

Phase II, Open-Label Study Evaluating the Activity of Imatinib in Treating Life-Threatening Malignancies Known to Be Associated with Imatinib-Sensitive Tyrosine Kinases

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Abstract Purpose: To evaluate the activity of imatinib in treating advanced, life-threatening malignancies expressing one or more imatinib-sensitive tyrosine kinases.

Experimental Design: This was a phase II, open-label, single arm study. Patients ≥ 15 years old with malignancies showing histologic or molecular evidence of expression/activation of imatinib-sensitive tyrosine kinases were enrolled. Patients were treated with 400 or 800 mg/d imatinib for hematologic malignancy and solid tumors, respectively. Treatment was continued until disease progression or unacceptable toxicity. The primary objective was to identify evidence of imatinib activity with tumor response as the primary end point.

Results: One hundred eighty-six patients with 40 different malignancies were enrolled (78.5% solid tumors, 21.5% hematologic malignancies). Confirmed response occurred in 8.9% of solid tumor patients (4 complete, 9 partial) and 27.5% of hematologic malignancy patients (8 complete, 3 partial). Notable activity of imatinib was observed in only five tumor types (aggressive fibromatosis, dermatofibrosarcoma protuberans, hypereosinophilic syndrome, myeloproliferative disorders, and systemic mastocytosis). A total of 106 tumors were screened for activating mutations: five *KIT* mutations and no platelet-derived growth factor receptor mutations were found. One patient with systemic mastocytosis and a partial response to therapy had a novel imatinib-sensitive *KIT* mutation (D816T). There was no clear relationship between expression or activation of wild-type imatinib-sensitive tyrosine kinases and clinical response.

Conclusion: Clinical benefit was largely confined to diseases with known genomic mechanisms of activation of imatinib target kinases. Our results indicate an important role for molecular characterization of tumors to identify patients likely to benefit from imatinib treatment.

Imatinib mesylate is a small-molecule selective inhibitor of the tyrosine kinases ABL, *Abl*-related gene product (ARG), KIT, CSF-1R, and platelet-derived growth factor receptors α and β (PDGFRA and PDGFRB, respectively; refs. 1–4). Dysregulation

of imatinib-sensitive tyrosine kinases is a key factor in the pathogenesis of several malignancies, and imatinib treatment dramatically benefits patients with such cancers (5). Notably, imatinib is effective in patients with Philadelphia chromosome-positive (Ph⁺) chronic myelogenous leukemia and metastatic gastrointestinal stromal tumors (6–9), with the latter commonly having pathogenic mutations of the imatinib-sensitive tyrosine kinases KIT (80–85% of cases) or PDGFRA (5% of cases; refs. 10–12).

To determine new malignancies that are responsive to imatinib therapy, we conducted a phase II, open-label, single arm study. A total of 186 patients with life-threatening malignancies known to express one or more imatinib-sensitive tyrosine kinases were enrolled between February 2001 and December 2004. Patients were eligible for enrollment if their disease had proven refractory to standard therapy or if no proven conventional therapy existed. Correlative studies were conducted to identify potential predictors of imatinib response.

Materials and Methods

Study design and patients. This was an open-label, multinational, multicenter exploratory phase II study (Imatinib Target Exploration

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Consortium Study B2225). Patients were eligible for enrollment if they had a life-threatening malignancy known to be associated with one or more imatinib-sensitive tyrosine kinases that had proven refractory to standard therapy or if no proven conventional therapy existed. Protein expression of imatinib-sensitive tyrosine kinases was determined by immunohistochemistry at local hospitals for KIT, PDGFRA, or PDGFRB and confirmed by a central laboratory (Institute of Pathology, Basel, Switzerland). Additional inclusion criteria were age ≥ 15 y, an Eastern Cooperative Oncology Group performance status of 0 to 2, and a life expectancy of >3 mo in the absence of any intervention. Patients with gastrointestinal stromal tumor, prostate cancer, small cell lung cancer, glioma, Ph+ acute lymphoblastic leukemia, or chronic myelogenous leukemia were excluded from this study. Adequate end organ function [defined as total bilirubin $<1.5 \times$ upper limit of normal (ULN), aspartate aminotransferase and alanine aminotransferase $<2.5 \times$ ULN (or $<5 \times$ ULN if hepatic disease involvement is present), creatinine $<1.5 \times$ ULN, neutrophils $>1.5 \times 10^9/L$, and platelets $>100 \times 10^9/L$] was required for enrollment eligibility. In addition, patients could not have other serious or uncontrolled medical conditions. Treatment with other investigational agents or chemotherapy within 4 wk of study entry was not permitted. Institutional review board approval at each participating center and written informed consent from each subject were obtained. Additional written consent was required for retrieval investigational use of tissue samples.

Treatment. Imatinib mesylate (Novartis) was supplied as 100-mg capsules that were taken orally. In patients with hematologic malignancies, the initial dose of imatinib was 400 mg/d, with escalation to 300 or 400 mg twice daily, if no significant disease improvement occurred after the first 4 to 8 wk on therapy. In patients with solid tumors, the initial dose of imatinib was 400 mg twice daily, with escalation to 500 mg twice daily, if no significant disease improvement occurred after the first 4 to 8 wk on therapy. Treatment continued until disease progression or unacceptable toxicity. Dose interruptions and reductions were allowed for prospectively defined hematologic and nonhematologic toxicities, as previously described (8).

Study objectives. The primary objective was to identify evidence of imatinib activity in any of the studied malignancies. The primary end point was efficacy as assessed by tumor response. Secondary objectives included evaluating the expression and activation status of the relevant tyrosine kinase or associated effector molecules, and evaluating the relationship between changes in tyrosine kinase or effector molecules and clinical outcomes. Safety was assessed by evaluating adverse events, clinical laboratory parameters, vital signs, and physical examination findings and by monitoring concomitant medication use.

