Kinase Mutations and Imatinib Response in Patients With Metastatic Gastrointestinal Stromal Tumor

By Michael C. Heinrich, Christopher L. Corless, George D. Demetri, Charles D. Blanke, Margaret von Mehren, Heikki Joensuu, Laura S. McGreevey, Chang-Jie Chen, Annick D. Van den Abbeele, Brian J. Druker, Beate Kiese, Burton Eisenberg, Peter J. Roberts, Samuel Singer, Christopher D.M. Fletcher, Sandra Silberman, Sasa Dimitrijevic, and Jonathan A. Fletcher

Purpose: **Most gastrointestinal stromal tumors (GISTs) express constitutively activated mutant isoforms of KIT or kinase platelet-derived growth factor receptor alpha (PDGFRA) that are potential therapeutic targets for imatinib mesylate. The relationship between mutations in these kinases and clinical response to imatinib was examined in a group of patients with advanced GIST.**

Patients and Methods: **GISTs from 127 patients enrolled onto a phase II clinical study of imatinib were examined for mutations of *KIT* or *PDGFRA*. Mutation types were correlated with clinical outcome.**

Results: **Activating mutations of *KIT* or *PDGFRA* were found in 112 (88.2%) and six (4.7%) GISTs, respectively. Most *KIT* mutations involved exon 9 (n = 23) or exon 11 (n = 85). All KIT mutant isoforms, but only a subset of PDGFRA mutant isoforms, were sensitive to imatinib, in vitro. In patients with GISTs harboring exon 11 *KIT* mutations, the partial response rate (PR) was 83.5%, whereas patients with tumors containing an exon 9 *KIT* mutation or no detectable mutation of *KIT* or *PDGFRA* had PR rates of 47.8% (P = .0006) and 0.0% (P < .0001), respectively. Patients whose tumors contained exon 11 *KIT* mutations had a longer event-free and overall survival than those whose tumors expressed either exon 9 *KIT* mutations or had no detectable kinase mutation.**

Conclusion: **Activating mutations of *KIT* or *PDGFRA* are found in the vast majority of GISTs, and the mutational status of these oncoproteins is predictive of clinical response to imatinib. *PDGFRA* mutations can explain response and sensitivity to imatinib in some GISTs lacking *KIT* mutations.**

J Clin Oncol 21:4342-4349.

GASTROINTESTINAL STROMAL tumors (GISTs) are mesenchymal neoplasms that arise primarily in the gut wall and are typically characterized by the expression of the receptor tyrosine kinase *KIT* (CD117).[1,2] Recent studies have established that activating mutations of KIT are present in up to 92% of GISTs and likely play a fundamental role in the development of these tumors.[3-10] The subset of GISTs that lack detectable *KIT* mutations can be divided into a group that has activating mutations in the related tyrosine kinase platelet-derived growth factor receptor alpha (*PDGFRA*) and a group without identified kinase mutations.[11]

Imatinib (formerly STI571; Gleevec in the United States and Glivec in Europe; Novartis Pharma, Basel, Switzerland) is a competitive inhibitor of BCR-ABL, ARG, KIT, PDGFRA, and PDGFRB tyrosine kinases.[12-15] In preclinical studies, imatinib was active against mutant isoforms of KIT commonly found in GIST.[13,16] Subsequent treatment of a patient with metastatic GIST resulted in marked clinical, radiologic, and pathologic improvement.[17] The clinical activity of imatinib for unresectable, metastatic GISTs has been documented in two clinical studies.[18,19]

In the present report, pretreatment GIST samples from patients enrolled onto a multicenter, open-label, randomized phase II study of imatinib treatment of metastatic GIST were analyzed for *KIT* or *PDGFRA* mutations with the aim of correlating clinical response to imatinib with tumor genotype.[19] In addition, the kinase activities of GIST-associated KIT and PDGFRA mutant isoforms were tested for sensitivity to imatinib in vitro in an effort to confirm the relevance of these molecular mechanisms to the observed clinical outcomes.

PATIENTS AND METHODS

Analysis of KIT *and* PDGFRA *Mutations*

Archival pretreatment pathology specimens were obtained from patients enrolled onto a randomized phase II trial of imatinib for metastatic GIST (CSTI571B 2222). The clinical design and primary clinical results have been previously published.[19] The study was approved by the local institutional review board of each participating institution, and written informed consent was obtained from each patient. In addition, informed consent for the

From the Oregon Health and Science University Cancer Institute, Oregon Health and Science University, and Portland Veterans Affairs Medical Center, Portland, OR; Dana-Farber Cancer Institute and Harvard Cancer Center, Boston, MA; Fox-Chase Cancer Center, Philadelphia, PA; University of Turku, Turku; University of Helsinki, Helsinki, Finland; and Novartis Oncology, Basel, Switzerland.

Submitted April 29, 2003; accepted September 15, 2003.

This work was supported by a research grant from Novartis Pharma AG, Basel, Switzerland, and grants from the Department of Veterans Affairs, Washington, DC (M.C.H.), Northwest Health Foundation, Portland, OR (M.C.H. and C.L.C.), a pilot grant from the Oregon Health and Science University Cancer Institute, Portland, OR (C.D.B., C.L.C., and M.C.H.), the Abraham J. and Phyllis Katz Foundation, Forest Hills, NY (G.D.D.), the Rubenstein Foundation, Newton, MA (G.D.D.), Leslie's Links and the Semonian Family Foundation, Boston, MA (G.D.D.).

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to Michael Heinrich, MD, R&D-19 3710 SW US Veterans Hospital Rd, Portland, OR 97207; e-mail: heinrich@ohsu.edu.

