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Dasatinib in Imatinib-Resistant Philadelphia Chromosome-Positive Leukemias

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ABSTRACT

BACKGROUND

The BCR-ABL tyrosine kinase inhibitor imatinib is effective in Philadelphia chromosome-positive (Ph-positive) leukemias, but relapse occurs, mainly as a result of the outgrowth of leukemic subclones with imatinib-resistant BCR-ABL mutations. We evaluated dasatinib, a BCR-ABL inhibitor that targets most imatinib-resistant BCR-ABL mutations, in patients with chronic myelogenous leukemia (CML) or Ph-positive acute lymphoblastic leukemia (ALL).

METHODS

Patients with various phases of CML or with Ph-positive ALL who could not tolerate or were resistant to imatinib were enrolled in a phase 1 dose-escalation study. Dasatinib (15 to 240 mg per day) was administered orally in four-week treatment cycles, once or twice daily.

RESULTS

A complete hematologic response was achieved in 37 of 40 patients with chronic-phase CML, and major hematologic responses were seen in 31 of 44 patients with accelerated-phase CML, CML with blast crisis, or Ph-positive ALL. In these two phases, the rates of major cytogenetic response were 45 percent and 25 percent, respectively. Responses were maintained in 95 percent of patients with chronic-phase disease and in 82 percent of patients with accelerated-phase disease, with a median follow-up more than 12 months and 5 months, respectively. Nearly all patients with lymphoid blast crisis and Ph-positive ALL had a relapse within six months. Responses occurred among all BCR-ABL genotypes, with the exception of the T315I mutation, which confers resistance to both dasatinib and imatinib in vitro. Myelosuppression was common but not dose-limiting.

CONCLUSIONS

Dasatinib induces hematologic and cytogenetic responses in patients with CML or Ph-positive ALL who cannot tolerate or are resistant to imatinib. (ClinicalTrials.gov number, NCT00064233.)

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CHRONIC MYELOGENOUS LEUKEMIA (CML) is associated with a chromosomal translocation that produces the Philadelphia chromosome (Ph).¹ This fusion gene encodes a chimeric protein, BCR-ABL, that is associated with uncontrolled activity of the ABL tyrosine kinase and can recapitulate features of CML in laboratory models.² Imatinib, an orally available ABL kinase inhibitor, can induce hematologic and cytogenetic remission in all stages of CML, as well as in Ph-positive acute lymphoblastic leukemia (ALL), with minimal toxicity.^{3,4} Imatinib is now first-line therapy for newly diagnosed CML.⁵

However, resistance to imatinib has become increasingly important.^{6,7} Furthermore, nearly all patients with chronic phase CML have persistent disease, measurable by polymerase chain reaction (PCR) and indicative of a reservoir of residual leukemia cells that may be a source of relapse.⁸⁻¹⁰ Relapse during imatinib treatment is most often caused by mutations in the kinase domain of BCR-ABL that interfere with imatinib binding.¹¹⁻¹³

Dasatinib (BMS-354825, Bristol-Myers Squibb) is an orally available ABL kinase inhibitor that differs from imatinib in that it can bind to both the active and inactive conformations of the ABL kinase domain.¹⁴⁻¹⁶ Dasatinib also inhibits a distinct spectrum of kinases that overlaps with the array of kinases that imatinib inhibits.¹⁷ Since it has less stringent binding requirements than does imatinib, dasatinib has activity against many imatinib-resistant kinase domain mutations of BCR-ABL. In cell-line models, dasatinib inhibited 18 of 19 imatinib-resistant BCR-ABL mutations within a narrow concentration range, similar to that required to block wild-type BCR-ABL.^{14,18} The only exception is a single mutation deep within the ATP-binding pocket of the ABL tyrosine kinase (T315I) that confers a high degree of resistance to imatinib and dasatinib and to the imatinib analogue AMN-107,¹⁹ presumably as a result of steric hindrance caused by replacement of threonine with the bulkier isoleucine residue.

We describe a clinical evaluation of dasatinib in a phase 1, open-label, dose-escalation study in patients with CML or Ph-positive ALL who could not tolerate or were resistant to imatinib. The ongoing study began in November 2003, and the last patient was enrolled in April 2005. The study was approved by the institutional review boards at the University of California at Los Angeles (UCLA) and the M.D. Anderson Cancer Center in Houston.