Assessments. Physical examination, evaluation of Eastern Cooperative Oncology Group performance status, body weight, hematology and blood chemistry testing, and disease measurement were done at baseline and repeated after 4 and 12 wk of therapy and at 12-wk intervals thereafter. Overall best response was measured according to modified Southwest Oncology Group criteria and the investigator's assessment of tumor response (13). In patients with solid tumors, the status of tumor lesions was assessed by computed tomography or magnetic resonance imaging whenever possible. Skin lesions were evaluated for surface area, depth, thickness, and consistency. For patients with hematologic malignancies, response was assessed by blood counts and bone marrow analyses. For patients with myelofibrosis, consensus criteria from the International Working Group were used (14). Efficacy results are based on the last formal evaluation done in December 2004 and supplemented with personal communication with individual investigators. Personal communication was used to obtain additional clinical and molecular data on patients with hypereosinophilic syndrome because many of these patients had prolonged drug treatment and had evolving clinical responses.

Evaluation of KIT expression and kinase activation. One entry criterion for enrollment of cases of solid tumors was KIT (CD117) positivity in at least 50% of the tumor cells, as detected by immunohistochemistry done by local pathology laboratories. Central

review of the CD117 stains was conducted at the Institute of Pathology, University Hospital Basel, Switzerland. In addition, parallel sections of the tumors were stained for CD117 using a standardized protocol, as previously described (15).

Patients were consented to undergo tumor biopsy procedures before and ~ 1 mo after beginning imatinib therapy. Consent for these biopsy procedures was obtained independently from consent to participate in the therapeutic study. Immunoblot analysis using snap-frozen biopsies was done to identify total and tyrosine-phosphorylated KIT, ABL1, ABL2, PDGFRA, and PDGFRB, using positive controls as previously described (16). Expression and phosphorylation of the signaling intermediates AKT, mitogen-activated protein kinase, and signal transducer and activator of transcription 3 were also evaluated (17). Thirty-five patient consented to a baseline, pretreatment biopsy. Twenty-five of these patients consented to an additional on-treatment biopsy.

Genomic mechanisms of oncogenic kinase activation were also investigated using available pathology specimens from 106 consenting patients. For these genotyping analyses, PCR amplification of genomic DNA for *KIT* (exons 9, 11, 13, and 17), *PDGFRA* (exons 12, 14, and 18), and *PDGFRB* (exons 11 and 17) was done, and amplicons were screened for mutations using denaturing, high-pressure liquid chromatography (WAVE, Transgenomic, Inc.), as previously described (10, 16, 18).

Mutant forms of *KIT* were cloned with the substitution D816V or D816T and transiently expressed by transfection in Chinese hamster ovary cells, as previously described. The transfected cells were exposed to varying concentrations of imatinib, and cell extracts were assessed for total and phosphorylated KIT protein by immunoblotting (18).

Statistical analysis. There was only one analysis population for efficacy and safety analyses, consisting of all patients who received at least one dose of study medication. The number of different indications analyzed was not predefined; similarly, the number of patients per indication was not prospectively stipulated. Enrollment of up to 10 patients per disease category was allowed initially, with enrollment of additional patients being contingent on suggestion of clinical efficacy. Because the study was intended to indicate proof of concept about the activity of imatinib in the studied diseases to support future clinical trials, no inferential methods were used and, therefore, power considerations did not apply to the choice of sample size. Data from all centers included in the study were pooled and summarized using descriptive statistics.

Results

Patient disposition. Between February 2001 and December 2004, 186 patients with 40 different pathologic diagnoses were enrolled across 13 centers in North America, Europe, and Australia.

At the time that this study was initiated, the original design was to enroll patients whose tumors expressed one or more imatinib-sensitive kinases. This was to be determined by immunohistochemistry at local hospitals for KIT, PDGFRA, or PDGFRB and confirmed by our central laboratory. However, as the trial progressed, several important operational issues were noted: (a) The central laboratory was unable to validate any immunohistochemical technique for assessing PDGFRA or PDGFRB. (b) Although KIT immunohistochemistry was beginning to be widely used for the diagnosis of gastrointestinal stromal tumor, it became apparent that many laboratories had problems with false-positive results on non-gastrointestinal stromal solid tumors (15, 16). (c) Some cases that lacked KIT expression as assessed by the central laboratory had clinical evidence of objective response or prolonged stable disease. (d) New imatinib-responsive diseases were being reported in the

literature but there was no rapid way to make the drug available for further clinical investigation, especially outside of North America (e.g., hypereosinophilic syndrome, chronic myelomonocytic leukemia with *PDGFRB* translocations, dermatofibrosarcoma protuberans, and chordoma; refs. 19–23).

In response to these issues, the study criteria were modified to allow patient entry based on published evidence of a potential role for KIT, PDGFRA, or PDGFRB in the biology of a given tumor. This evidence could include (a) preclinical studies of protein expression by tumor cell lines or by cells in archival pathology specimens; (b) cell models indicating imatinib responsiveness of a tumor type; (c) report of a genomic mechanism for activation of an imatinib-sensitive kinase (e.g., COL1A1-PDGFB fusion in dermatofibrosarcoma protuberans); or (d) publication of a report of an objective patient response to imatinib treatment. Following revision of the entry criteria, we limited the enrollment to ≤ 10 patients for any given disease indication, unless at least one of the first 10 patients had an objective response.

For descriptive purposes, patients were retrospectively divided into two groups: those with solid tumors (78.5%) and those with hematologic malignancies (21.5%). Of the enrolled patients, 172 (92.5%) discontinued treatment before the planned 2 years of imatinib therapy. The most common reason for discontinuation was an unsatisfactory therapeutic effect (59.7% of patients).