0732-183X/03/2123-4342/$20.00

DOI: 10.1200/JCO.2003.04.190

analysis of tumor-associated genetic alterations was obtained independently of patient consent for participation in the clinical study. Sections were prepared from formalin-fixed, paraffin-embedded pretreatment specimens trimmed to enrich for tumor cells. Polymerase chain reaction amplification of genomic DNA for *KIT* and *PDGFRA* was performed, and amplicons were analyzed for mutations as previously described.[10,11,20,21] In 15 cases with available frozen material, the entire *KIT* cDNA was sequenced.[4]

In Vitro Studies

KIT and *PDGFRA* mutations were cloned by site-directed mutagenesis of the respective wild-type cDNA.[11,22] All mutations were confirmed by bidirectional sequencing. Chinese hamster ovary cells were transiently transfected with plasmids encoding cDNAs for wild-type or mutant proteins.[11,23] Twenty-four hours after transfection, the cells were treated with control media or media containing various concentrations of imatinib for 90 minutes.[13] The cells were then collected, and protein lysates were prepared and analyzed for KIT or PDGFRA activation as previously described.[4,11] Experiments involving recombinant DNA were performed using BL2 procedures in accordance with National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules.

Statistical Analysis

Best clinical response to imatinib were classified as partial responses (PR), stable disease, progressive disease (PD), or nonassessable (NA) determined using standard Southwest Oncology Group response criteria[24] as listed in the report of the primary clinical results of this trial.[19] Response rates were calculated using an intention-to-treat analysis. Seven patients were classified as NA for response. Five of these patients had early adverse events, and therefore clinical response could not be assessed or confirmed (two patients with exon 11 mutations, two patients with exon 9 mutations, and one patient with no mutation). After central radiology review, two patients were deemed to have assessable but not measurable disease (one patient with a *KIT* exon 11 mutation and one patient with *PDGFRA* D842V mutation). Patients with NA disease were included in the calculations of event-free survival and survival. Tumor response rates were compared among mutation groups using Fisher's exact test.[25] Event-free survival and overall survival was estimated using the Kaplan-Meier method and the differences among mutation groups were compared using a log-rank test.[26] For the event-free survival analysis, end point clinical events were defined as PD, patient death from any cause, withdrawal of patient consent, or discontinuation of therapy as a result of toxicity. The current report includes all available patient follow-up data through August 27, 2002.

Logistic and proportional hazards models were fitted to the response, event-free survival, and overall survival data to assess the possible interactions of mutational status and clinical outcome. The following patient characteristics were assessed: daily imatinib dose, sex, Eastern Cooperative Oncology Group performance status at baseline, prior chemotherapy, age greater than 65 years, presence of liver metastases, decreased pretreatment serum albumin, elevated pretreatment liver transaminases, elevated pretreatment creatinine, presence or absence of exon 9 *KIT* mutation, presence or absence exon 11 *KIT* mutation, absence of any *KIT* or *PDGFRA* mutation, presence or absence of other genotype (mutation of *KIT* exon 13 or mutation of *KIT* exon 17 or any *PDGFRA* mutation), and no specimen available for genotyping. A step-wise regression procedure was conducted to retain those variables with a $P < .1$.[27]

RESULTS

Spectrum of Mutations in GIST Patients Enrolled Onto the Phase II Trial

Tumor specimens suitable for genetic analysis were available from 127 (86.4%) of the 147 patients enrolled in this study. In four additional patients, a sample was obtained but proved unsuitable because of an insufficient amount of GIST in the specimen. The results of the genotyping studies are graphically depicted in Fig 1. Overall, 112 (88.2%) of the 127 GISTs evaluated had activating mutations of *KIT* exon 9, 11, 13, or 17. No GIST had an activating mutation in more than one *KIT* exon. The most common type of mutation (71 patients) was in-frame deletion of a portion of the juxtamembrane domain (exon 11). These deletion mutations were sometimes accompanied by point mutations or small insertions involving amino acid residues immediately preceding or after the deletion, or both. Isolated point mutations of *KIT* exon 11 were confined to codons 557 (three cases), 559 (three cases), 560 (six cases), and 576 (two cases). The second most common mutation type was in-frame duplication of nucleotides in *KIT* exon 9 resulting in the

