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ORIGINAL REPORT

Primary and Secondary Kinase Genotypes Correlate With the Biological and Clinical Activity of Sunitinib in Imatinib-Resistant Gastrointestinal Stromal Tumor

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ABSTRACT

Purpose
Most gastrointestinal stromal tumors (GISTs) harbor mutant KIT or platelet-derived growth factor receptor α (PDGFRA) kinases, which are imatinib targets. Sunitinib, which targets KIT, PDGFRs, and several other kinases, has demonstrated efficacy in patients with GIST after they experience imatinib failure. We evaluated the impact of primary and secondary kinase genotype on sunitinib activity.

Patients and Methods
Tumor responses were assessed radiologically in a phase I/II trial of sunitinib in 97 patients with metastatic, imatinib-resistant/intolerant GIST. *KIT*/*PDGFRA* mutational status was determined for 78 patients by using tumor specimens obtained before and after prior imatinib therapy. Kinase mutants were biochemically profiled for sunitinib and imatinib sensitivity.

Results
Clinical benefit (partial response or stable disease for $\geq$ 6 months) with sunitinib was observed for the three most common primary GIST genotypes: *KIT* exon 9 (58%), *KIT* exon 11 (34%), and wild-type *KIT*/*PDGFRA* (56%). Progression-free survival (PFS) was significantly longer for patients with primary *KIT* exon 9 mutations ($P = .0005$) or with a wild-type genotype ($P = .0356$) than for those with *KIT* exon 11 mutations. The same pattern was observed for overall survival (OS). PFS and OS were longer for patients with secondary *KIT* exon 13 or 14 mutations (which involve the KIT-adenosine triphosphate binding pocket) than for those with exon 17 or 18 mutations (which involve the KIT activation loop). Biochemical profiling studies confirmed the clinical results.

Conclusion
The clinical activity of sunitinib after imatinib failure is significantly influenced by both primary and secondary mutations in the predominant pathogenic kinases, which has implications for optimization of the treatment of patients with GIST.

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INTRODUCTION

The pathogenesis of most gastrointestinal stromal tumors (GISTs) results from activating mutations of KIT or of platelet-derived growth factor receptor α (PDGFRA). More than 80% of GISTs express mutated, constitutively active KIT, and another 5% to 7% express mutated PDGFRA; 10% to 15% of tumors have no associated mutations in these kinases.[1-3]

Imatinib mesylate, a selective inhibitor of KIT and PDGFRA (and of platelet-derived growth factor receptor β [PDGFRB] and BCR-ABL kinase), has revolutionized the treatment of GIST; however, up to 14% of GISTs exhibit primary resistance to imatinib (defined as progression within 3 to 6 months of initiating therapy),[4-6] and another 40% to 50% develop resistance within 2 years of beginning therapy (ie, secondary resistance).[5,6] Sunitinib malate (SUTENT; Pfizer, New York, NY), another small-molecule tyrosine kinase inhibitor (TKI) with selectivity for KIT and PDGFRA (and for PDGFRB, all three isoforms of vascular endothelial growth factor receptor [VEGFR], FMS-like tyrosine kinase 3 [FLT3], colony-stimulating factor 1 receptor [CSF-1R], and glial cell line-derived neurotrophic factor receptor [rearranged during transfection; RET; Pfizer, New York, NY; data on file]),[7-11] has demonstrated clinical benefit in phase I to phase III

trials of patients with imatinib-resistant or -intolerant GIST.[12,13] Sunitinib has been approved multinationally for the treatment of patients with GIST for whom prior imatinib therapy failed because of disease progression or drug intolerance.

GIST responsiveness to imatinib varies by primary *KIT* genotype; exon 11-mutant GISTs are more sensitive than exon 9-mutant or wild-type GISTs (ie, those that lack *KIT* or *PDGFRA* mutations).[3,14,15] Exons 11 and 9 are the most common sites of *KIT* mutation in GIST (approximately 70% and 15% of tumors, respectively).[3,14] Secondary kinase mutations are common in GISTs that exhibit secondary resistance but not in those that exhibit primary resistance.[16,17] Secondary point mutations associated with imatinib resistance usually are located in the drug/adenosine triphosphate (ATP) binding pocket of the receptor (encoded by exons 13 and 14) or in the activation loop (encoded by exon 17).[16-28] Two recent studies that used cell-based assays reported that sunitinib inhibited the kinase activity of KIT receptors that contained mutations in the drug/ATP binding pocket that confer resistance to imatinib.[29,30] Because these mutations (ie, T670I and V654A [substitutions of isoleucine for threonine at position 670 and alanine for valine at position 654, respectively]) are commonly found in patients with GIST who have secondary imatinib resistance, the results provide a possible basis for sunitinib antitumor activity in patients with imatinib-refractory GIST.

To further explore the relationship between primary and secondary GIST kinase mutations and the response to sunitinib, we determined primary and secondary *KIT* or *PDGFRA* mutations in biopsied tissue from patients with imatinib-refractory GIST who received sunitinib as part of a phase I/II trial,[12] and we correlated the presence of these mutations with clinical benefit. In addition, in vitro studies assessed the sensitivity of KIT and PDGFRA mutants to sunitinib and imatinib directly.

PATIENTS AND METHODS

Biopsies for genotype analyses were obtained from patients enrolled on a sunitinib phase I/II trial that was described in an earlier report of efficacy/safety results from the study.[12] Patients were adults who had histologically confirmed metastatic/unresectable GIST and documented failure of imatinib caused by resistance or intolerance. Most patients (55 of 97) received sunitinib 50 mg/d in 6-week cycles that comprised 4 weeks on, followed by 2 weeks off, treatment. Additional information about methods is listed in the Appendix (online only).