Baseline demographics and disease characteristics. The mean age of the population was 47.9 years, with most patients (82.3%) < 65 years old. Gender was relatively balanced for the patient population as a whole and most patients (94.6%) were Caucasian. Most patients (76.9%) were diagnosed at least 12 months before study enrollment; 60.8% were diagnosed at least 24 months before study enrollment. Patients with solid tumors tended to be suffering from recurrence or progression of disease, with the first recurrence most often having occurred ≥ 3 months before study entry.

Clinical results: solid tumors. The mean (\pm SD) imatinib dose intensity administered to patients with solid tumors was 648.1 ± 192.4 mg/d, and the median duration of treatment was 2.5 months (range, 0–67.3 months). Overall, 28.8% and 8.2% of solid tumor patients received imatinib for ≥ 5 and ≥ 20 months, respectively. The median time to progression in patients with solid tumors was 2.5 months (Fig. 1). Patients with solid tumors receiving imatinib for ≥ 20 months had the following tumor types: adenoid cystic carcinoma (1 of 12, 8.3%), aggressive fibromatosis (3 of 20, 15.0%), chordoma (2 of 5, 40.0%), dermatofibrosarcoma protuberans (3 of 12, 25.0%), liposarcoma (2 of 11, 18.2%), and synovial sarcoma (1 of 16, 6.2%).

Response data for each disease category are shown in Table 1. Confirmed responses occurred in 8.9% of the patients with solid tumors [four complete responses (CR) and nine partial responses (PR)]. Four confirmed CRs were seen in patients with dermatofibrosarcoma protuberans—two of these patients had an initial PR to imatinib therapy but obtained a CR after surgery and have remained disease free. Confirmed PRs occurred in solid tumor patients with aggressive fibromatosis, dermatofibrosarcoma protuberans, and synovial sarcoma. No confirmed CRs or PRs were observed in patients with adenoid cystic carcinoma, chondrosarcoma, chordoma, leiomyosarcoma, liposarcoma, melanoma, Ewing's sarcoma, rhabdomyosarcoma, desmoplastic round cell tumor, malignant schwannoma, osteosarcoma, breast cancer, or mesothelioma. In addition, no confirmed responses were observed for 16 other disease conditions for which only a single patient was enrolled (Table 1). Among the chordoma patients, the majority showed stable disease (4 of 5, 80.0%), with no patients having progressive disease.

Clinical results: hematologic malignancies. The mean (\pm SD) imatinib dose intensity administered to patients with hematologic malignancies was 463.3 ± 233.7 mg/d, and the median duration of treatment was 5.6 months (range,

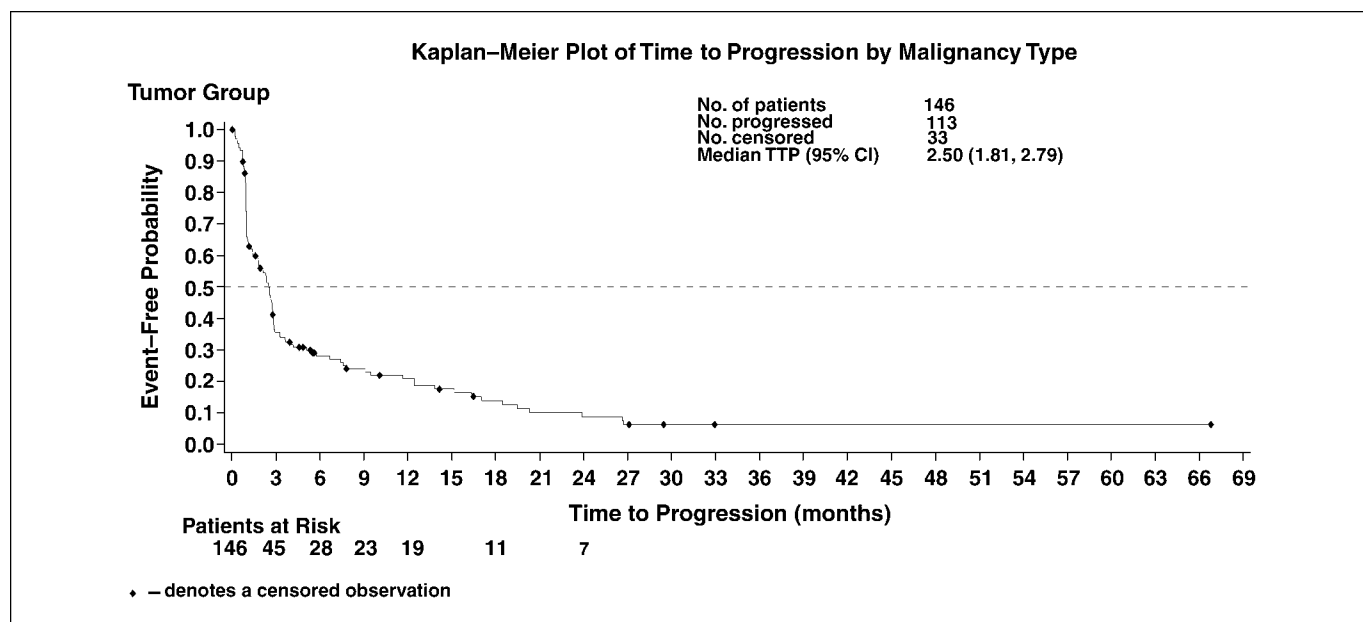


Fig. 1. Kaplan–Meier plot of time to progression in patients with solid tumors.