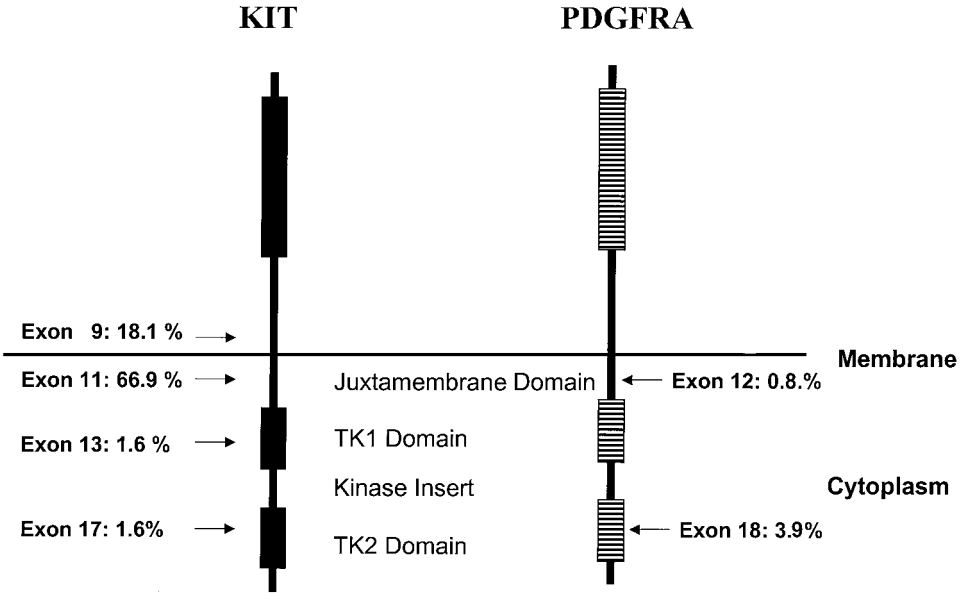

Fig 1. Structure of KIT and platelet-derived growth factor receptor alpha (PDGFRA). The locations of gastrointestinal stromal tumor (GIST)-associated kinase mutations are shown in relationship to the structural features of the proteins. GISTs from 9 (7.1%) of 127 patients had no detectable *KIT* or *PDGFRA* mutation.

previously described insertion of AY residues at codon 502 (22 patients) or a novel insertion of FAF residues at position 506 (one patient).[4]

The entire coding region of *KIT* mRNA was analyzed in 15 tumors for which frozen tumor was available, using a combination of reverse transcriptase polymerase chain reaction and direct sequencing. In all cases, including one case with no detectable mutation, these results confirmed the genomic DNA analyses; no additional mutations were discovered. In 10 (7.9%) of the 127 cases, no wild-type *KIT* allele was detected, indicating a homozygous or hemizygous genotype. Because of the presence of normal tissue elements in most of the GIST specimens analyzed, 7.9% represents a minimum estimate of the fraction of GISTs that express only mutant KIT.

Fifteen of 127 cases had no detectable *KIT* mutation (*KIT* wild-type [WT]). Given our recent finding of gain-of-function PDGFRA mutations in *KIT*-WT GISTs, we tested these cases for *PDGFRA* mutations in the proximal extracellular (exon 10), juxtamembrane (exon 12), TK1 (exon 14), and activation loop (exon 18) domains. Six of the *KIT*-WT tumors had a *PDGFRA* mutation (40.0% of *KIT*-WT). Five of these mutations were in the kinase activation loop, including the previously described point mutation D842V (three patients) and deletion DIMH842–845 (one patient), as well as a novel deletion of I843 (one patient).[11] The remaining tumor contained the previously described point mutation V561D in the PDGFRA juxtamembrane domain. None of these cases was included in our original report of *PDGFRA* mutations in GISTs.[11] Screening of 97 cases from this study that had a documented *KIT* mutations failed to yield any *PDGFRA* exon 18 mutations, supporting our previous observation that gain-of-function mutations in KIT and PDGFRA are mutually exclusive in GISTs.[11]

In Vitro Activity of Imatinib Against Representative KIT and PDGFRA Oncoproteins Associated With GISTs

Imatinib binds reversibly to the ATP-binding pocket of ABL, ARG, KIT, PDGFRA, and PDGFRB but not other tyrosine kinases.[9,12,15] To assess the drug sensitivity of KIT oncoproteins associated with GISTs, the mutant isoforms were expressed in Chinese hamster ovary cells, and inhibition of autophosphorylation was examined using varying concentrations of imatinib. The KIT isoforms that were tested included the most commonly identified codons altered by point mutation (V560G) or deletion mutation (del 557 to 558 WK) in exon 11, the exon 9 insertion (ins AY at codon 503), and the point mutations observed in exon 13 (K642E) and exon 17 (N822H, N822K). As shown in Fig 2A, all of these mutant isoforms were as sensitive to imatinib as wild-type KIT (concentration that inhibits phosphorylation by 50% [IC_{50}], 100 to 200 nmol/L). In contrast, and as previously described,[28] the mastocytosis-associated D816V isoform was resistant to imatinib up to 10 μmol/L.[28] The sensitivity of another exon 11 deletion (del codon 579) was similar to that of the other GIST-associated KIT mutations (data not shown).

Similar assays were performed to test the potency of imatinib against wild-type and mutant isoforms of PDGFRA. In contrast to native PDGFRA, the mutant PDGFRA isoforms were strongly phosphorylated in the absence of PDGF-AA ligand (Fig 2B). Phosphorylation of ligand-stimulated native PDGFRA was potently inhibited by imatinib (IC_{50}, 100 to 200 nmol/L), consistent with earlier reports.[12] Imatinib was similarly effective against the V561D, del DIMH842–845, and delI843 PDGFRA isoforms (IC_{50}, 100 to 200 nmol/L), whereas inhibition of the D842V mutant required 10- to 20-fold higher drug levels (IC_{50}, approximately 1 to 2 μmol/L).

Correlation of KIT *Mutational Status With Clinical Response to Imatinib*

Best clinical response to imatinib were classified as PR, stable disease, PD, or NA determined using standard Southwest Oncology Group response criteria[24] as listed in the report of the primary clinical results of this trial.[19] No patient in the study had a complete response.[19] Response rates were calculated using an intention-to-treat analysis. Response data for the various tumor genotypes are listed in Table 1. Patients whose tumor expressed an exon 11 mutant KIT protein were much more likely to have a PR with imatinib therapy (83.5%) than patients whose tumor expressed either an exon 9 mutant isoform protein (47.8%; $P = .0006$) or contained no detectable mutation of KIT or PDGFRA (0.0%; $P < .0001$). The frequency of a PR was also significantly different between patients with an exon 9 mutation versus no detectable mutation ($P = .013$). There was no statistically significant difference in the response rates between the group of patients with *KIT* exon 11 point mutations and the group with exon 11 deletion mutations or between the group with heterozygous tumors and the group with tumors homozygous/hemizygous for exon 11 deletion mutation (data not shown). No statistically significant difference in the response rates between the two doses of imatinib for any of the genotype subgroups was found. A step-wise logistical regression analysis was performed to identify other clinical factors that might predict response to imatinib. The strongest predictor of response was the presence of a *KIT* exon 11 mutation (hazard ratio, 7.85; 95% CI, 3.55 to 17.37). The only other variable noted in this regression analysis to predict lack of response to imatinib was an elevated creatinine at baseline (hazard ratio, 0.27; 95% CI, 0.08 to 0.92).

