial growth factor receptor (VEGFR). SRC kinases have also been found to have oncogenic activities in cancer cell lines from tumors involving the colon, breast, pancreas, lung, and brain. Dasatinib has been found to inhibit kinases that include BCR-ABL, SRC, LCK, YES, FYN, c-KIT, ephrin (EPH) receptor-A2, and PDGFR. Dasatinib is capable of binding to these kinases, inhibiting their autophosphorylation and downstream phosphorylation of additional targets, and thus blocking the oncogenic activities that would otherwise result.
IN VITRO ACTIVITY

The therapeutic limitations of imatinib (development of resistant BCR-ABL variants, intolerable adverse effects) pointed to a need for therapeutic alternatives in patients with CML or Ph-positive ALL and led to in vitro testing of dasatinib against imatinib-resistant mutations. Shah et al examined the ability of dasatinib to inhibit proliferation of imatinib-resistant mutants, as well as of the wild-type form of the kinase. Kinase activity in Ba/F3 cells expressing each of the 15 BCR-ABL variants was assessed after exposure to dasatinib at concentrations ranging from 1 to 10,000 nmol/L. Fourteen of the mutants were sensitive to dasatinib within the range from 1 to 10 nmol/L. Activity at these low concentrations suggests good potency at serum levels achievable with oral administration. Despite this broad activity against many mutants, kinase activity of the T315I mutant was preserved at the much higher dasatinib concentration of 10,000 nmol/L. The imatinib-resistant mutation involving substitution of threonine for isoleucine at position 315 of the BCR-ABL peptide chain was therefore thought to confer resistance to dasatinib as well. These investigators also observed variation in the sensitivity of various other mutations to dasatinib. For example, compared with the wild-type form of BCR-ABL, the F317L mutant required 3- to 5-fold higher concentrations of dasatinib, whereas the Q252R mutant was more sensitive to dasatinib activity. Moving from cell culture to a mouse model, the investigators injected Ba/F3 cells containing the same BCR-ABL mutants into severe combined immunodeficiency mice. After confirmation of extensive disease, a 2-week course of dasatinib 10 mg/kg was administered. Consistent with the results of the in vitro studies, all BCR-ABL mutants were susceptible to dasatinib except those containing the T315I mutation, which did not respond significantly. In a follow-up experiment, mice prepared in the same manner but injected with either wild-type BCR-ABL or the T315I or M351T mutant were assessed for survival after exposure to dasatinib. Dasatinib prolonged survival, except in the mice injected with the T315I mutant.

O’Hare et al compared the ability of dasatinib and imatinib to block the phosphorylating activity of BCR-ABL using Ba/F3 cells transfected with mutations in the kinase domain that imparted known imatinib resistance. They found that dasatinib had a phosphorylation-inhibiting potency ~325-fold that of imatinib in the wild-type BCR-ABL isoform. Dasatinib inhibited all mutants except T315I at half-maximal inhibitory concentrations (IC50) <1.7 nmol/L. Therefore, dasatinib was thought to be a potent, concentration-dependent inhibitor of BCR-ABL autophosphorylation and of BCR-ABL phosphorylation of other substrates. When these investigators evaluated the ability of dasatinib to block proliferation of Ba/F3 cells expressing these same mutations, dasatinib was found to have similar potency against all mutants except T315I, which was resistant to both imatinib and dasatinib.

To explain the observed resistance of the T315I mutant and better understand the interaction of dasatinib with BCR-ABL, it is helpful to consider the structure that results from the amino acid variation. As stated earlier, the T315I mutant is created by a nucleotide substitution of isoleucine for threonine at position 315 of the peptide chain. The original threonine residue provides an oxygen atom critical to the formation of a hydrogen bond with imatinib, allowing subsequent inhibition of the kinase activity of BCR-ABL. Isoleucine contains a hydrogen atom in the side chain, resulting in a steric clash with imatinib that interferes with imatinib binding and prevents enzyme inhibition and continued kinase activity. The alteration in atomic interactions occurring in the T315I mutant is probably associated with the observed interruption in binding and resistance to TK inhibitors, including dasatinib.

The in vitro activity of dasatinib extends beyond that of simply inhibiting BCR-ABL. Dasatinib has also been tested for its ability to inhibit the SRC kinase family, which is believed to be responsible for regulating signals from cell surface proteins, including integrins, growth factors, and others. SRC is also believed to play a role in mediating malignant transformation, proliferation, survival, angiogenesis, and motility. Based on the finding that SRC is present in epithelial tumors, Johnson et al sought to determine the potential for dasatinib to induce antitumor effects in head and neck squamous cell carcinoma and non-small cell lung cancer (NSCLC) cell lines. These investigators found that exposing the cells to dasatinib 100 nmol/L for 24 hours resulted in moderately impaired levels of apoptosis, inhibition of cell migration and invasion, and potent suppression of SRC activation. They suggested that dasatinib blocked SRC from sending downstream signals to other proteins, such as focal adhesion kinase (FAK), paxillin, p130Cas, MAPK, signal transducer and activator of transcription (STAT) 5, STAT3, AKT, and p27, that are responsible...
for the malignant activities characteristic of cancer cells. Although the in vitro results seemed promising, IC₅₀ values >10,000 nmol/L were required to block SRC and produce the desired effects in some of the NSCLC cell lines. Therefore, although dasatinib has an ability to inhibit multiple kinases, the high concentrations required to affect some of these enzymes may limit its therapeutic role because of the multiple pathways responsible for the characteristics of many cancer cell lines.

The structural similarity of the active conformations of the ABL and SRC family kinases led Nam et al. to evaluate the antikinase activity of dasatinib in an androgen-independent human prostate cancer cell line, DU-145. They found that dasatinib 100 nmol/L was able to inhibit the in vitro phosphorylation of FAK by LYN and SRC kinases in these prostate cancer cells. The researchers suggested that dasatinib's inhibitory activity prevents downstream signaling of malignant cell function, such as that which occurs with FAK-mediated secretion of matrix metalloproteinase-9, which is involved in facilitating cell invasion.