Table 1. Response to imatinib by indication (analysis population, *N* = 186)

Main indication	CR, n (%)	PR, n (%)	SD, n (%)	PD, n (%)	Unknown, n (%)	Median TTP (95% CI), mo	Putative target
Solid tumors: sarcoma							
Synovial sarcoma (<i>n</i> = 16)	0	1 (6.3)	3 (18.8)	11 (68.8)	1 (6.3)	1.1 (1.0-4.1)	KIT
Dermatofibrosarcoma protuberans (<i>n</i> = 12)	4 (33.3)	6 (50.0)	0	1 (8.3)	1 (8.3)	23.9 (7.7-∞)	PDGFRB
Leiomyosarcoma (<i>n</i> = 11)	0	0	1 (9.1)	8 (72.7)	2 (18.2)	1.1 (1.0-2.9)	KIT
Liposarcoma (<i>n</i> = 11)	0	0	5 (45.5)	6 (54.5)	0	2.6 (2.1-12.5)	KIT
Chondrosarcoma (<i>n</i> = 7)	0	0	1 (14.3)	5 (71.4)	1 (14.3)	[0.03 ⁺ -4.6 ⁺]	KIT
Ewing's sarcoma (<i>n</i> = 4)	0	0	0	3 (75.0)	1 (25.0)	[1.0-2.6]	KIT/PDGFR
Angiosarcoma (<i>n</i> = 2)	0	0	0	2 (100)	0	[0.7-0.8]	KIT/PDGFR
Rhabdomyosarcoma (<i>n</i> = 2)	0	0	0	2 (100)	0	[0.3-0.4]*	PDGFR
Aggressive fibromatosis (<i>n</i> = 20)	0	2 (10.0)	8 (40.0)	7 (35.0)	3 (15.0)	9.1 (2.9-17.0)	PDGFRA
Chordoma (<i>n</i> = 5)	0	0	4 (80.0)	0	1 (20.0)	[2.7-33.0 ⁺]	PDGFRB
Desmoplastic small round cell tumor (<i>n</i> = 5)	0	0	0	5 (100)	0	[0.5-1.4]	KIT, PDGFR
Neurofibrosarcoma (<i>n</i> = 3)	0	0	2 (66.7)	0	1 (33.3)	[0.03 ⁺ -16.4]	PDGFR
Malignant schwannoma (<i>n</i> = 2)	0	0	0	1 (50.0)	1 (50.0)	[0.9 ⁺ -1.0]*	PDGFR
Osteosarcoma (<i>n</i> = 2)	0	0	0	2 (100)	0	[1.0-2.8]*	PDGFR
Solid tumors: nonsarcoma							
Adenoid cystic carcinoma (<i>n</i> = 12)	0	0	6 (50.0)	5 (41.7)	1 (8.3)	5.7 (1.0-11.7)	KIT
Mesothelioma (<i>n</i> = 6)	0	0	0	3 (50.0)	3 (50.0)	[0.03 ⁺ -2.3]	PDGFRB
Malignant melanoma (<i>n</i> = 5)	0	0	0	4 (80.0)	1 (20.0)	[0.2-2.8]	KIT
Intraocular melanoma (<i>n</i> = 3)	0	0	0	3 (100)	0	[1.0-2.8]	KIT
Breast carcinoma (<i>n</i> = 2)	0	0	0	1 (50.0)	1 (50.0)	[0.03 ⁺ -0.6]*	KIT/PDGFR
Hematologic malignancies							
Hyper eosinophilic syndrome (<i>n</i> = 14)	5 (35.7)	1 (7.1)	1 (7.1)	6 (42.9)	1 (7.1)	8.4 (2.33-∞)	PDGFRA/KIT
Multiple myeloma (<i>n</i> = 6)	0	0	0	6 (100)	0	[1.0-3.0]	KIT
Myelofibrosis (<i>n</i> = 8)	0	0	5 (62.5)	1 (12.5)	2 (25)	[0.03 ⁺ -25.2 ⁺]	PDGFRB
Myeloproliferative disorders (<i>n</i> = 7)	3 (42.9)	1 (14.3)	0	2 (28.6)	1 (14.3)	[0.03 ⁺ -26.7 ⁺]	PDGFRB
Systemic mastocytosis (<i>n</i> = 5)	0	1 (20.0)	1 (20.0)	0	3 (60.0)	[0.03 ⁺ -22.3 ⁺]	KIT/PDGFR

NOTE: Other indications included only one patient: endometrial sarcoma (PD), fibrosarcoma breast (SD), fibrosarcoma ovary (PD), ovarian sarcoma (PD), pleural sarcoma (SD), adenocarcinoma (unknown), hemangiopericytoma (PD), malignant fibrous histiocytoma (PD), malignant mesenchymoma (PD), ovarian stromal tumor (PD), ovarian carcinoma (PD), rectal carcinoma (PD), renal cell carcinoma (unknown), seminoma (PD), small cell lung cancer (PD), and thymic carcinoma (PD). The symbol "*" means that indications have only two patients each. The symbol "+" indicates a censored time to event. "∞" indicates that the upper bound of the median confidence interval is infinity. For those indications with <10 patients, only the range of time to progression is reported in the form "[min-max]." A month is calculated as 365.25 divided by 12. Abbreviations: TTP, time to progression; SD, stable disease; PD, progressive disease.

0.5-26.7 months). Overall, 55.0% and 32.5% of hematologic malignancy patients received imatinib for ≥ 5 and ≥ 20 months, respectively. The median time to progression in patients with hematologic malignancies was 13.1 months (Fig. 2). The discrepancy between median treatment duration and median time to progression may be attributable in some cases to an adverse effect of imatinib on peripheral blood counts (see "Adverse events"). Alternatively, in some cases, the lack of perceived clinical benefit may have led investigators to discontinue treatment despite the absence of confirmed protocol-defined progressive disease (i.e., patient response classified as stable disease). Patients with hematologic malignancies receiving imatinib for ≥ 20 months had the following tumor types: hyper eosinophilic syndrome (6 of 14, 42.9%), myelofibrosis (2 of 8, 25.0%), Ph- myeloproliferative disorders (4 of 7, 57.1%), and systemic mastocytosis (1 of 5, 20.0%).