The number of *PDGFRA*-mutant GISTs in the study was too small to define a relationship between *PDGFRA* mutations and response to imatinib. Nevertheless, none of the patients with the imatinib-resistant D842V mutation responded to drug (two patients with PD, one patient classified as NA as a result of technical difficulties in disease measurement), whereas two of three patients with imatinib-sensitive PDGFRA oncoproteins achieved a PR with imatinib therapy.

Correlation of Tumor Genotype With Event-Free and Overall Survival

Event-free survival for the entire patient population was estimated by Kaplan-Meier analysis. With a median follow-up of approximately 19 months (594 days), 81 (55.1%) of 147 patients had experienced one or more end point clinical events, and the median event-free survival was approximately 17 months (Fig 3A). Event-free survival was also analyzed for the three largest

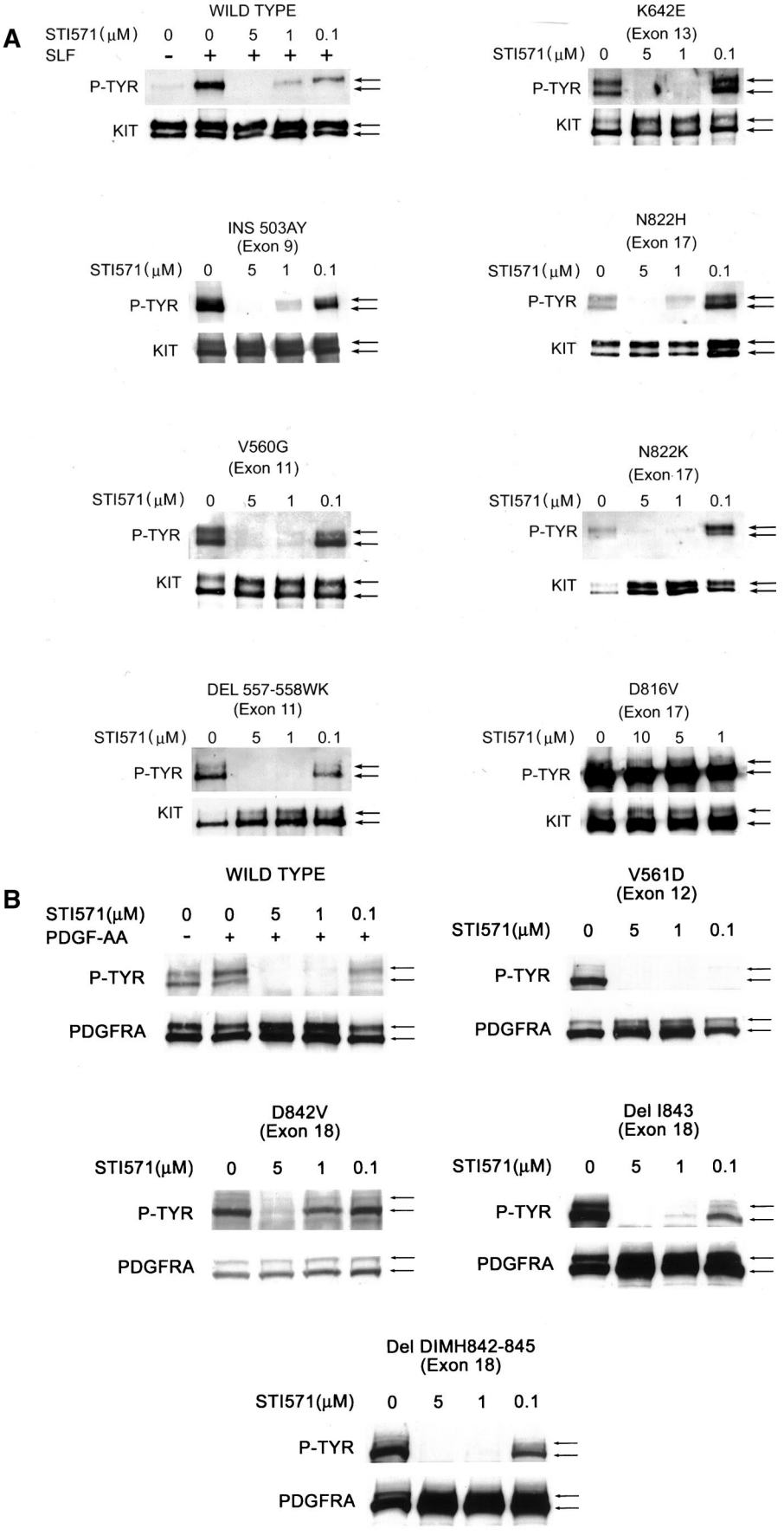

Fig 2. (A) In vitro sensitivity of wild-type and mutant KIT isoforms to imatinib. All gastrointestinal stromal tumor-associated KIT mutant isoforms were inhibited by imatinib with a similar sensitivity as ligand-activated wild-type KIT. In contrast, the mastocytosis-associated D816V mutant isoform was not inhibited by imatinib. (B) In vitro sensitivity of wild-type and mutant platelet-derived growth factor receptor alpha (PDGFRA) isoforms to imatinib. The V561D, deletion I843, and deletion DIMH 842-845 mutant isoforms had similar sensitivity to imatinib as ligand-activated wild-type PDGFRA. In contrast, the D842V mutant isoform was 10- to 20-fold more resistant to imatinib.