Dasatinib has been applied to other lung cancer cell activities. For example, the EGFR contains a kinase domain that selectively activates AKT and STAT pathways that are an integral component of lung cancer development. These EGFR-dependent actions are present in many mutant lung cancer cell lines, but are not present in the wild type. Inhibition of these EGFR-dependent cells can facilitate apoptosis and thus may be a mechanism of cancer management. Song et al. evaluated the potential of dasatinib, as a known SRC inhibitor, to provide antitumor activities in human NSCLC cell lines containing EGFR mutations. Although the wild-type cell lines were found to have IC₅₀ values >10 mmol/L and were deemed resistant to dasatinib, all mutants had IC₅₀ concentrations of between 100 and 250 nmol/L, indicating the potential for in vivo susceptibility to dasatinib. These authors found that dasatinib induced apoptosis and inhibited DNA synthesis in these mutant cells that required EGFR activity. This effect may be the result of direct action of dasatinib on EGFR or of kinase inhibition of SRC imparted by dasatinib that blocks EGFR activation.

Schittenhelm et al. studied the activity of dasatinib against the KIT-receptor TK. Activated mutations of KIT have been found in some human cancers, such as mast cell disorders, seminomas, acute myelogenous leukemia, and gastrointestinal stromal tumors (GIST). Imatinib, which is a KIT inhibitor, has been used to treat patients with GIST, but mutations in the activation loop may cause resistance. Using concentrations achievable with oral administration, these authors found that dasatinib inhibited wild-type KIT and imatinib-resistant forms that are dependent on KIT activity. Therefore, dasatinib may play a role in managing malignancies involving mutations in the KIT activation loop.

The role of dasatinib has also been studied in the treatment of multiple myeloma. In addition to PDGFR and SRC kinases, EPH-receptor kinases are believed to have roles in tumor cell activities. Deng et al. exposed in vitro myeloma cells to dasatinib. IC₅₀ values were <100 nmol/L in 7 of 15 myeloma cell lines tested. Cells were likely to be sensitive to dasatinib if they had increased baseline expression of genes responsible for proliferation and anti-apoptosis, such as MAF, MAFF, NFYC, PML, and YY1. Dasatinib responsiveness was also predicted by the presence of other genes for cell surface receptors (eg, EPH receptor-B4 and CXCR4) and the proteosome subunits PSMC3, PSMD12, and PSME2, as well as the apoptotic regulators CIAP1 and IKKe. These studies emphasize the myriad factors guiding tumor growth, and the effect kinase inhibition may play in disease management.

Aside from the role of dasatinib in treating various malignancies, the compound has also been found to block the PDGFR kinase in vascular smooth muscle cells. PDGF is believed to play a pathologic role in causing restenosis of coronary arteries after vascular injury. Either inhibiting PDGF or blocking the PDGFR is thought to suppress restenosis. Chen et al. studied the ability of dasatinib to inhibit PDGFRB TK and reduce PDGF-stimulated migration and proliferation of vascular smooth muscle cells. A dasatinib IC₅₀ of 3 nmol/L was found to reversibly inhibit the activity of PDGF-stimulated A10 cells. The reversibility of this action is important, as PDGF-mediated restenosis activity peaks shortly after vascular injury and then slowly declines. Selective exposure to dasatinib for a period after such procedures as coronary angioplasty and stenting may have therapeutic value, while reducing the risk of vascular damage caused by excessive PDGFR inhibition.

**Pharmacokinetics**

After oral administration of dasatinib, Cmax is reached within 0.5 to 6 hours. Dose-proportional increases in AUC are observed at doses between 15 and
240 mg/d. Although a 14% increase in AUC may occur in patients taking dasatinib with a high-fat meal, this effect does not appear to be clinically relevant. Concomitant administration of antacids containing aluminum hydroxide or magnesium hydroxide may result in ~60% reductions in the dasatinib AUC and C\textsubscript{max}, as a result of the pH-dependent solubility of dasatinib. Administering dasatinib 2 hours after an antacid does not affect the AUC, but the C\textsubscript{max} may be increased by as much as 26%. The distribution of dasatinib is extensive, with a V\textsubscript{d} of ~2500 L. Approximately 96% of dasatinib is bound to plasma proteins.\textsuperscript{26}

Dasatinib is metabolized to an active metabolite and inactive metabolites by the cytochrome P450 (CYP) 3A4 isozyme.\textsuperscript{26} The active metabolite does not appear to play a significant role in dasatinib's total therapeutic activity. Dasatinib has weak inhibitory activity against CYP3A4, which carries the potential for decreased clearance of other drugs metabolized by this enzyme. The overall mean terminal t\textsubscript{1/2} of dasatinib is 3 to 5 hours. Concomitant administration of drugs that inhibit CYP3A4, such as azole antifungals, macrolides, and antiretrovirals, may lead to an increase in dasatinib exposure that could increase the risk of toxicity. Similarly, concomitant administration of CYP3A4 inducers may lead to a >80% reduction in dasatinib exposure. The impact of these pharmacokinetic alterations on the therapeutic activity of dasatinib is unknown. Dasatinib is primarily eliminated in feces as products of metabolism.\textsuperscript{26}

The prescribing information for dasatinib states that there are no clinically relevant data to suggest that dose changes are necessary based on sex or advanced age, or that there may be pharmacokinetic differences based on race.\textsuperscript{26} No clinical studies have been published regarding the use of dasatinib in patients with renal or hepatic insufficiency.