METHODS

PATIENTS AND STUDY DESIGN

Patients who were at least 14 years of age were eligible if they had Ph-positive chronic-phase or accelerated-phase CML or blast crisis or Ph-positive ALL and hematologic resistance or intolerance to imatinib, and all patients provided written informed consent. Chronic-phase CML was defined by the presence of less than 15 percent blasts, less than 20 percent basophils, and less than 30 percent blasts plus promyelocytes in peripheral blood or bone marrow and a platelet count of at least 100,000 per cubic millimeter, with no extramedullary involvement. Blast crisis was defined by the presence of at least 30 percent blasts in peripheral blood or bone marrow or extramedullary infiltrates of leukemic cells (other than the spleen or liver). Patients were classified as having accelerated-phase disease if they did not fulfill criteria for chronic-phase disease or blast crisis but did meet any of the following criteria: the presence of at least 15 percent but less than 30 percent blasts in peripheral blood or bone marrow, the presence of at least 20 percent basophils in peripheral blood or bone marrow, the presence of at least 30 percent blasts plus promyelocytes (but less than 30 percent blasts) in peripheral blood or bone marrow, or a platelet count of less than 100,000 per cubic millimeter unrelated to therapy. Patients with accelerated-phase disease or blast crisis who had met the criteria for chronic-phase disease at the time of entry were enrolled as having chronic-phase CML. Patients with Ph-positive ALL had at least 30 percent lymphoblasts in peripheral blood or bone marrow without previous evidence of chronic-phase CML.

Hematologic resistance to imatinib was classified as primary (a lack of adequate response) or acquired (a relapse after an initial response), as detailed in Table 1 of the Supplementary Appendix (available with the full text of this article at www.nejm.org). Patients with cytogenetic or molecular resistance, but without hematologic resistance, to imatinib were not eligible. Patients were considered to be unable to tolerate imatinib if they had discontinued imatinib treatment as a result of nonhematologic toxic effects of any grade.

The primary objective of this study was to define the tolerability and safety of dasatinib. Secondary end points were the determination of pharmacokinetic properties and antileukemic activity of

dasatinib and the examination of potential correlates of clinical response to the *BCR-ABL* genotype. Initially, dasatinib was administered only to patients with chronic-phase disease on a once-daily schedule for five consecutive days, followed by two days without treatment (a regimen of five days "on" and two days "off") every week. The study protocol permitted progression to the administration of continuous daily doses of dasatinib and dose escalation. After obtaining pharmacokinetic data on the initial chronic-phase cohorts, we amended the protocol to include twice-daily administration of dasatinib for patients following the regimen of five days on and two days off and the regimen of continuous daily doses. After clinical activity was observed in chronic-phase CML, the protocol was amended to include patients with the other phases of CML or with Ph-positive ALL, all of whom were treated twice daily.

ASSESSMENT OF TOXIC EFFECTS AND RESPONSE

Patients were seen weekly for the first 12 weeks, monthly for the next 12 weeks, and then every 3 months. Complete and differential blood counts were obtained twice weekly for the first 12 weeks, every 2 weeks for 12 weeks, and every 6 weeks thereafter. Morphologic and cytogenetic analyses of bone marrow were performed every three months or more frequently in patients with accelerated-phase CML, CML with blast crisis, or Ph-positive ALL if such analysis was clinically indicated. Adverse events were evaluated throughout the study and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0.²⁰

In patients with chronic-phase disease, hematologic responses were scored as either a complete response or no response (Table 2 of the Supplementary Appendix). A complete response was defined by a white-cell count of no more than the upper limit of normal; a platelet count of less than 450,000 per cubic millimeter; the absence of blasts or promyelocytes in peripheral blood; the presence of less than 5 percent myelocytes plus metamyelocytes in peripheral blood; and the absence of extramedullary involvement (including a normal-size liver and spleen on physical examination). Progressive disease was defined as a progression to accelerated-phase disease or blast crisis or an inability to maintain a complete response even with dose escalation.

In patients with accelerated-phase disease,

CML with blast crisis, or Ph-positive ALL, hematologic responses were scored as a major response (<5 percent blasts), a minor response, or no response (Table 2 of the Supplementary Appendix). Two subgroups were included in a major hematologic response: no evidence of leukemia and complete hematologic response, on the basis of whether full recovery of blood counts was achieved. Confirmed responses were those that persisted for four weeks. Any patient with a major or minor response who subsequently did not meet these criteria during a two-week period was considered to have progressive disease. The disease of patients with accelerated-phase CML was considered to have progressed if blast crisis developed. The disease of patients with blast crisis or Ph-positive ALL was considered to have progressed if the number of blasts in peripheral blood or bone marrow increased despite at least four weeks of treatment. Cytogenetic responses were defined as follows, on the basis of the percentage of Ph-positive cells in metaphase in bone marrow: complete response, 0; partial response, 1 to 35 percent; minor response, 36 to 65 percent; minimal response, 66 to 95 percent; and no response, 96 to 100 percent. The rate of major cytogenetic response included patients with complete or partial cytogenetic response.