Table 1. *KIT* and *PDGFRA* Genotype Versus Clinical Response in the CSTI571B 2222 Phase II Trial

Genotype	Partial Response		Stable Disease		Progressive Disease		Nonassessable	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
KIT exon 11, n = 85	71	83.5	7	8.2	4	4.7	3	3.5
KIT exon 9, n = 23	11	47.8	6	26.1	4	17.4	2	8.7
No *PDGFRA* or *KIT* mutation, n = 9	0	0.0	3	33.3	5	55.6	1	11.1
PDGFRA-sensitive, n = 3	2	66.7	0	0.0	1	33.3	0	0.0
PDGFRA-resistant D842V, n = 3	0	0.0	0	0.0	2	66.7	1	33.3
KIT exon 13, n = 2	2	100.0	0	0.0	0	0.0	0	0.0
KIT exon 17, n = 2	1	50.0	0	0.0	1	50.0	0	0.0

NOTE. Each of the *KIT* genotypes is categorized by the exon location of the mutation (all are imatinib-sensitive), whereas *PDGFRA* genotypes are categorized as either sensitive (*PDGFRA*-sensitive) or resistant (D842V) to imatinib.

Abbreviation: *PDGFRA*, platelet-derived growth factor receptor alpha.

groups of kinase genotypes represented in this study: mutation of *KIT* exon 11, *KIT* exon 9, or no detectable mutation of *KIT* or *PDGFRA*. As depicted in Fig 3C, patients whose tumors expressed an exon 11 mutant KIT isoform were much less likely to experience treatment failure than patients whose tumors expressed an exon 9 mutant KIT isoform ($P < .0001$) or were without a detectable mutation in *KIT* or *PDGFRA* ($P < .0001$). There was no significant difference in the rate of treatment failure for the group with *KIT* exon 9 mutation compared with those with no detectable *KIT* or *PDGFRA* mutation ($P = .14$). In patients without detectable *KIT* or *PDGFRA* mutation, the median event-free survival was 82 days. In contrast, the median event-free survival for patients with a *KIT* mutation of exons 9 or 11 was 200 days and 687 days, respectively.

A proportional hazards model for event-free survival was fitted with the potential prognostic factors described above (Table 2).[27] In the stepwise regression analysis, several variables were noted to be correlated with the risk of experiencing an adverse clinical event; these included exon 11 mutation status, daily imatinib dose, poor baseline Eastern Cooperative Oncology Group performance status, and having no specimen available for genotyping (unknown kinase mutational status). The presence of a *KIT* exon 11 mutation was the strongest prognostic factor and reduced the risk of adverse clinical events by more than 80%. The protective effect of an unknown mutational status is likely due to the fact that approximately 67% of such patients would be expected to have a *KIT* exon 11 mutation and are therefore at a reduced risk of adverse clinical events. Clearly, it is better to have a 67% chance of a favorable genotype than to have a documented unfavorable genotype (eg, no kinase mutation of *KIT* or *PDGFRA*).

On the basis of Kaplan-Meier analysis, the overall survival for the entire patient population at 76 weeks was 85% (Fig 3B). As depicted in Fig 3D, patients whose tumors expressed an exon 11 mutant KIT isoform had improved survival compared with patients whose tumor expressed an exon 9 mutant KIT isoform ($P = .0034$) or whose tumor had no detectable mutation of KIT or PDGFRA ($P < .0001$). There was also a significant difference in survival in favor of the *KIT* exon 9 mutation subgroup compared with those patients with no detectable *KIT* or *PDGFRA* mutation ($P = .0067$). As with event-free survival, a proportional hazards model was fitted with the potential prognostic factors described above (Table 2). The presence of a *KIT* exon 11 mutation was the strongest prognostic factor, reducing the risk of death by more than 95%.

DISCUSSION

Therapeutic responses to targeted inhibition of activated tyrosine kinases have been demonstrated in certain types of leukemia, sarcoma, and breast cancer.[29-32] The mechanisms of kinase activation vary considerably among these cancers, but the influence of these mechanisms on drug response has not been well studied. GISTs, in particular, present a variety of genomic mutations across two different receptor tyrosine kinase genes. We report here that the type of *KIT* or *PDGFRA* mutation in clinically advanced GISTs is predictive of the response to imatinib therapy. We show that most GISTs express kinase oncoproteins that are intrinsically sensitive to imatinib, accounting for the excellent overall clinical response to imatinib. Nonetheless, a minority of GISTs express kinase oncoproteins that are either intrinsically resistant to imatinib, or are associated with poor clinical response despite in vitro sensitivity to imatinib. These findings highlight the relevance of molecular oncogenic mechanisms in determining response to targeted therapies in cancer.