**PHARMACODYNAMICS**

The dasatinib molecule was synthesized with the intention of targeting kinases involved in signaling pathways responsible for oncogenesis. Inhibition of these kinases may impede cellular activities that favor malignant processes. Because dasatinib is considered a targeting molecule, numerous analyses have been performed to predict and explain its actions. In generating the dasatinib molecule, Lombardo et al\textsuperscript{3} considered the structure of other kinases (eg, LCK) and the actions of other kinase inhibitors (eg, imatinib). Therefore, using 2-acylamino-5-cardoxamidothiazoles as their base, they were able to evaluate a variety of compounds, looking for one that would maximize the assumed potential for antiproliferation via inhibition of SRC/ABL kinases. To best understand the mechanism by which dasatinib performs its inhibitory functions, Tokarski et al\textsuperscript{4} used x-ray crystallography to visualize the binding of dasatinib to ABL kinase within the P-loop. This P-loop is the site of phosphate transfer for the kinase and a target binding site for TK inhibitors such as dasatinib (Figure 2).\textsuperscript{4} These investigators suggested that based on interactions between the 2 molecules, dasatinib had an advantage in binding to ABL. Whereas interactions with imatinib cause the P-loop to fold back on itself toward the ATP binding site, resulting in the need for an inhibitory conformation for imatinib docking, bound dasatinib has different interactions that allow the agent to bind to either the inactive or active form of the kinase. These differences in steric interactions are important, not only because they increase dasatinib’s availability to bind to ABL in the active or inactive states, but also because variations created by substitution of amino acids through mutations occurring in the kinase domain are less likely to affect dasatinib than they are to affect imatinib. The reduction in restrictions for adequate dasatinib binding help explain dasatinib’s potential as a broad-acting kinase inhibitor with enhanced potency relative to other known ABL inhibitors.\textsuperscript{4} Currently, the T315I mutation is the only known mutation of BCR-ABL with resistance to dasatinib. Computer models have predicted this resistance to occur based on the structural alterations that impair critical binding of dasatinib to BCR-ABL and may result in increased steric bulk.\textsuperscript{4}

Computer imaging of dasatinib bound to different conformations of ABL has been used to assess the predicted roles of particular amino acids and structural features of the kinase. Gambacorti-Passerini et al\textsuperscript{34} found that dasatinib binds the inactive, or closed-loop, conformation of ABL, forming several hydrogen bonds with peptide residues, resulting in a snug fit. These investigators also predicted the ability of dasatinib to bind effectively to another form of ABL, termed the intermediate conformation, which contains an open activation loop similar to that in the active enzyme. The binding model of Gambacorti-Passerini et al predicted that dasatinib was likely to be more active against the inactive and intermediate forms of ABL than against the active open-looped form. This was because of the
Figure 2. ABL kinase-dasatinib complex. Part A shows ABL kinase (the 3-dimensional ribbon structure) binding dasatinib (the stick structure). Part B shows a cutaway view of dasatinib and nearby residues of the adenosine triphosphate binding site. Reprinted with permission from Tokarski et al. Cancer Res. 2006; 66:5790–5797.

electrostatic characteristics of residues contained in a sequence of amino acids termed the DFG motif, found in many protein kinases, that can alter the fit and sensitivity to dasatinib. These investigators cited the T315I mutant to support this theory, noting the lack of involvement of T315 in binding ATP. However, the importance of the T315I mutation in dasatinib resistance suggests that dasatinib’s activity is against the intermediate and inactive forms rather than against the active kinase, because dasatinib does not act as a true inhibitor of ATP binding. Therefore, the additional interaction with the intermediate form of ABL may explain dasatinib’s advanced activity against imatinib-resistant mutants.

Lombardo et al. conducted affinity studies as part of their evaluation of dasatinib’s ability to bind and inhibit BCR-ABL and SRC. Measured inhibition constant (SD) values against BCR-ABL and SRC were 30 (22) pmol/L and 16 (1.0) pmol/L, respectively. To assess dasatinib’s broader potential, the group also studied the selectivity of dasatinib for a variety of other kinases. Significant activity (IC50 values <30 nmol/L) was found against LCK, c-KIT, and PDGFRβ, with >100-fold selectivity against additional kinases, such as p28, human epidermal growth factor receptor (Her) 1, Her2, FGFR-1, MEK, VEGFR-2, cyclin-dependent kinase 2, IKK, AKT, FAK, insulin-like growth factor–1 receptor, IR, MK2, and protein kinase C (PKC)–α, PKC-δ, PKC-τ, and PKC-ζ.

EFFICACY

Although dasatinib is purported to have broad-spectrum kinase-inhibiting activity, clinical research has centered mainly on its inhibition of BCR-ABL TK in Ph-positive CML and ALL. The treatment of patients with CML has 2 aims: to achieve a hematologic response and to achieve a cytogenetic response. During the CP, a complete hematologic response (CHR) is defined as normalization of peripheral blood counts and absence of all signs and symptoms of
leukemia; the absence of a hematologic response is termed no response. During the AP, myeloid BC, or Ph-positive ALL, a major hematologic response (MHR) is considered a CHR if there are <5% blasts in the bone marrow, normalization of blood counts, and no other signs and symptoms of leukemia; an MHR may also include patients with no evidence of leukemia but without normalization of platelet or white blood cell counts. A minor hematologic response consists of <15% of blasts in bone marrow or peripheral blood, <30% blasts plus promyelocytes in bone marrow and peripheral blood, <20% basophils in peripheral blood, and no extramedullary involvement other than the spleen or liver. Progression refers to movement from the AP to BC, or when the number of blasts increases after at least 4 weeks of treatment in patients who are in BC. A complete cytogenetic response (CCR) requires the absence of Ph in the bone marrow. A partial cytogenetic response (PCR) is defined as 1% to 35% Ph-positive metaphases, a minor cytogenetic response is 36% to 65% Ph-positive metaphases, a minimal cytogenetic response is 66% to 95% Ph-positive metaphases, and no response is >95% Ph-positive metaphases. A major cytogenetic response (MCR) includes those patients who have achieved either a CCR or PCR.

Abruzzese et al reported the case of a 67-year-old man with Ph-positive ALL who relapsed with extramedullary disease after he had received chemotherapy plus imatinib. At the time of the original diagnosis, the patient had 70% BCR-ABL-positive metaphases, but achieved remission with standard chemotherapy that included intrathecal treatment. Seven months after the diagnosis, during the first remission, imatinib therapy was initiated at 400 mg/d. Four months later, the dose was increased to 600 mg/d after lymphoblasts were identified in a lumbar puncture specimen. A second remission was achieved within 3 months, but was lost 6 months later with the development of a scalp lesion containing infiltrates of precursor B lymphoblastic leukemia. Examination of the bone marrow at that time noted 45% blasts with 40% Ph-positive metaphases. Dasatinib was started at this point (dose not reported), and the scalp lesion was completely eliminated within 4 weeks. By month 5 of dasatinib treatment, the patient was deemed to be in complete remission, with no signs of even extramedullary disease and an absence of Ph in the bone marrow. Despite its value as an early case of a response to dasatinib after the failure of imatinib, this report was limited by a lack of data on the duration of the response to dasatinib.