Response data for each disease category are shown in Table 1. Confirmed response was observed in 27.5% of patients with hematologic malignancies (eight CRs and three PRs). Confirmed CRs were confined to a subset of patients with hyper eosinophilic syndrome or myeloproliferative disorders. Confirmed PRs occurred in patients with hyper eosinophilic syndrome, myeloproliferative disorders, and systemic mastocytosis. No confirmed CRs or PRs were observed in patients with multiple myeloma or myelofibrosis. As of October 2007, five patients with hyper eosinophilic syndrome remained on imatinib with a CR or PR (minimum duration of therapy >48 months).

Molecular analyses. The presumed imatinib-sensitive target kinase expressed by tumor type at the time of study enrollment is shown in Table 1 for the 24 disease categories enrolling two or more patients. Fresh frozen tumor biopsies were available from 35 patients and were analyzed for expression of total and

activated (tyrosine-phosphorylated) KIT, PDGFRA, and PDGFRB by immunoblot analysis (Table 2). Despite positive immunohistochemistry results, KIT expression was only detected in a minority of tumor samples examined by immunoblotting and was only phosphorylated in one case each of mast cell leukemia, melanoma, and myeloma. PDGFRA was expressed by three of four analyzed cases of dermatofibrosarcoma protuberans and two of four cases of leiomyosarcoma, but there was no evidence of phosphorylated PDGFRA in any of these cases. A number of analyzed sarcomas expressed PDGFRB, including cases of aggressive fibromatosis, dermatofibrosarcoma protuberans, liposarcoma, leiomyosarcoma, and myxoid liposarcoma. However, phosphorylation of PDGFRB was infrequently detected in mesenchymal tumors. The majority of tumors had biochemical evidence of activation of the AKT, mitogen-activated protein kinase, and/or signal transducer and activator of transcription-3 pathways. There was no correlation between expression or activation status of imatinib target kinases and clinical response, nor between activation of the AKT, mitogen-activated protein kinase, or signal transducer and activator of transcription-3 pathways and clinical response.

Genotyping studies for *KIT*, *PDGFRA*, and *PDGFRB* were done on 106 cases. Activating point mutations in exon 17 of *KIT* were found in four cases of systemic mastocytosis (D816V, three cases; D816T, one case; Fig. 3) and one case of mast cell leukemia (D816V). No specimens were available for analysis from the other three patients with systemic mastocytosis. No other mutations of *KIT* were present in the other tumors ($n = 99$), nor were any activating mutations found in *PDGFRA* ($n = 97$) or *PDGFRB* ($n = 85$). The *KIT* D816T mutation has previously not been reported in human mast cell neoplasms. This patient had a PR with a reduction in serum tryptase from 106 to 11.8 $\mu\text{g/L}$ (normal $<13.5 \mu\text{g/L}$) and a marked reduction in the frequency of clinically significant anaphylactoid reactions that were occurring on a daily basis before start of

imatinib therapy. In contrast, no significant response was seen in three cases of systemic mastocytosis or one case of mast cell leukemia with documented D816V mutation. In our *in vitro* model system, the D816T mutant kinase was significantly more sensitive to imatinib than the D816V protein (biochemical IC_{50} of 0.25-0.5 versus >10 micro molar or 250-500 vs 10,000 nanomolar/L, respectively; Fig. 3).

A subset of patient samples was also screened for genomic rearrangement and/or amplification of imatinib-sensitive tyrosine kinases using standard metaphase cytogenetics and/or fluorescent *in situ* hybridization (19, 24). Results were negative except for patients with diseases already known to be associated with gain-of-function rearrangements: dermatofibrosarcoma protuberans (9 of 10 analyzed cases), myeloproliferative disorders (4 of 7 cases positive for t[5;12]), and hyper-eosinophilic syndrome (3 of 7 cases positive for *FIP1L1-PDGFRB* rearrangement). Clinical responses (CR or PR) were observed in 9 of 9 cases of dermatofibrosarcoma protuberans with 17;22 translocation, 4 of 4 cases of myeloproliferative disorder with 5;12 translocation, and 3 of 3 cases with *FIP1L1-PDGFRB* rearrangement. In contrast, the one case of dermatofibrosarcoma protuberans lacking an associated t(17;22) had progressive disease (time to progression, 1 month), the three cases of myeloproliferative disorder without t(5;12) had stable disease ($n = 1$; time to progression, 6 months) or PD ($n = 2$; mean time to progression, 0.9 months). None of the four cases of cytogenetically analyzed hyper-eosinophilic syndrome lacking *FIP1L1-PDGFRB* rearrangement had an objective response. Based on personal communication with treating physicians, we determined that all of these patients had PD, although in some cases the progression was slow enough that the patients were originally classified as having stable disease in December of 2004.

Adverse events. Imatinib was generally well tolerated and the nature and incidence of adverse events reported were similar to those reported in previous studies in gastrointestinal