Gain-of-function mutations of *PDGFRA* were only recently discovered in GISTs.[11] The current series of tumors is the largest that has been examined for *PDGFRA* mutations and confirms that *PDGFRA* and *KIT* mutations are mutually exclusive. *PDGFRA* mutations were demonstrated in 4.7% of the genotyped GISTs in this clinical trial and involved domains homologous to those often mutated in *KIT*. The data from this study support a mechanistic link between PDGFRA activation and imatinib activity in GIST patients whose disease does not express a mutant KIT protein. Whereas all GIST-associated mutant KIT isoforms examined had in vitro sensitivity to imatinib similar to that of wild-type KIT protein, the PDGFRA D842V mutant was substantially more resistant to the drug. The imatinib-resistant KIT D816V mutation in human mastocytosis and the PDGFRA D842V mutation involve the same conserved aspartic acid residue in the kinase activation loop, suggesting a common basis for imatinib resistance in KIT and PDGFRA oncoproteins activated by this mechanism.[28] On the basis of studies of ABL kinase mutations that are correlated with clinical resistance to imatinib in chronic myelogenous leukemia, the level of in vitro resistance manifested by the PDGFRA D842V isoform

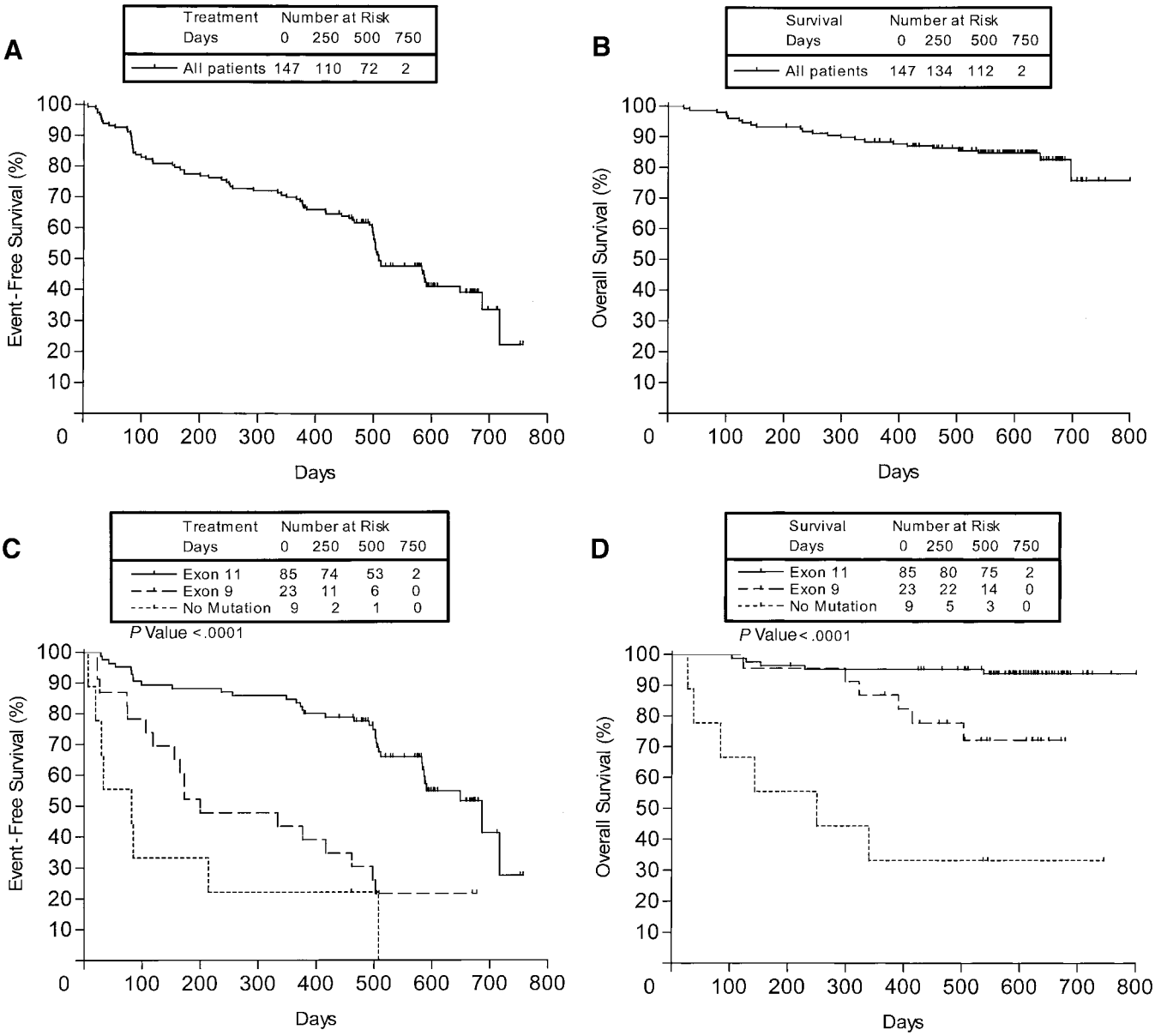

Fig 3. Gastrointestinal stromal tumor (GIST) kinase genotype correlates with event-free survival and overall survival. Kaplan-Meier estimate of the probability of event-free survival (A) and overall survival for all patients in the CSTI571B 2222 phase II GIST study (B).[19] Kaplan-Meier estimate of the probability of event-free survival (C) and overall survival (D) for patients with *KIT* exon 11 mutation, *KIT* exon 9 mutation, or no mutation of *KIT* or platelet-derived growth factor receptor alpha (*PDGFRA*). The log-rank *P* value is listed above each graph.

would be predicted to result in clinical resistance.[33] Consistent with this prediction, none of the three patients whose tumor harbored the D842V mutation showed a clinical response. The other mutant isoforms of PDGFRA were sensitive to imatinib in vitro, and two of three patients bearing tumors with these mutations responded well to therapy. These findings provide a basis for imatinib response in some KIT-WT GISTs and suggest that imatinib can be used successfully in the treatment of GISTs driven by imatinib-sensitive PDGFRA oncoproteins. These data are complementary to the data by Cools et al,[34] who reported the efficacy of imatinib in treating patients with hypereosinophilic syndrome associated with the oncogenic FIP1L1-PDGFRA fusion protein. It remains to be proven whether imatinib will have therapeutic activity against other solid tumors driven by wild-type or oncogenic PDGFRA kinase.