Talpaz et al performed a Phase I, open-label, prospective study evaluating the activity of various doses of dasatinib (range, 15 mg QD–120 mg BID) in the treatment of adult patients with CML or Ph-positive ALL who were resistant to or unable to tolerate imatinib treatment. The primary objective of the study was to determine the safety and tolerability of dasatinib, with evaluation of its pharmacokinetics and antileukemic activity as secondary objectives. Patients were evaluated for the specified outcomes for at least the first 12 weeks of therapy and potentially every 3 months thereafter while treatment continued. The study enrolled 84 patients (40 CP, 11 AP, 23 BC, and 10 Ph-positive ALL) ranging in age from 15 to 79 years; 72 had imatinib-resistant disease. Sixty patients received imatinib doses >600 mg/d, and 12 of these patients developed intolerance related to either abnormal results on liver function testing, rash, bone pain, fatigue, or depression. Of the 40 patients in the CP, 37 achieved a CHR and 18 achieved an MCR. Of note is that 13 of 16 patients (81%) who had an MCR to imatinib before relapsing also had an MCR to dasatinib. In addition, 18 patients who did not have an MCR to imatinib did have a cytogenetic response to dasatinib, and 9 patients with only a minor cytogenetic response or PCR to imatinib had a CCR to dasatinib. Typically, doses of at least 50 mg/d were required for a hematologic response, and higher doses were required for achievement of an MCR. These responses occurred whether dasatinib was given once daily or in 2 divided doses. In patients with more advanced disease or Ph-positive ALL, an MHR was achieved in 6 of 9 patients (67%) in the AP, 7 of 14 (50%) in myeloid BC, and 5 of 8 (63%) in either lymphoid BC or Ph-positive ALL. The duration of response in patients in the CP or AP was between 2 and 19 months; 6 of 14 patients (43%) in myeloid BC had been in MHR for 5 to 12 months at the time the data were published, and 3 of these patients had been in CCR for 10 to 12 months. Conversely, patients in lymphoid BC or Ph-positive ALL who originally responded to dasatinib relapsed within a median of 4 months. Of the 60 patients who had mutations in BCR-ABL at baseline, dasatinib invoked either a hematologic or cytogenetic response in all except those who carried the T315I mutation. These results
suggest that dasatinib offers antileukemic activity in patients with CML or Ph-positive ALL, regardless of the phase, and that the response is dependent on the BCR-ABL genotype.

In an effort to evaluate the response to dasatinib in patients identified with 1 of 13 different BCR-ABL point mutations, Jabbour et al \(^3\) assessed dasatinib activity in 26 patients with imatinib-resistant CML. All patients received open-label dasatinib, and patients' previous therapy was not reported. Three patients (1 each in the AP with mutations T315I, L364I, and G250; 2 with T315I in the CP; 1 with F317L in the CP) did not respond to dasatinib. The most commonly seen mutations in the 20 patients who did respond were F317L, M351T, E355G, F486S, E255V, and G250E. Three patients with mutations F317L, M351T, and E355G lost their response to dasatinib within 3 months, while the median duration of response in patients with other mutations was at least 5 months. Of the 12 patients with mutations in the P-loop region of BCR-ABL, 11 (92%) responded to dasatinib for a median of at least 5 months. Overall survival or terminal response data were not reported, nor was the dose of dasatinib or schedule of administration. The results of this study suggest that dasatinib has activity in patients with a wide variety of mutations in the BCR-ABL kinase domain, which would appear to constitute an advantage relative to imatinib.

Although mutations within the ATP binding site are of most concern, other mutations in BCR-ABL also occur. Chu et al \(^3\) described the activity of dasatinib in a 39-year-old patient with CML in the CP and imatinib resistance related to genetic alteration outside the ATP binding site. Resistance developed after a cytogenetic response to 9 months of imatinib treatment. Examination of the amino acid sequence before initiation of dasatinib therapy found a 35 base pair insertion into intron 8 of the ABL junction, a site distinct from the region that binds imatinib. The patient was then enrolled in a Phase II trial and received dasatinib 70 mg BID. A CCR was attained within 3 months. How this genetic abnormality affects imatinib resistance is not understood, although the findings of this report suggest that dasatinib may have additional actions to eliminate BCR-ABL-containing clones.

Branford et al \(^3\) sought to determine the effect of dasatinib on BCR-ABL clones in patients with imatinib-resistant CML or Ph-positive ALL. BCR-ABL levels, as measured by real-time quantitative polymerase chain reaction (RQ-PCR) testing, were evaluated for drug effect. Patients with CML in the AP or BC and those with Ph-positive ALL received dasatinib for a median of 5 months, and those in the CP received dasatinib for a median of 12 months. RQ-PCR analysis was performed from 2 to 17 times in each patient. Six of 14 patients (43%) in the AP or BC and 7 of 19 patients (37%) in CP achieved a >2-log reduction, which was considered a CCR. A major molecular response (>3-log reduction in BCR-ABL) was seen in 4 AP/BC and 4 CP patients. In patients whose disease progressed during therapy, all contained mutations in BCR-ABL. Six of the 7 patients who progressed during dasatinib therapy had the T315I mutation, which was detected at baseline in half of them. These results suggest that patients in various phases of CML containing different mutations in BCR-ABL can respond to dasatinib. In this study, the occurrence of mutations was also found to be a strong predictor of progression and relapse, and the T315I mutation was the most frequently detected mutation during dasatinib treatment. The variations in response in studies such as this suggest that although dasatinib has potential, factors other than inhibition of BCR-ABL play a role in the overall pathology of CML.