ANALYSIS OF MUTATIONAL STATUS

Blood samples were collected from all patients for analysis of *BCR-ABL* mutations at baseline, and sequencing of the *BCR-ABL* kinase domain was conducted at a central laboratory, as described previously.¹² Approximately 1500 nucleotides spanning the *BCR-ABL* catalytic domain were sequenced, with the use of *BCR-ABL* complementary DNA (cDNA) amplified by reverse-transcriptase PCR (RT-PCR) from peripheral blood as a template. At least 10 independent *BCR-ABL* clones were sequenced from each patient.

STUDY RESPONSIBILITIES

The study was designed by academic investigators and a representative of the sponsor, Bristol-Myers Squibb. Data were collected at UCLA and at the M.D. Anderson Cancer Center under the supervision of academic investigators and then were analyzed at Bristol-Myers Squibb by company representatives and by Drs. Sawyers and Talpaz; the latter academic investigators vouch for the accuracy and completeness of the data analysis.

RESULTS

PATIENTS

A total of 84 patients were enrolled in the study: 40 with chronic-phase CML, 11 with accelerated-phase CML, 23 with myeloid blast crisis, and 10 with lymphoid blast crisis or Ph-positive ALL (Table 1). Of these 84 patients, 50 (60 percent) had received previous chemotherapy (excluding hydroxyurea) and 12 (14 percent) had undergone bone marrow or stem-cell transplantation. Seventy-two patients (86 percent) were resistant to imatinib, and all but 12 had received imatinib in daily doses of 600 mg or greater. Twelve patients (14 percent) could not tolerate imatinib because of abnormal liver-function tests, rash, bone pain, fatigue, or depression. Mutations in the BCR-ABL kinase domain were detected in 60 patients (71 percent) (more detail is available in Tables 3 and 4 of the Supplementary Appendix).

TOXIC EFFECTS

Grade 3 or 4 neutropenia occurred in 45 percent of patients with chronic-phase disease and in 89 percent of patients with accelerated-phase disease, CML with blast crisis, or Ph-positive ALL (Table 2). Among the patients with accelerated-phase CML, CML with blast crisis, or Ph-positive ALL, 55 percent had grade 3 or 4 myelosuppression at the start of the study, as is typical in this population of patients (Table 5 of the Supplementary Appendix). Grade 3 or 4 thrombocytopenia occurred in 35 percent of patients with chronic-phase disease and in 80 percent of patients with accelerated-phase disease, CML with blast crisis, or Ph-positive ALL. Myelosuppression required the interruption of treatment in about 60 percent of patients and generally resolved within three months, often in association with a cytogenetic response. Twenty-five percent of patients required a reduction in the dose of dasatinib (Table 6 of the Supplementary Appendix).

Fifteen patients had pleural effusions that could not be attributed to known causes and were therefore deemed to be treatment-related. The effusions were managed with diuretics, thoracentesis, or pleurodesis. Other adverse events were grade 1 or 2 diarrhea (23 percent), peripheral edema (19 percent), and headache (10 percent). In seven patients, grade 3 or 4 abnormalities in liver-function tests developed, but these effects resolved within two to three weeks without a modifica-

tion in the dose. Grade 1 or 2 hypocalcemia was noted in about 60 percent of patients but was asymptomatic and did not worsen with continued therapy (Table 7 of the Supplementary Appendix). A maximum tolerated dose was not determined, and no patient withdrew from the study as a result of toxic effects. Patients who entered the study as a result of imatinib intolerance did not have similar complications with dasatinib.

HEMATOLOGIC AND CYTOGENETIC RESPONSES

The rates of complete hematologic response and major cytogenetic response in the 40 patients with chronic-phase CML were 92 percent and 45 percent, respectively (Table 3). Most of the complete hematologic responses occurred at doses of dasatinib of 50 mg per day or more, whereas most cytogenetic responses required higher doses (Table 8 of the Supplementary Appendix). A cytogenetic response was more common in patients with chronic-phase disease who had had a previous cytogenetic response while receiving imatinib. Of 16 patients who had had a previous major cytogenetic response while receiving imatinib, 13 had a major cytogenetic response while receiving dasatinib. However, of 18 patients who had had no cytogenetic response while receiving imatinib, 5 had a cytogenetic response while receiving dasatinib, and 9 patients who had had only minor or partial responses while receiving imatinib had a complete cytogenetic response while receiving dasatinib. The rates of hematologic and cytogenetic response among patients who were treated with the once-daily regimen were similar to the rates among those treated twice daily.