A subset of GIST tumors in this study lacked detectable *KIT* or *PDGFRA* mutations. Although such GISTs lack apparent genomic mutations, they can express phosphorylated KIT or PDGFRA proteins that likely contribute to tumor proliferation or survival.[35,36] In the present study, GISTs lacking a detectable kinase mutation had a lower overall response to imatinib (0.0%) than tumors with an exon 11 mutation (83.5%) or an exon 9 mutation (47.8%). Event-free and overall survival were also significantly shorter in patients whose GISTs lacked a detectable kinase mutation. These results suggest that GISTs lacking a *KIT* or

Table 2. Independent Factors Predictive of Event-Free and Overall Survival

Factor	Event-Free Survival			Overall Survival		
	Hazard Ratio	95% CI	*P*	Hazard Ratio	95% CI	*P*
Poor performance status at baseline	1.57	1.18 to 2.1	.0022	2.8	1.57 to 5.02	.0005
Daily dose of 600 mg	0.57	0.36 to 0.91	.018	NI		NI
KIT Exon 11 mutation	0.17	0.10 to 0.29	< .0001	0.04	0.01 to 0.12	< .0001
No specimen for genotyping	0.31	0.16 to 0.62	.0008	0.14	0.03 to 0.57	.0065
Elevated creatinine at baseline	NI		NI	2.9	0.86 to 9.80	.0866
Prior chemotherapy	NI		NI	2.6	0.98 to 7.06	.0558
Exon 9 mutation	NI		NI	0.31	0.11 to 0.89	.0289

NOTE. *P* values were derived from the Cox proportional hazards model, with simultaneous inclusion of all factors shown.
Abbreviation: NI, variables not included in the model after step-wise selection procedure.

PDGFRA mutation are biologically distinct and might be less dependent on these kinases than GISTs expressing mutant kinases.

The PR rate and event-free survival also differed between the groups of patients whose GISTs had *KIT* exon 9 versus exon 11 mutations. This finding is notable, because the KIT oncoproteins encoded by exon 9 and exon 11 mutations were equally sensitive to imatinib in vitro (Fig 2A). Preliminary studies suggest differences in downstream signaling in exon 9 versus exon 11 KIT-mutant GISTs,[37] and such biologic differences might influence the susceptibility of the tumor cells to apoptosis in response to kinase suppression by imatinib. Alternatively, the activation mechanisms for KIT exon 9 mutants might vary between the in vitro and in vivo settings. It is also noteworthy that in many patients, disease progression did not occur until 12 months after initiating treatment with imatinib. Preliminary studies suggest that the molecular mechanisms for late resistance to imatinib in GISTs may be analogous to those described in patients with chronic myelogenous leukemia.[33,36,38-41]

These data provide strong evidence of a mechanistic link between expression of an imatinib-sensitive mutant KIT or PDGFRA kinase in GISTs and clinical response to imatinib. Overall, the PR rate of patients with an imatinib-sensitive mutation of KIT or PDGFRA was 75.7% (87 of 115 patients), whereas the PR rate in patients with no kinase mutation or an imatinib-resistant mutation was 0.0% (zero of 12 patients). Expressed differently, 87 of the 87 genotyped patients (100.0%) who achieved a PR during imatinib therapy had GISTs that expressed an imatinib-sensitive mutant kinase.

In conclusion, we provide evidence that *KIT* and *PDGFRA* mutational status predicts clinical response to imatinib in patients with metastatic GIST. In a subset of GISTs lacking *KIT* mutations, gain-of-function *PDGFRA* mutations can account for imatinib clinical response. Therefore, imatinib therapy should not be withheld from patients whose GISTs lack *KIT* mutations or whose GISTs do not express the KIT protein.[11] These findings emphasize that molecular subclassification of GISTs is crucial in the design and interpretation of clinical trials and in identifying patients who are at high risk for early treatment failure.

ACKNOWLEDGMENT

The acknowledgment is included in the full-text version of this article, available on-line at www.jco.org. It is not included in the PDF (via Adobe® Acrobat Reader®) version.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Acted as a consultant within the last 2 years: George D. Demetri, Novartis; Brian J. Druker, Novartis. Received more than $2,000 a year from a company for either of the last 2 years: George D. Demetri, Novartis; Margaret von Mehren, Novartis; Charles D. Blanke, Novartis; Heikki Joensuu, Novartis; Sandra Silberman, Novartis; Sasa Dimitrijevic, Novartis; Deate Kiese, Novartis; Michael C. Heinrich, Novartis; Christopher L. Corless, Novartis; Jonathan A. Fletcher, Novartis; and Burton Eisenberg, Novartis.