To test the potential for cross-resistance among different TK inhibitors, Quintas-Cardama et al \(^1\) conducted an open-label, prospective trial of dasatinib in 23 patients with CML (median age, 58 years; age range, 19–76 years) who had failed to respond to imatinib and nilotinib therapy. Nine patients had also failed previous treatment with interferon alfa, and 2 had undergone allogeneic stem-cell transplantation. Although it is not clear how the regimens were assigned, 3 dosing regimens were studied. Thirteen patients (1 CP, 7 AP, 5 BC) received dasatinib 70 mg BID, 9 patients (2 CP, 1 second CP, 3 AP, and 3 BP) received dasatinib 140 mg QD, and 1 patient in BC received dasatinib 120 mg BID. Patients received dasatinib for 1 to 10 months, with a median duration of 4 months. Thirteen patients had an observed response. Ten patients (43%) (1 CP, 8 AP, and 1 BP) achieved a CHR, with disappearance of all signs and symptoms of leukemia. In terms of cytogenetic response, 2 patients (9%) had a CCR, 4 (17%) had a PCR, and 1 (4%) had a minor cytogenetic response. Seven of the 10 patients who had a hematologic response also had a cytogenetic response. Of the 10 patients who did not have a cytogenetic response, 9 (39%) had a hematolog-
logic response and 1 (4%) had disease progression. The activity of dasatinib in this group of patients who were either refractory to or relapsed during previous imatinib and nilotinib therapy may indicate the absence of cross-resistance between kinase-inhibiting agents. Therefore, stepwise use of agents may be possible. Furthermore, resistance is likely to depend more on the individual interactions of each agent with the molecular target than on the mechanism of action of the class as a whole.

The Phase II START (SRC/ABL Tyrosine kinase inhibition Activity Research Trials of dasatinib) program involves the largest and most comprehensive study of dasatinib in the treatment of Ph-positive leukemias to date.\(^{39-43}\) It included 5 separate arms (C, A, B, L, and R) that evaluated the effects of dasatinib in patients at different stages of CML or Ph-positive ALL who were either resistant to or unable to tolerate imatinib treatment. In the open-label, nonrandomized, noncomparative arms (START-C, A, B, and L), dasatinib treatment was initiated at 70 mg BID. The dose could be escalated to 90 or 100 mg BID in patients who had a poor initial response, or decreased to 50 or 40 mg BID in the event of persistent drug-related toxicity.

Ottmann et al\(^{40}\) reported a preliminary analysis of the START-L trial, which involved 28 patients (61% male; median age, 44 years; age range, 20–84 years) with CML in lymphoid BC or Ph-positive ALL who had developed imatinib resistance (n = 27) or intolerance (n = 1). Seventeen patients (61%) had received imatinib doses >600 mg/d, and 15 (54%) had been taking imatinib for >12 months. Twelve patients (43%) had undergone stem-cell transplantation. Dose escalation of dasatinib occurred in 8 patients (29%), and dose reduction occurred in 3 patients (11%). Thirteen patients (46%) attained a CHR, 7 (25%) with a return to normal blood counts. Nine (32%) of these patients maintained a response after a median follow-up of at least 14 weeks. Eleven patients (39%) had a CCR, and 1 (4%) had a minor CR.

Guilhot et al\(^{41}\) reported a preliminary analysis of the first 107 patients in the START-A trial, which focused on those with CML in the AP who were resistant to imatinib (n = 99) or were unable to tolerate it (n = 8). Fifty-one percent of patients were male, and the median age was 57 years (age range, 23–86 years). Sixty-eight percent of patients had received imatinib for at least 3 years, 24% for 1 to 3 years, and 8% for <1 year. Fifty-nine percent had received imatinib at doses >600 mg/d. Additional previous therapies included interferon (75%), stem-cell transplantation (18%), and other unspecified chemotherapy (67%). All patients were started on dasatinib 70 mg; the median duration of therapy was 8.3 months (range, 0.2–12.9 months). At the time of publication, 67 patients (63%) remained on dasatinib. At 8-month follow-up, 69 (64%) had an MCR, and 18 (17%) had a minor hematologic response. In the patients with an MCR, 27 (25%) had no evidence of leukemia. Of note is that 65% of the patients who were resistant to imatinib achieved a CHR with dasatinib, with no evidence of leukemia in 25%. At 8 months, 26 patients (24%) had a CCR, 9 (8%) had a PCR, 6 (6%) had a minor CR, and 20 (18%) had a minimal cytogenetic response. The cytogenetic response could not be determined for 13 patients (12%).

Hochhaus et al\(^{39}\) reported preliminary results of the START-C trial in 186 of the planned 387 patients with CML in the CP with either imatinib resistance (n = 127) or inability to tolerate imatinib (n = 59). This arm evaluated the hematologic, cytogenetic, and molecular responses to dasatinib, as well as overall survival. Fifty-four percent of patients were female, and the median age was 59 years (age range, 24–79 years). Eighty percent of patients had received imatinib for at least 1 year, and 54% had received it for >3 years. Fifty-two percent were receiving an imatinib dose >600 mg/d. Additional previous therapies included interferon (70%) and stem-cell transplantation (9%). Patients were started on dasatinib 70 mg BID, with dose adjustment as described earlier. A CHR to dasatinib was observed in 168 patients (90%). A higher proportion of hematologic responders was seen among patients who had been unable to tolerate imatinib (97%) compared with those who had developed resistant disease (87%). Response times ranged from 1.1 to >10.6 months. In the 186 patients evaluated for cytogenetic response after 8 months of dasatinib treatment, 97 (52%) achieved an MCR, of whom 50 (39%) were originally imatinib resistant and 47 (80%) were originally imatinib intolerant. Seven patients (4%) achieved a minor cytogenetic response and 16 (9%) achieved a minimal cytogenetic response. When the molecular response was measured using real-time quantitative reverse-transcriptase PCR testing, there was found to be a drop in the BCR-ABL:ABL ratio from a median of 66% at baseline to 3% at 9-month follow-up. The rate of progression-free survival was 92% in patients with at least 8 months of follow-up data.
Talpaz et al reported on the first 34 patients in START-B, which enrolled patients with CML in myeloid BC with either imatinib resistance or intolerance. Seventy-one percent of patients were male, and the median age was 54 years (age range, 21–71 years). Five patients (15%) had previously undergone stem-cell transplantation, and 18 (53%) had received interferon. Forty-four percent of patients had received an imatinib dose >600 mg/d, and 41% had received imatinib for at least 3 years. Escalation of the dasatinib dose to 100 mg BID occurred in 32% of patients, and reductions to 50 or 40 mg BID occurred in 21% of patients. Sixteen patients (47%) achieved an MHR; of these, 7 (44%) had a return of blood counts to normal levels and 9 (56%) had no evidence of leukemia. There were 13 cytogenetic responses (6 [18%] CCR; 5 PCR [15%]).