The rate of major hematologic response in patients with CML in the accelerated or blast phase or those with Ph-positive ALL was 70 percent. These responses were confirmed after four weeks in 6 of 9 patients with accelerated-phase disease, 7 of 14 patients with myeloid blast crisis, and 5 of 8 patients with either lymphoid blast crisis or Ph-positive ALL. Major cytogenetic responses were observed in all three groups of patients with accelerated-phase disease (Table 3).

In patients with chronic-phase or accelerated-phase CML, responses were maintained after 2 to 19 months of follow-up (Fig. 1). All patients with chronic-phase or accelerated-phase disease who had a complete or major hematologic response remain in the study, with the exception of one patient with chronic-phase disease who chose to withdraw

Table 1. Baseline Characteristics of the Patients.

Characteristic	Chronic-Phase CML (N=40)	Accelerated-Phase CML (N=11)	CML with Myeloid Blast Crisis (N=23)	CML with Lymphoid Blast Crisis or Ph-Positive ALL (N=10)	Total (N=84)
Sex — no. (%)					
Male	21 (52)	3 (27)	14 (61)	9 (90)	47 (56)
Female	19 (48)	8 (73)	9 (39)	1 (10)	37 (44)
Age — yr					
Median	61	63	53	50	56
Range	28–79	40–73	30–70	15–73	15–79
Hematologic analysis — cells/mm ³					
White-cell count ($\times 10^{-3}$)					
Median	33	21	20	12	23
Range	3–243	1–108	<1–117	1–198	<1–243
Platelet count ($\times 10^{-3}$)					
Median	310	279	39	40	216
Range	52–2166*	4–1710	7–1057	22–375	4–2166
Disease history†					
Duration of disease — mo					
Median	90	67	44	26	71
Range	13–207	22–139	5–216	9–70	5–216
Previous complete hematologic response with imatinib — no. (%)	30 (75)	8 (73)	15 (65)	9 (90)	62 (74)
Previous cytogenetic response with imatinib — no. (%)	20 (50)	4 (36)	5 (22)	3 (30)	32 (38)
Treatment history — no. (%)					
Previous bone marrow or stem-cell transplantation	2 (5)	0	5 (22)	5 (50)	12 (14)
Previous chemotherapy	22 (55)	4 (36)	15 (65)	9 (90)	50 (60)
Previous interferon	37 (92)	9 (82)	12 (52)	2 (20)	60 (71)
History of imatinib resistance or intolerance — no. (%)					
Any resistance	32 (80)	9 (82)	22 (96)	9 (90)	72 (86)
Primary hematologic resistance	8 (20)	1 (9)	6 (26)	1 (10)	16 (19)
Acquired hematologic resistance	23 (58)	8 (73)	15 (65)	8 (80)	54 (64)
Primary or acquired resistance unknown	1 (2)	0	1 (4)	0	2 (2)
Intolerance — no. (%)	8 (20)	2 (18)	1 (4)	1 (10)	12 (14)
Maximum previous imatinib dose — no. (%)					
400 to <600 mg/day	7 (18)	1 (9)	3 (13)	1 (10)	12 (14)
600 mg/day	7 (18)	3 (27)	7 (30)	2 (20)	19 (23)
>600 mg/day	26 (65)	7 (64)	13 (57)	7 (70)	53 (63)
BCR-ABL mutation detected — no. (%)	33 (82)	8 (73)	13 (57)	6 (60)	60 (71)

* Two patients had platelet counts of less than 100,000 per cubic millimeter.

† Two patients with chronic-phase disease had prior blast crisis but reverted to a second chronic phase while receiving imatinib therapy.

from the study to undergo allogeneic marrow transplantation when a donor became available. In contrast, responses in patients with CML with myeloid or lymphoid blast crisis or with Ph-posi-

tive ALL were generally short-lived. Despite the high rate of major hematologic response, all but one of the patients with lymphoid blast crisis or Ph-positive ALL had a relapse after a median fol-

Table 2. Highest Grade of Adverse Hematologic and Nonhematologic Events during the Study.