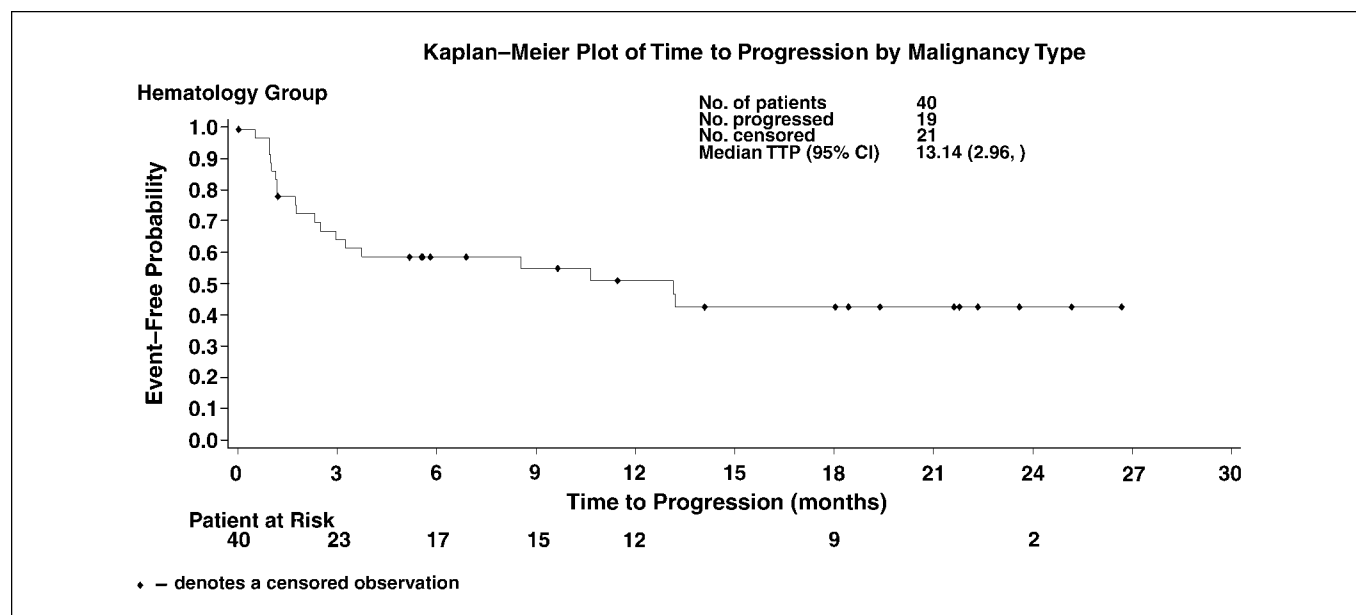


Fig. 2. Kaplan–Meier plot of time to progression in patients with hematologic malignancies.

Table 2. Immunohistochemistry/immunoblotting evaluations

Diagnosis	Clinical response/ TTP, mo	KIT IHC (LL), %	KIT IHC (CL), %	KIT	pKIT	PDGFRA	pPDGFRA	PDGFRB	pPDGFRB	pAKT	pMAPK	pSTAT3
AF	PD/2.8	>50	5	-	-	-	-	+++	-	+++	+++	++
AF	SD/12.5	>50	>50	-	-	-	-	+++	-	+++	+++	++
AF	PR/17.0	>50	>50	-	-	-	-	+	-	+	-	-
AF	PD/1.0	>50	>50	-	-	-	-	++	-	+++	+++	+
Chondrosarcoma	PD/1.2	>50	ND	+	-	-	-	-	-	+	++	++
DFSP	CR/33.0+	ND	0	-	-	++	-	+++	+	++	++	+++
DFSP	UNK/0.0+	ND	ND	-	-	+	-	+++	-	++	++	+
DFSP	PR/23.9	0	0	-	-	-	-	+	-	+	++	+++
DFSP	PR/1.9+	ND	0	-	-	+	-	++	-	++	++	+
Hemangiopericytoma	PD/1.5	50	ND	-	-	-	-	-	-	++	+	+
Liposarcoma	SD/15.2	>50	ND	++	-	++	-	++	++	++	+++	++
Liposarcoma	PD/1.2	>50	0	+	-	-	-	++	+	+++	++	+++
Liposarcoma	SD/16.5+	>50	0	-	-	-	-	-	-	+	-	+
Liposarcoma—dediff	PD/0.8	>50	0	-	-	-	-	+	+	+++	++	++
Liposarcoma—dediff	SD/2.8+	>50	ND	-	-	-	-	-	-	-	+	++
Liposarcoma	PD/2.6	0	ND	-	-	-	-	++	-	++	-	-
Leiomyosarcoma	PD/2.6	ND	ND	-	-	+++	-	++	-	++	++	+++
Leiomyosarcoma	UNK/5.1	>50	0	-	-	-	-	-	-	-	-	-
Leiomyosarcoma	PD/1.0	>50	0	-	-	-	-	+	-	+	+++	++
Leiomyosarcoma	PD/1.0	>50	ND	-	-	+	-	+++	-	+++	+++	+++
Malignant Schwannoma	UNK/1.0+	0	ND	-	-	-	-	-	-	+	-	-
Malignant Schwannoma	PD/1.0	>50	0	-	-	-	-	-	-	+	-	-
Mast cell leukemia	SD/1.4	>50	>50	+++	++	-	-	-	-	-	-	+
Melanoma	PD/1.0	>50	>50	+++	+	-	-	-	-	++	+	-
Intraocular Melanoma	PD/2.3	>50	ND	-	-	-	-	-	-	-	-	-
Mesenchymoma	PD/1.4	>50	ND	-	-	-	-	-	-	+	+	+
Mesothelioma	UNK/0.0+	>50	ND	-	-	-	-	-	-	+	+	+
Myeloma	PD/3.0	>50	ND	+	-	-	-	-	-	+	-	-
Myeloma	PD/1.0	>50	ND	++	+	-	-	-	+	+	-	++
Myeloma	PD/1.0	>50	ND	-	-	-	-	-	-	+	+	-
Renal cell carcinoma	PD/1.4	>50	ND	-	-	-	-	-	-	+	+	++
Rhabdomyosarcoma	PD/1.0	>50	>50	-	-	-	-	-	-	-	-	-
Rhabdomyosarcoma	PD/1.0	>50	20	-	-	-	-	+	-	+++	++	+
Rhabdomyosarcoma	PD/1.0	>50	20	++	+	-	-	++	-	++	+++	++
Rhabdomyosarcoma	PD/2.7	>50	5	-	-	-	-	+++	-	++	++	+++

NOTE: A time to progression value with the symbol "+" indicates a censored time to event.