Because of the preliminary nature of the findings from the first 4 treatment arms of START, information on the overall duration of treatment and the responses is not yet available. However, the results support the use of dasatinib in all phases of CML and Ph-positive ALL in patients who have failed imatinib treatment. No explanation for the absence of more widespread and sustained hematologic and cytogenetic responses is currently available. This may be another indication that factors other than BCR-ABL are responsible for controlling the rate of progression of Ph-positive disease.

The final arm, START-R, was the first randomized comparative trial of dasatinib and imatinib. In this arm, patients with CML in the CP who were deemed resistant to imatinib at doses ranging from 400 to 600 mg were randomized in a 2:1 ratio to receive dasatinib 70 mg BID or high-dose imatinib (800 mg/d). The primary end point was an MCR after 12 weeks of therapy. Treatment crossover was allowed for patients with no response or inability to tolerate the treatment to which they were originally randomized. In addition, escalation of the dasatinib dose to 90 mg BID was allowed in those with an inadequate response at week 12. A reduction in the dose of either drug (dasatinib to 50 or 40 mg BID; imatinib to 600 mg/d) could be made in the event of toxic effects. Although 150 patients were randomized to treatment, preliminary data are available for only the first 36 patients (22 dasatinib, 14 imatinib). Sixty-seven percent of patients were female, and the median age of patients was 57 years. Sixty-four percent of the dasatinib group and 79% of the imatinib group had received interferon previously. Ten patients in the dasatinib group and only 1 in the imatinib group had a documented BCR-ABL mutation. Toxic effects requiring a dose reduction occurred in 8 of the 22 patients (36%) receiving dasatinib and 1 of the 14 patients (7%) receiving imatinib. Twenty-one (95%) patients in the dasatinib group and 13 (93%) in the imatinib group achieved a CHR. At 12 weeks, the primary outcome of an MCR was achieved by a respective 7 (32%) and 1 (7%) patients. A total of 13 patients (2 [9%] dasatinib, 11 [79%] imatinib) required a therapeutic crossover as a result of adverse effects, but data on the specific responses in these patients are not available. These results suggest that dasatinib may be associated with a hematologic or cytogenetic response in patients with resistance to imatinib doses ≤600 mg/d. However, the results are limited by the lack of data on progression-free and overall survival.

RESISTANCE

Targeted therapy is based on the identification of a pathogenetic focal point. The quandary inherent in the use of targeted therapy becomes apparent when the target changes, when multiple targets are involved in the pathogenesis of disease, or when the therapy cannot arrive at its target in sufficiently high quantities to impart its actions. Factors that have been hypothesized to result in resistance to imatinib include mutations in the BCR-ABL gene, amplification of BCR-ABL, the existence of BCR-ABL-independent pathways, and cellular efflux pumps. Primary resistance includes mechanisms that are independent of BCR-ABL, as well as existing mutations in BCR-ABL before the initiation of targeted therapy. Secondary, or acquired, resistance involves changes occurring after the start of therapy and may also include BCR-ABL-independent and BCR-ABL-dependent mechanisms. Although multiple mechanisms of resistance to TK inhibitors have been theorized, mutations occurring in the amino acid structure of BCR-ABL have been most studied.

BCR-ABL represents an important target in CML and Ph-positive ALL because of its significant impact on disease development. Studies have found that inhibition of the kinase activity of BCR-ABL may disrupt some disease-provoking mechanisms. However, just as genetic alteration creates BCR-ABL through chromosomal translocation, it also gives dasatinib its strength, as additional mutations to the amino acid
structure of the enzyme may disrupt the activity of man-made targeted therapies. Many of the studies discussed previously have indicated that resistance to imatinib occurs through several mutations in various regions of BCR-ABL.\textsuperscript{5,13-17} Although many mutations have been discovered in the BCR-ABL kinase domain that confer resistance to imatinib, only the T315I mutant has shown resistance to dasatinib.\textsuperscript{13}

A report by Soret et al\textsuperscript{45} described a case of the T315I mutation appearing during dasatinib therapy, providing an example of acquired resistance. A 68-year-old patient with CML had been treated with imatinib for 34 months. Imatinib therapy was stopped when the disease relapsed, at which time only the V379I mutation was identified. The patient was started on dasatinib therapy and within 7 months, achieved a minor cytogenetic response. At 10 months, the patient again had increasing levels of Ph-positive cells. This time, the T315I mutant was identified as the culprit clone. The authors suggested that imatinib therapy may have induced selection of the V379I mutant, after which dasatinib therapy selected out the T315I mutant. They also suggested that to improve the selection of therapy, patients should be screened for existing BCR-ABL mutations when imatinib resistance is observed. Furthermore, use of multiple agents that target different mutants might be helpful in preventing the selection of resistant clones.

To facilitate the identification of clones with mutations that confer resistance to available therapies, Bradeen et al\textsuperscript{14} developed an assay that allowed preparation of resistance profiles for 3 TK inhibitors, nilotinib, imatinib, and dasatinib. They based their work on the reports of resistance to kinase inhibitors caused by mutations in BCR-ABL in hopes that the availability of more pretreatment information might improve clinical outcomes through proper selection of therapy.\textsuperscript{16}

On the assumption that use of multiple TK inhibitors might reduce the expansion of mutant clones through the ability of at least one of the agents to overcome resistance to the others, O’Hare et al\textsuperscript{22} investigated the potential for combining multiple kinase inhibitors in the management of Ph-positive malignancy. One concern with the use of multiple kinase inhibitors is the potential for antagonism between agents. To test their hypothesis, O’Hare et al exposed imatinib and dasatinib to Ba/F3 cells expressing the wild-type or BCR-ABL mutants Y253F, E255H, T315I, or M351T for 3 hours. No interference between agents in reducing cell proliferation was observed in any strain except the T315I mutant, which was unaffected under experimental conditions. In fact, a minor reduction in the IC\textsubscript{50} was observed for the sensitive mutants, suggesting that not only was anti-BCR-ABL activity preserved when dasatinib and imatinib were used together, but it may have been slightly enhanced. This finding may contribute to the use of reduced doses of combination agents, thus diminishing the incidence of dose-related adverse effects while improving therapeutic outcomes.