Adverse Event	Chronic-Phase CML (N=40)	Accelerated-Phase CML (N=11)	CML with Myeloid Blast Crisis (N=23)	CML with Lymphoid Blast Crisis or Ph-Positive ALL (N=10)
	<i>number (percent)</i>			
Hematologic				
Neutropenia*				
Grade 3	9 (22)	3 (27)	2 (9)	2 (20)
Grade 4	9 (22)	6 (55)	20 (87)	6 (60)
Thrombocytopenia†				
Grade 3	8 (20)	3 (27)	2 (9)	0
Grade 4	6 (15)	6 (55)	17 (74)	7 (70)
Nonhematologic				
Diarrhea				
Grade 1–4	7 (18)	5 (45)	5 (22)	2 (20)
Grade 3 or 4	0	0	1 (4)	0
Vomiting				
Grade 1–4	0	1 (9)	2 (9)	1 (10)
Grade 3 or 4	0	0	0	0
Nausea				
Grade 1–4	2 (5)	1 (9)	4 (17)	1 (10)
Grade 3 or 4	0	0	0	0
Gastrointestinal hemorrhage				
Grade 1–4	4 (10)	0	3 (13)	0
Grade 3 or 4	2 (5)	0	3 (13)	0
Pleural effusion				
Grade 1–4	5 (13)	0	8 (35)	2 (20)
Grade 3 or 4	0	0	3 (13)	0
Peripheral edema				
Grade 1–4	7 (18)	3 (27)	5 (22)	1 (10)
Grade 3 or 4	0	0	0	0
Periorbital edema				
Grade 1–4	2 (5)	1 (9)	2 (9)	1 (10)
Grade 3 or 4	0	0	0	0

low-up of four months (range, one to eight). In a similar manner, only 6 of 14 patients (43 percent) with CML with myeloid blast crisis who had a major hematologic response are still participating in the study, with follow-up ranging from 5 to 12 months. However, three of these patients have

sustained complete cytogenetic remission at 10, 11, and 12 months each.

CLINICAL RESPONSE AND BCR-ABL GENOTYPE

Of 84 study patients, 60 (71 percent) had BCR-ABL mutations detected at baseline (Fig. 2B). The BCR-

Table 2. (Continued.)

Adverse Event	Chronic-Phase CML (N=40)	Accelerated-Phase CML (N=11)	CML with Myeloid Blast Crisis (N=23)	CML with Lymphoid Blast Crisis or Ph-Positive ALL (N=10)
	<i>number (percent)</i>			
Pericardial effusion				
Grade 1–4	1 (2)	0	3 (13)	0
Grade 3 or 4	0	0	2 (9)	0
Generalized edema				
Grade 1–4	2 (5)	1 (9)	0	1 (10)
Grade 3 or 4	0	0	0	0
Dyspnea or pulmonary edema				
Grade 1–4	4 (10)	3 (27)	2 (9)	1 (10)
Grade 3 or 4	0	0	2 (9)	0
Rash				
Grade 1–4	1 (2)	5 (45)	2 (9)	1 (10)
Grade 3 or 4	0	0	0	0
Flushing				
Grade 1–4	0	3 (27)	1 (4)	0
Grade 3 or 4	0	0	0	0
Headache				
Grade 1–4	4 (10)	3 (27)	1 (4)	0
Grade 3 or 4	0	0	0	0
Fatigue				
Grade 1–4	3 (8)	0	0	1 (10)
Grade 3 or 4	1 (2)	0	0	0
Tumor lysis syndrome				
Grade 1–4	0	0	2 (9)	0
Grade 3 or 4	0	0	2 (9)	0

* Before treatment, grade 3 or 4 neutropenia occurred in no patients with chronic-phase disease, in 18 percent of patients with accelerated-phase disease, in 30 percent of patients with myeloid blast crisis, and in 20 percent of patients with lymphoid blast crisis or Ph-positive ALL.

† Before treatment, grade 3 or 4 thrombocytopenia occurred in no patients with chronic-phase disease, in 27 percent of patients with accelerated-phase disease, in 60 percent of patients with myeloid blast crisis, and in 70 percent of patients with lymphoid blast crisis or Ph-positive ALL.

ABL mutations in 56 of these 60 patients had been reported previously in association with imatinib resistance, but 4 patients had new *BCR-ABL* mutations identified within the kinase domain (Tables 3 and 4 of the Supplementary Appendix). The contribution of these mutations to imatinib resistance will need to be assessed in vitro and confirmed in other clinical isolates.