Abbreviations: ND, not done; UNK, unknown; IHC, immunohistochemistry; LL, local pathology laboratory; CL, central pathology laboratory; AF, aggressive fibromatosis; DFSP, dermatofibrosarcoma protuberans; MAPK, mitogen-activated protein kinase; STAT3, signal transducer and activator of transcription 3.

stromal tumor or chronic myelogenous leukemia (6–9). Almost all patients reported at least one drug-related adverse event, with gastrointestinal symptoms, general systemic symptoms, skin disorders, and musculoskeletal and connective tissue disorders reported most frequently (all >60% incidence). Patients with hematologic malignancies had a higher incidence of hematologic system adverse events than patients with solid tumors (46.7% versus 23.4%). Nausea, vomiting, and diarrhea were the most commonly reported adverse events in both groups. Although many adverse events were mild to moderate in severity, study drug-related grade 3 and 4 adverse events were reported by 28.5% and 3.8% of patients, respectively. The most common grade 3 to 4 study drug-related adverse events affected the hematologic (8.1%) or the gastrointestinal (7.5%) systems.

Discussion

This proof of concept phase II open-label study evaluated the activity of imatinib in treating 40 different life-threatening malignancies known to be associated with one or more

imatinib-sensitive tyrosine kinases in patients who had exhausted all existing treatment options. Of the 186 patients evaluated in the current study, 146 (78.5%) had solid tumors and 40 (20.5%) had hematologic malignancies. Response to imatinib was higher in the hematologic malignancy group than in the solid tumor group (overall response, 27.5% versus 8.9%). Six malignancies were identified in which imatinib therapy was associated with one or more objective clinical responses. Responses to imatinib in patients with dermatofibrosarcoma protuberans, aggressive fibromatosis, hypereosinophilic syndrome, and myeloproliferative disorders were frequent enough to be of potential clinical utility, and more detailed clinical and molecular characterization of some patients from this study with these specific diagnoses has previously been published (16, 19, 24–26). In addition, our current results are consistent with the published literature documenting the activity of imatinib for these tumor types (20, 21, 23, 24, 26–30). Notably, applications for the use of imatinib to treat malignancies such as dermatofibrosarcoma protuberans, hypereosinophilic syndrome, myeloproliferative

disorder, as well as systemic mastocytosis have already been approved by health authorities worldwide, partially on the basis of results from the current study.

For the remaining malignancies studied, imatinib had no significant activity, with no CRs, low to zero frequency of PRs, and a short median time to disease progression. The majority of solid tumor patients had metastatic non-gastrointestinal stromal tumor sarcoma. The lack of efficacy of imatinib for this heterogeneous group of patients confirms previous reports (8, 31). Although no confirmed responses were observed in the chordoma group, the majority of patients (80.0%) achieved stable disease, with no patient having progressive disease. Notably, several other studies of advanced chordoma patients have reported activity of imatinib alone or in combination with chemotherapy (22, 32, 33). Further investigation of imatinib for this indication is warranted.

In previous studies of non-gastrointestinal stromal solid tumors, there was no correlation between KIT-expression as assessed by immunohistochemistry and response to imatinib therapy. For example, imatinib showed no activity in two phase II studies enrolling a total of 42 malignant melanoma patients (17 patients had KIT-positive tumors; refs. 34, 35). Similarly, despite selection for patients with KIT-positive tumors, none of the eight melanoma patients in the current study responded to imatinib treatment. Although mutations in KIT have recently been described in melanoma, none of the seven melanomas tested in our study harbored a KIT mutation (36). The efficacy of imatinib for treatment of advanced KIT-mutated melanoma is currently under investigation.

Several case reports of objective responses of metastatic adenoid cystic carcinoma to imatinib treatment have been published (37, 38). However, in two separate phase II studies of adenoid cystic carcinoma, no objective responses were noted in a total of 30 patients (26 patients had confirmed KIT-positive tumors; refs. 39, 40). In the current study, none of the 12 adenoid cystic carcinoma patients with confirmed expression of KIT had a CR or PR. Therefore, detection of KIT by immunohistochemistry is not necessarily predictive of a response to imatinib therapy. The lack of a clinical response associated with imatinib treatment of patients with desmoplastic small round cell tumor, mesothelioma, and chordoma in the current study also suggests that tumor-specific expression of PDGF (desmoplastic small round cell tumor and mesothelioma) or activation of PDGFR (chordoma) is not by itself predictive of response to imatinib therapy (41–43).

Mutational screening in this study confirms the hypothesis that intragenic activating mutations of KIT or PDGFR are rare outside of gastrointestinal stromal tumor, seminoma, melanoma, acute myeloid leukemia, and systemic mastocytosis (15, 36, 44–48). Notably, almost all favorable responses to imatinib in the current trial were in malignancies for which activation of an imatinib-sensitive tyrosine kinase (predominantly PDGFR) was shown to occur via genomic mutation and/or rearrangement (dermatofibrosarcoma protuberans, hypereosinophilic syndrome, PDGFRB-rearranged myeloproliferative disorders, and systemic mastocytosis)—the only exceptions being two patients with aggressive fibromatosis and one