The clinical application of targeted therapies such as dasatinib differs from that of traditional chemotherapies in being based on an in-depth understanding of the mechanisms of these therapies against the various mutations of BCR-ABL. Whereas determination of tumor sensitivity or resistance to cytotoxic agents was not clinically feasible in the past, the identification of patients carrying various molecular mutations and assigning therapy accordingly can now be considered a reality. The National Institutes of Health has suggested factors to consider in the molecular monitoring of patients with CML.\textsuperscript{46} Among these considerations is the need for timely detection and reporting of BCR-ABL mutations in individual patients. Patients with advanced disease or those in any disease phase who have not responded to treatment with a TK inhibitor should have mutational analysis performed. Patients in the CP who have not yet been started on a kinase inhibitor may not benefit from mutational analysis because of the low incidence of mutations developing within the kinase domain at this stage of disease. Although these patients are Ph positive, the probability of finding a clone that is resistant to initial therapy (eg, imatinib) is low.\textsuperscript{46}

Resistance created by BCR-ABL mutations appears to be a limitation of all known TK inhibitors. Even dasatinib, which seems to have the broadest spectrum of activity, has little effect against the T315I mutation. The most challenging goal in the treatment of CML, however, may be elimination of the pathologic stem cells. The quiescent nature of these cells may make them inherently resistant to TK inhibitors.\textsuperscript{47,48} In vitro analysis has indicated that dasatinib has only moderate ability to eradicate cells within the stem-cell compartment. Combining TK inhibitors with agents that work by different mechanisms may ultimately offer greater potential for cure through the targeting of multiple malignant functions in CML cells.\textsuperscript{49-51} TK-independent
pathways may also play an important role in the pathology of CML, allowing cells to proliferate despite adequate exposure to TK inhibitors. This may also explain why patients with more advanced stages of CML are less likely to respond to TK-inhibitor treatment.52

ADVERSE EFFECTS

Even the most effective drug therapy is limited if it is not well tolerated. The promise of targeted therapy lies not only in its specificity, but also in its selectivity in avoiding contact with non–disease-related processes and cells; however, even highly selective drugs can have significant adverse effects. In the Phase I study by Talpaz et al13 involving 84 patients with CML or Ph-positive ALL who were treated with dasatinib 15 to 240 mg/d, grade 3/4 neutropenia was observed in 45% of patients in the CP at the start of therapy and 89% of patients with CML in the AP or BC, or with Ph-positive ALL. However, grade 3/4 neutropenia was present in 55% of patients with advanced CML or ALL before the start of dasatinib therapy. Grade 3/4 thrombocytopenia was seen in 35% of patients in the CP and 80% of patients in the AP or BC, or with Ph-positive ALL. Approximately 66% of patients required dasatinib-free periods because of myelosuppression, which typically took ~3 months to resolve; 25% had a reduction in the dasatinib dose because of myelosuppression. An interesting nonhematologic adverse effect was the pleural effusions seen in 15 patients; these effects were believed to be treatment related. Other nonhematologic adverse effects included grade 1/2 diarrhea (23%), peripheral edema (19%), headache (10%), grade 3/4 abnormalities on liver function tests (8%), and grade 1/2 hypocalcemia (60%). The authors noted that a maximum tolerated dose was not determined and that no patients withdrew from the study due to adverse effects. In those patients who had been unable to tolerate previous imatinib therapy, the same adverse effects were not observed with dasatinib. Although myelosuppression was the most common adverse effect observed, the authors noted that it was unclear whether this was related to anti-Ph therapy or general hematopoietic toxicity.

In the START-C trial in 186 patients with CML in CP,39 adverse effects occurring within the first 8 months of treatment that were considered related to dasatinib included the following grade 3/4 cytopenias: neutropenia in 92 patients (49%), thrombocytopenia in 88 (47%), leukopenia in 46 (25%), and anemia in 40 (22%). The most common nonhematologic adverse effects of all grades were elevated aspartate aminotransferase (60%), diarrhea (56%), elevated alanine aminotransferase (52%), fatigue (38%), headache (34%), dyspnea (27%), rash (22%), asthenia (20%), nausea (19%), peripheral edema (18%), pleural effusion (19%), and elevated bilirubin (14%). No grade 3/4 nonhematologic adverse effects occurred at an incidence >3%.

In the START-R trial,42 patients with CML in CP received either dasatinib or high-dose imatinib after having been found resistant to lower doses of imatinib. Dose reductions secondary to toxicity were required in 36% of patients receiving dasatinib. Grade 3/4 neutropenia was observed in 36% of dasatinib patients and grade 3/4 thrombocytopenia in 41%. Grade 1/2 nonhematologic toxicities included facial/peripheral edema (36%), diarrhea (32%), and nausea (32%).

In the START-A trial,41 myelosuppression was the most common toxicity in patients receiving dasatinib. All patients developed grade 3/4 cytopenias, including thrombocytopenia (82%), neutropenia (76%), anemia (69%), and leukopenia (61%). The most common (>20%) nonhematologic toxicities of all grades were diarrhea (50%), headache (28%), fatigue (23%), pleural effusions (23%), pyrexia (23%), nausea (22%), and peripheral edema (22%). The most common (>1%) grade 3/4 nonhematologic adverse effects included gastrointestinal bleeding (7%), diarrhea (6%), asthenia (4%), dyspnea (4%), fatigue (4%), pyrexia (4%), and pleural effusions (3%). Although dose reduction was allowed in this study, the numbers of patients who had a dose reduction or an interruption of treatment were not reported.