Hematologic and cytogenetic responses were observed broadly across all *BCR-ABL* genotypes, with the exception of T315I, the single mutation predicted to confer cross-resistance to dasatinib and imatinib in preclinical studies.^{14,18} In two patients who did not have a response to treatment, this mutation was the dominant pretreatment genotype and was one of four distinct imatinib-

Table 3. Treatment Response.*

Response	Chronic-Phase CML (N=40)	Accelerated-Phase CML (N=11)	CML with Myeloid Blast Crisis (N=23) <i>number (percent)</i>	CML with Lymphoid Blast Crisis or Ph-Positive ALL (N=10)	Total (N=84)
Hematologic response†					
Major	37 (92)‡	9 (82)	14 (61)	8 (80)	68 (81)
Complete	37 (92)‡	5 (45)	8 (35)	7 (70)	57 (68)
No evidence of leukemia	NA	4 (36)	6 (26)	1 (10)	11 (25)§
Minor	NA	0	4 (17)	0	4 (9)§
Cytogenetic response¶					
Overall	25 (62)	4 (36)	12 (52)	9 (90)	50 (60)
Major	18 (45)	3 (27)	8 (35)	8 (80)	37 (44)
Complete	14 (35)	2 (18)	6 (26)	3 (30)	25 (30)
Partial	4 (10)	1 (9)	2 (9)	5 (50)	12 (14)
Minor	0	0	2 (9)	1 (10)	3 (4)
Minimal	7 (18)	1 (9)	2 (9)	0	10 (12)

* NA denotes not applicable.

† Major hematologic response, which was defined as less than 5 percent blasts in bone marrow, had two subgroups: complete hematologic response (less than 5 percent blasts in bone marrow and a return of peripheral blood to normal measures) and no evidence of leukemia (same definition as complete hematologic response but without hematopoietic recovery of the peripheral-blood measures).

‡ One patient met all the criteria for complete hematologic response but had a platelet count of more than 450,000 per cubic millimeter owing to a concurrent diagnosis of essential thrombocytosis. This patient had a complete cytogenetic response and was counted as having had a complete hematologic response in this analysis.

§ This group excluded patients with chronic-phase CML.

¶ Major cytogenetic response consisted of complete response (no Ph-positive cells in bone marrow) and partial response (1 to 35 percent Ph-positive cells). Other categories were minor response (36 to 65 percent Ph-positive cells) and minimal response (66 to 95 percent Ph-positive cells). One patient in the cohort with lymphoid blast crisis or Ph-positive ALL had a partial cytogenetic response, with 3 of 30 Ph-positive cells in metaphase after four weeks of treatment with dasatinib, but had 91 percent blasts in bone marrow.

resistant subclones detected at baseline in a third patient, who did not have a response. This case is of particular interest because the three clones expressing *BCR-ABL* alleles F359V, M244V, and G250E, all of which were predicted to retain sensitivity to dasatinib on the basis of preclinical studies, were gradually extinguished during 60 days of treatment (Fig. 2C). In contrast, the T315I clone, which initially represented a minor fraction of the imatinib-resistant population, expanded during therapy and was the only clone detected at clinical relapse. A fourth patient also had disease progression with T315I, but the mutation was not detected among the 10 clones sequenced before dasatinib treatment.

DISCUSSION

Imatinib can produce durable cytogenetic remission with minimal toxic effects in most cases of

chronic-phase CML.⁵ Yet additional therapies are clearly needed to address the growing problem of relapse. A detailed understanding of the molecular basis of imatinib resistance, primarily as a result of mutations in the *BCR-ABL* gene, led to the evaluation of dasatinib. Some imatinib-resistance mutations encode amino acid substitutions in the *BCR-ABL* kinase domain at residues that directly contact imatinib. More common are mutations that alter the flexibility of the *BCR-ABL* kinase domain and destabilize the specific inactive conformation to which imatinib binds.²¹ Dasatinib binds to the *ABL* kinase domain in a manner distinct from that of imatinib and thereby retains activity against nearly all imatinib-resistance mutations (Fig. 2A). In addition to point mutations, amplification of the *BCR-ABL* gene is associated with imatinib-related relapse in advanced-phase CML.¹¹ *BCR-ABL*-independent pathways have also been implicated in some cases.^{22,23}

Our results demonstrate that dasatinib has clinical activity in all stages of imatinib-resistant CML and Ph-positive ALL, including resistance caused by *BCR-ABL* gene mutation. Furthermore, the correlation of the *BCR-ABL* genotype with a clinical response to dasatinib mirrors predictions from preclinical findings, in that none of the three patients with a T315I mutation that was detected before treatment had a response to dasatinib.