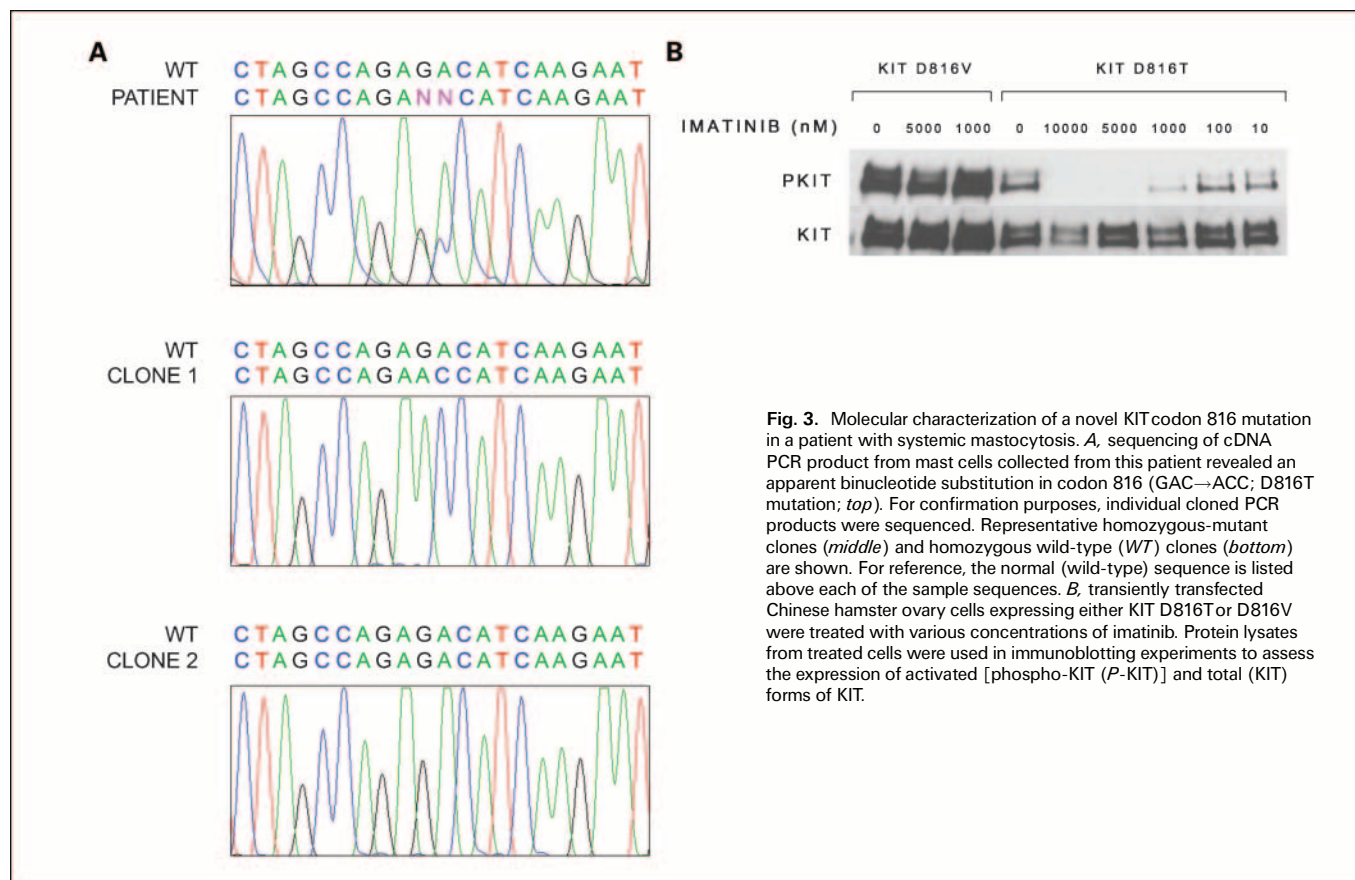


Fig. 3. Molecular characterization of a novel KIT codon 816 mutation in a patient with systemic mastocytosis. **A**, sequencing of cDNA PCR product from mast cells collected from this patient revealed an apparent binucleotide substitution in codon 816 (GAC→ACC; D816T mutation; *top*). For confirmation purposes, individual cloned PCR products were sequenced. Representative homozygous-mutant clones (*middle*) and homozygous wild-type (WT) clones (*bottom*) are shown. For reference, the normal (wild-type) sequence is listed above each of the sample sequences. **B**, transiently transfected Chinese hamster ovary cells expressing either KIT D816T or D816V were treated with various concentrations of imatinib. Protein lysates from treated cells were used in immunoblotting experiments to assess the expression of activated [phospho-KIT (P-KIT)] and total (KIT) forms of KIT.

patient with synovial sarcoma. Isolated cases of prolonged stable disease were observed in a few other tumor categories. Based on these data, it is not clear if the activity of imatinib in these tumors is due to inhibition of imatinib-sensitive tyrosine kinase signaling in tumors or occurs by other mechanisms.

In this study, one patient with systemic mastocytosis had a favorable response to imatinib and was found to have a novel imatinib-sensitive KIT mutation (D816T). This result is consistent with other reports of favorable responses of systemic mastocytosis patients with imatinib-sensitive kinase mutations to imatinib treatment (30, 49). Therefore, molecular characterization of systemic mastocytosis cases may be useful in identifying patients for treatment with imatinib or other KIT kinase inhibitors (50).

Immunoblot analyses for total and tyrosine-phosphorylated KIT, PDGFRA, and PDGFRB were done in cases where adequate tumor samples were available. These experiments failed to show a relationship between activation of wild-type imatinib-sensitive tyrosine kinases and clinical response to imatinib; however, this approach may not have been sensitive enough to detect low-level, but biologically relevant, activation of these kinases (24).

In summary, imatinib treatment in this study was associated with favorable outcomes in patients with dermatofi-

brosarcoma protuberans, hypereosinophilic syndrome, myeloproliferative disorders, and systemic mastocytosis, adding to data showing the therapeutic efficacy of imatinib in tumor types with genomic mechanisms of activating imatinib-sensitive tyrosine kinases. In addition, imatinib treatment was associated with clinical benefit in some patients with chordoma or aggressive fibromatosis, two diseases in which genomic activation of imatinib-sensitive tyrosine kinases has not been shown. Imatinib showed no appreciable anticancer activity against the remaining malignancy types studied. Further investigation is required to determine whether the activity of imatinib in tumor types with apparently wild-type KIT and PDGFR signaling pathways occurs via inhibition of target kinase activity in the tumor cells or by other mechanisms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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