The START B trial42 also reported significant myelosuppression, with 59% of patients having neutropenia and 56% having thrombocytopenia. Nonhematologic toxicities included grade 1/2 diarrhea (24%), rash (12%), and peripheral edema (9%). Nausea was reported in 9% of patients (1 case of grade 3) and pleural effusions in 12% of patients.

In the interim analysis of the START-L trial,40 of the 28 patients (11%) evaluated required a dose reduction as a result of inability to tolerate dasatinib. Forty-four percent of patients had grade 3/4 neutropenia, and 71% had grade 3/4 thrombocytopenia; neutropenia and thrombocytopenia were present in a respective 63% and 58% of these patients before the initiation of dasatinib treatment. Grade 1/2 peripheral

November 2007

M. Steinberg 2303
edema was observed in 3 patients (11%), and 1 patient (4%) had grade 1 facial edema. Gastrointestinal intolerance occurred at a low incidence (frequency not reported). In comparing the different arms of the START trial, it should be noted that patients in advanced stages of CML (beyond the CP) appeared more likely to develop myelosuppression during dasatinib treatment. It is difficult to ascertain whether this higher incidence of neutropenia, thrombocytopenia, and anemia constitutes an adverse effect of dasatinib or is related to the negative hematologic sequelae of CML.

The most frequently reported serious adverse effects associated with dasatinib use are pyrexia (9%), pleural effusion (8%), febrile neutropenia (7%), gastrointestinal bleeding (6%), pneumonia (6%), thrombocytopenia (5%), dyspnea (4%), anemia (3%), cardiac failure (3%), and diarrhea (2%). Abnormal laboratory values may also occur, including hypocalcemia and increased transaminases and bilirubin. Table II includes a complete list of the adverse effects observed in clinical trials of dasatinib.

**DOSAGE AND ADMINISTRATION**

Dasatinib is approved by the US Food and Drug Administration for use in the treatment of patients with CML (CP, AP, or myeloid or lymphoid BC) or Ph-positive ALL who are resistant to or unable to tolerate previous therapy. The recommended starting dosage is 140 mg/d, administered as two 70-mg doses, one in the morning and one in the evening, without regard to meals. Dasatinib is available as 20-, 50-, or 70-mg oral tablets that should not be crushed, cut, or chewed, but swallowed whole. The dose of dasatinib may be increased or decreased in 20-mg increments per dose based on the response and tolerability. In light of the potential for myelosuppression associated with dasatinib use, a stepwise approach to dose titration is recommended based on laboratory values (Table III).

**CONCLUSIONS**

Dasatinib offers a new treatment option for patients with CML or Ph-positive ALL who are either unable to tolerate or resistant to previous therapy, including imatinib. Dasatinib has been found to be more effective in eliciting a cytogenetic or hematologic response and better tolerated than high-dose imatinib. However, many factors play a role in determining whether dasatinib may provide benefit to patients. Some of

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**Table II. Adverse effects reported in >10% of patients during clinical trials of dasatinib (N = 911).**

<table>
<thead>
<tr>
<th>Adverse Effect</th>
<th>Overall Frequency, %</th>
<th>Grade 3/4, %</th>
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<td>2</td>
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Table III. Adjustment of the dasatinib dose in the case of treatment-related cytopenias.26

Chronic-phase CML
Starting dose: 70 mg BID
First episode of neutropenia (absolute neutrophil count <0.5 × 10⁹/L) and/or thrombocytopenia (platelet count <50 × 10⁹/L)
Hold dasatinib
Restart dasatinib at original dose on neutrophil recovery (absolute neutrophil count >1.0 × 10⁹/L) and platelet recovery (platelet count >50 × 10⁹/L)
Second episode of neutropenia and/or thrombocytopenia for >7 days
Hold dasatinib
Restart dasatinib at 50 mg BID on neutrophil recovery (absolute neutrophil count >1.0 × 10⁹/L) and platelet recovery (platelet count >50 × 10⁹/L)
Third episode of neutropenia and/or thrombocytopenia for >7 days
Hold dasatinib
Restart dasatinib at 40 mg BID on neutrophil recovery (absolute neutrophil count >1.0 × 10⁹/L) and platelet recovery (platelet count >50 × 10⁹/L)

Accelerated or blast phase of CML or Ph+ ALL
Starting dose: 70 mg BID
First episode of neutropenia (absolute neutrophil count <0.5 × 10⁹/L) and/or thrombocytopenia (platelet count <10 × 10⁹/L)
Rule out leukemia as cause of cytopenia
If cytopenia is related to leukemia, consider dose increase to 100 mg BID
Hold dasatinib if treatment is believed to be responsible for cytopenia
Restart dasatinib at original dose on neutrophil recovery (absolute neutrophil count >1.0 × 10⁹/L) and platelet recovery (platelet count >20 × 10⁹/L)
Second episode of neutropenia (absolute neutrophil count <0.5 × 10⁹/L) and/or thrombocytopenia (platelet count <10 × 10⁹/L)
Rule out leukemia as cause of cytopenia
Hold dasatinib if complete investigations and comparative studies, the actual survival benefit of dasatinib remains unclear. There is also a lack of studies comparing the impact of dasatinib as first-line therapy with that of imatinib or allogeneic stem-cell transplantation. Whether the high cost of dasatinib is warranted by the therapeutic benefits remains to be determined in pharmacoeconomic, quality-of-life, cost–benefit, and cost-effectiveness studies.

Our current understanding of the genetic component in the pathogenesis of CML and its effect on drug pharmacodynamics makes CML a model for the management of other malignancies. Dasatinib appears to offer a therapeutic alternative for patients with CML, potentially filling some of the gaps in disease management created by the many mutations that occur in the BCR-ABL amino acid sequence. Additional investiga-
tion into the genetic mechanisms of the disease and identification of targets in addition to BCR-ABL may yield additional benefits. Directions for future research include the evaluation of dasatinib as first-line therapy in malignancies involving the Ph chromosome, as well as pharmacoeconomic comparisons with other treatments for these conditions. The role of sensitivity testing in targeted therapy to identify the presence of resistant variations remains to be clarified.

REFERENCES


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