In addition to the induction of remission in patients with a broad range of imatinib-resistant *BCR-ABL* mutations, dasatinib had activity in patients who had received little or no cytogenetic benefit from imatinib. The ability of dasatinib to induce cytogenetic responses in patients with imatinib resistance raises the possibility of clinical benefit in other patients with CML who have a suboptimal response to imatinib (i.e., residual Ph-positive cells in the marrow) but who have no evidence of frank hematologic resistance to imatinib. Dasatinib may have this additional activity because it inhibits *BCR-ABL* kinase activity more effectively than does imatinib. Another possibility relates to the differential susceptibility of imatinib and dasatinib to drug efflux pumps, such as multidrug resistance protein 1 (P-glycoprotein), which are highly expressed in hematopoietic stem cells. Imatinib is a substrate of P-glycoprotein, whereas dasatinib is not. Dasatinib may therefore achieve a higher intracellular concentration than imatinib. A third possibility is that the many additional kinases that are targeted by dasatinib contribute to a cytogenetic response.

The major adverse effect of dasatinib was reversible myelosuppression. We cannot tell whether the myelosuppression was solely the result of the action of dasatinib against Ph-positive leukemia cells or of a more general hematopoietic toxic effect. Myelosuppression typically resolved in patients who had a cytogenetic remission and has not been a complication of dasatinib treatment in patients with solid tumors,²⁴ but further studies are needed to clarify the nature of dasatinib-associated myelosuppression. Nonmalignant pleural effusions developed in 15 patients, often in the absence of edema, whereas the common imatinib-related side effect of periorbital edema was less frequent. Other frequent imatinib-associated side effects, such as muscle cramps and nausea, were rarely observed. An important finding is that patients who could not tolerate imatinib and were

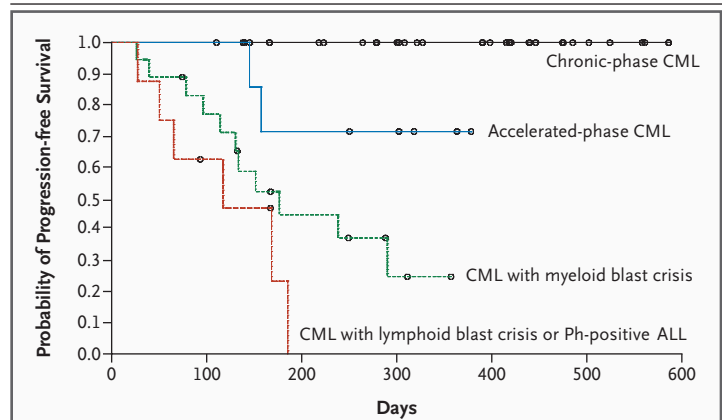


Figure 1. Kaplan-Meier Analysis of Progression-free Survival among Patients with CML or ALL Associated with Dasatinib.

The time to progression of disease is shown among patients with CML who had a complete hematologic response and among patients with accelerated-phase CML, myeloid blast crisis, lymphoid blast crisis, or Ph-positive ALL who met the criteria for a minor or major hematologic response. Circles represent patients who had a response and continued to be treated or who withdrew from the study to undergo stem-cell transplantation. One patient in the cohort with lymphoid blast crisis or Ph-positive ALL who had a complete cytogenetic response withdrew from the study on day 167 to undergo allogeneic transplantation.

treated with dasatinib did not have a recurrence of nonhematologic toxic effects (e.g., liver-function abnormalities and rash) that were associated with imatinib.

The rationale underlying the use of dasatinib for imatinib-resistant CML may have implications for other kinase-dependent cancers. Kinase-domain mutations, which were initially discovered as a resistance mechanism in CML, have been described in lung cancer, gastrointestinal stromal tumor, and the hypereosinophilic syndrome with resistance to kinase inhibitors.²⁵⁻²⁹ The activity of dasatinib in cases of CML with imatinib resistance is likely the result of the drug's less stringent conformational binding requirements relative to imatinib and suggests a general approach in which kinase-dependent cancers are treated with a combination of inhibitors that differ in their kinase-binding properties.