REFERENCES

1. Fletcher C, Berman J, Corless C, et al: Diagnosis of gastrointestinal stromal tumors: A consensus approach. Hum Pathol 33:459, 2002

2. Miettinen M, Lasota J: Gastrointestinal stromal tumors: Definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. Virchows Arch 438:1-12, 2001

3. Lasota J, Wozniak A, Sarlomo-Rikala M, et al: Mutations in exons 9 and 13 of KIT gene are rare events in gastrointestinal stromal tumors: A study of 200 cases. Am J Pathol 157:1091-1095, 2000

4. Lux ML, Rubin BP, Biase TL, et al: KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. Am J Pathol 156:791-795, 2000

5. Lasota J, Jasinski M, Sarlomo-Rikala M, et al: Mutations in exon 11 of c-Kit occur preferentially in malignant versus benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. Am J Pathol 154:53-60, 1999

6. Moskaluk CA, Tian Q, Marshall CR, et al: Mutations of c-kit JM domain are found in a minority of human gastrointestinal stromal tumors. Oncogene 18:1897-1902, 1999

7. Taniguchi M, Nishida T, Hirota S, et al : Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. Cancer Res 59:4297-4300, 1999

8. Hirota S, Isozaki K, Moriyama Y, et al: Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 279:577-580, 1998

9. Buchdunger E, Zimmermann J, Mett H, et al: Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res 56:100-104, 1996

10. Corless CL, McGreevey L, Haley A, et al: KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. Am J Pathol 160:1567-1572, 2002

11. Heinrich MC, Corless CL, Duensing A, et al: PDGFRA Activating mutations in gastrointestinal stromal tumors. Science 299:708-710, 2003

12. Buchdunger E, Cioffi CL, Law N, et al: Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-Kit and

platelet-derived growth factor receptors. J Pharmacol Exp Ther 295:139-145, 2000

13. Heinrich MC, Griffith DJ, Druker BJ, et al: Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. Blood 96:925-932, 2000

14. Druker BJ, Tamura S, Buchdunger E, et al: Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2:561-566, 1996

15. Okuda K, Weisberg E, Gilliland DG, et al: ARG tyrosine kinase activity is inhibited by STI571. Blood 97:2440-2448, 2001

16. Tuveson DA, Willis NA, Jacks T, et al: STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: Biologic and clinical implications. Oncogene 20:5054-5058, 2001

17. Joensuu H, Roberts PJ, Sarlomo-Rikala M, et al: Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N Engl J Med 344:1052-1056, 2001

18. Van Oosterom AT, Judson I, Verweij J, et al: Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumors: A phase I study. Lancet 358:1421-1423, 2001

19. Demetri GD, von Mehren M, Blanke CD, et al : Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 347:472-480, 2002

20. Rader A, Avery A, Wait CL, et al: Fine-needle aspiration biopsy diagnosis of gastrointestinal stromal tumors utilizing morphology, immunocytochemistry and mutational analysis of c-kit. Cancer 93:269-275, 2001

21. Choy YS, Dabora SL, Hall F, et al: Superiority of denaturing high performance liquid chromatography over single-stranded conformation and conformation-sensitive gel electrophoresis for mutation detection in TSC2. Ann Hum Genet 63:383-391, 1999

22. Yarden Y, Kuang W-J, Yang-Feng T, et al: Human proto-oncogene c-kit: A new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 6:3341-3351, 1987

23. Bold G, Altmann KH, Frei J, et al: New anilinophthalazines as potent and orally well absorbed inhibitors of the VEGF receptor tyrosine kinases useful as antagonists of tumor-driven angiogenesis (published erratum appears in J Med Chem 43:3200, 2000). J Med Chem 43:2310-2323, 2000

24. Green S, Weiss GR: Southwest Oncology Group standard response criteria, end point definitions and toxicity criteria. Invest New Drugs 10:239-253, 1992

25. Mehta CR, Patel NR: A network algorithm for performing Fisher's exact test in r x c contingency tables. J Am Stat Assoc 78:427-434, 1983

26. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958

27. Cox DR: Regression models and life-tables. J R Stat Soc B 34:187-220, 1972

28. Ma Y, Zeng S, Metcalfe DD, et al: The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors: Kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. Blood 99:1741-1744, 2002

29. Druker BJ, Talpaz M, Resta DJ, et al: Efficacy and safety of a specific inhibitor of the bcr-abl tyrosine kinase in chronic myeloid leukemia. N Engl J Med 344:1031-1037, 2001

30. Apperley JF, Gardembas M, Melo JV, et al: Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med 347:481-487, 2002

31. Rubin BP, Schuetze SM, Eary JF, et al: Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. J Clin Oncol 20:3586-3591, 2002

32. Piccart M, Lohrisch C, Di Leo A, et al: The predictive value of HER2 in breast cancer. Oncology 61:73-82, 2001 (suppl)

33. Shah N, Nicoll J, Nagar B, et al: Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2:117-125, 2002

34. Cools J, DeAngelo DJ, Gotlib J, et al: A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 348:1201-1214, 2003

35. Rubin BP, Singer S, Tsao C, et al: KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res 61:8118-8121, 2001

36. Fletcher JA, Corless CL, Dimitrijevic S, et al: Mechanisms of resistance to imatinib mesylate (IM) in advanced gastrointestinal stromal tumor (GIST). Proc Am Soc Clin Oncol 22:815, 2003 (abstr 3275)

37. Heinrich MC, Rubin BP, Longley BJ, et al: Biology and genetic aspects of gastrointestinal stromal tumors: KIT activation and cytogenetic alterations. Hum Pathol 33:484-495, 2002

38. Gorre ME, Mohammed M, Ellwood K et al: Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293:876-880, 2001

39. Druker BJ, Sawyers CL, Kantarjian H, et al: Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 344:1038-1042, 2001

40. Branford S, Rudzki Z, Walsh S, et al: High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood 99:3472-3475, 2002

41. Hochhaus A, Kreil S, Corbin AS, et al: Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. Leukemia 16:2190-2196, 2002