Although the clinical results reported here support the use of dasatinib as single-agent therapy for imatinib-resistant CML and Ph-positive ALL, acquired resistance to dasatinib may be the eventual outcome, as already observed in many of the patients with blast crisis and Ph-positive ALL who were treated in this trial. Preclinical studies have identified several *BCR-ABL* mutations

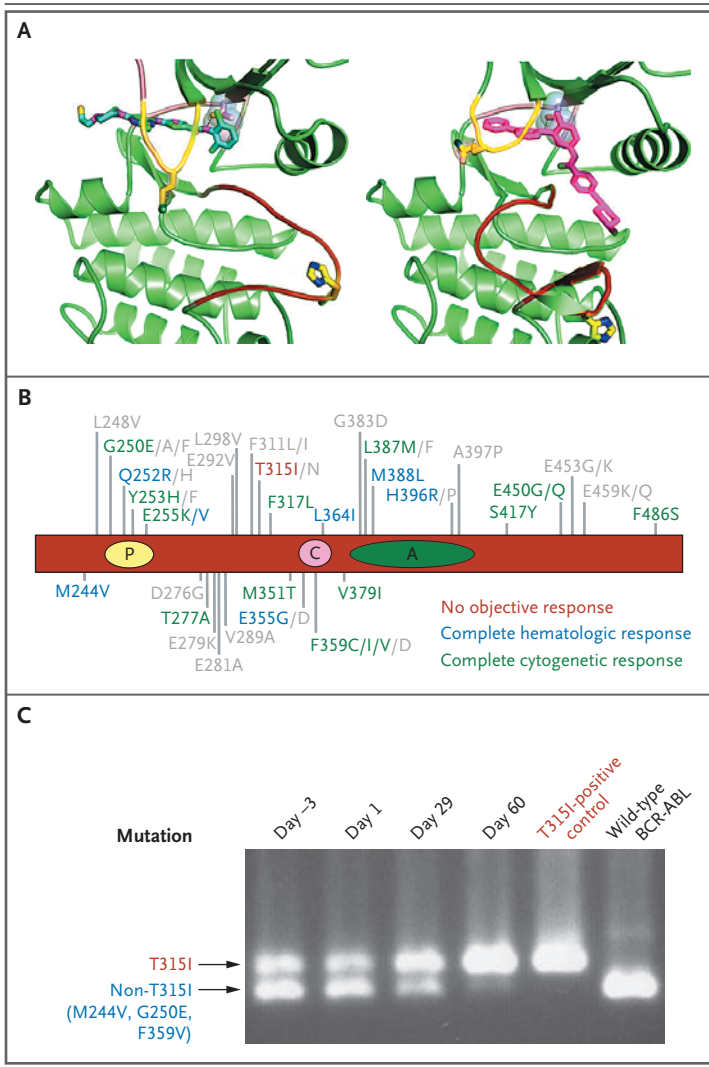


Figure 2. Correlation of the BCR-ABL Genotype with Hematologic and Cytogenetic Responses.

Panel A shows the three-dimensional structure of the ABL kinase bound with dasatinib and imatinib. Dasatinib and imatinib appear with carbon atoms in aqua and magenta, respectively. The ATP phosphate-binding loop (P loop) of ABL is colored yellow, and the activation loop is colored red, and the diverging direction of the activation loop in the two structures is shown. Imatinib could not bind to the active conformation of ABL because of a clash with the activation loop when positioned in the dasatinib-bound ABL conformation. Dasatinib, on the other hand, could bind with the activation loop in either conformation. T315 is a contact residue for both dasatinib and imatinib and is shown with carbon atoms in pink and oxygen atoms in red. The hydrogen bond between the inhibitors and T315 is shown as a dashed line. T315I is modeled and shown in a blue surface representation highlighting the increased bulk of the residue and the loss of a hydrogen bond, as compared with T315. An animated video of the differential binding of dasatinib and imatinib is available with the full text of this article at www.nejm.org. In Panel B, amino acid substitutions in the kinase domain of BCR-ABL that were detected in patients in this study are shown in red for no response, blue for complete hematologic response, or green for complete cytogenetic response. Mutations depicted in gray have been previously reported in patients with imatinib-resistant CML but were not detected in patients in this study. P denotes P loop, C catalytic domain, and A activation loop. In Panel C, a region of BCR-ABL was amplified by RT-PCR from peripheral-blood samples obtained from Patient 27 before starting dasatinib and at three time points during therapy. The T315I mutation was detected by a loss of unique DdeI restriction digest site, which alters DNA fragment size. Wild-type and T315I BCR-ABL plasmid are included as controls.

that confer resistance to dasatinib but not to imatinib, providing a rationale to explore combination therapy as initial treatment for CML.³⁰ For these reasons, successful long-term treatment of CML may require a cocktail of kinase inhibitors with activity against all drug-resistant BCR-ABL mutations (including T315I), analogous to the success of highly active antiretroviral therapy in the treatment of infection with the human immunodeficiency virus. Elsewhere in this issue of the *Journal*, Kantarjian et al.³¹ report that the kinase inhibitor nilotinib has a relatively favorable safety profile and is active in imatinib-resistant CML.

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