RESULTS

Primary Tumor Genotype and Efficacy

Tissue for pre-imatinib genotype analysis was available for 78 of 97 patients on the trial. These patients overall had bulky metastatic disease and had received a median of 78 weeks of prior imatinib therapy (Table 1). Primary *KIT* mutations were identified in 83% of tumors, whereas 5% had *PDGFRA* mutations, and 12% contained wild-type *KIT* and *PDGFRA* (Appendix Table A1, online only). The most *KIT* mutations (69%) were located in exon 11, then in exon 9 (30% of *KIT* mutations), and then in exon 13 (2% of *KIT* mutations). *PDGFRA* mutations were located in exon 12 in one patient's tumor and in exon 18 in the tumors of three patients.

Clinical benefit (partial response [PR] or stable disease [SD] for ≥ 6 months) was observed for the three most common GIST genotypes (Table 2). The clinical benefit rate was 58% for tumors with primary *KIT* exon 9 mutations, 34% for those with exon 11 mutations, and 56% for those with wild-type *KIT* and *PDGFRA* before imatinib therapy. Objective responses (ie, PRs) were significantly more common in patients with *KIT* exon 9 than exon 11 mutant GISTs (37% *v* 5%; $P = .002$). Of the four patients with *PDGFRA* mutations, none experienced clinical benefit. Among patients classified as imatinib-intolerant (n = 4), tumor genotyping revealed a primary *KIT* exon 9 mutation in one (who achieved a PR) and a wild-type genotype in the other three patients (who achieved SD, two for > 6 months).

Median progression-free survival (PFS) was significantly longer for patients with primary *KIT* exon 9 mutations (19.4 months; 95% CI, 11.1 to not yet attained [NA]; $P = .0005$) or a wild-type genotype (19.0 months; 95% CI, 3.9 to NA; $P = .0356$) than for those with *KIT* exon 11 mutations (5.1 months; 95% CI, 4.5 to 7.8; Fig 1A). PFS did not differ significantly between patients with exon 9 mutations and a wild-type genotype. Median overall survival (OS) was also significantly longer for patients with exon 9 mutations (26.9 months; 95% CI, 12.2 to NA; $P = .012$) or a wild-type genotype (30.5 months; 95% CI, 19.8 to NA; $P = .0132$) than for those with exon 11 mutations (12.3

Table 1. Baseline Characteristics and Prior Imatinib Treatment of Patients With Pre-Imatinib Genotyping Data

Characteristic	No. of Patients (N = 78)	% of Patients
Sex		
Male	53	68
Female	25	32
Age, years		
Median	55	
Range	26-76	
ECOG performance status		
0	38	49
1	24	44
2	6	8
Time since initial diagnosis, weeks		
Median	139	
Range	23-664	
Most common disease present at screening		
Liver metastases	72	92
Soft tissue	37	47
Peritoneal metastases	36	46
Local recurrence	28	36
Prior therapy other than imatinib		
Surgery	78	100
Radiotherapy	10	13
Systemic therapy	34	44
Prior imatinib therapy		
Maximum dose, mg		
Median	600	
Range	400-1,000	
Duration of treatment, weeks		
Median	78	
Range	10-151	
Reason for discontinuation		
Tumor progression	74	95
Intolerance	4	5

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Table 2. Response to Sunitinib by Primary and Secondary Tumor Genotype

Response by Tumor Genotype												
Primary (n = 77)*							Secondary (n = 65)*†					
Mutation Status	No.	Median Duration of Prior IM (months)	RECIST Response No.	RECIST Response %	Clinical Benefit‡ No.	Clinical Benefit‡ %	Mutation Status§	No.	Median Duration of Prior IM (months)	RECIST Response No.	RECIST Response %	Clinical Benefit‡ No.	Clinical Benefit‡ %
KIT mutation	64		9	14	27	42							
KIT exon 9	19	12.5	7	37‖	11	58¶	*KIT* 9 → 9	13	12.2	5	38	8	62
							KIT 9 → 9 + 13	1	17.3	1	100	1	100
							KIT 9 → 9 + 17	2	17.7	0	0	0	0
KIT exon 11	44	22.8	2	5	15	34	*KIT* 11 → 11	10	22.1	1	10	1	10
							KIT 11 → 11 + (13 or 14)	17	20.0	1	6	10	59
							KIT 11 → 11 + (17 or 18)	10	23.3	0	0	1	10
KIT exon 13	1	14.0	0	0	1	100	*KIT* 13 → 13 + 17	1	14.0	0	0	1	100
PDGFRA mutation	4		0	0	0	0							
PDGFRA exon 12	1	18.6	0	0	0	0	*PDGFRA* 12 → 12 + 18	1	18.6	0	0	0	0
PDGFRA exon 18	3	7.9	0	0	0	0	*PDGFRA* 18 → 18	2	8.5	0	0	0	0
No *KIT*/*PDGFRA* mutation	9	10.5	0	0	5	56	No mutation → no mutation	8	10.8	0	0	4	50

Abbreviations: IM, imatinib; RECIST, Response Evaluation Criteria in Solid Tumors; PDGFRA, platelet-derived growth factor receptor α.
*One additional patient had baseline pre-imatinib mutations of *KIT* in both exons 13 and 17 and was excluded from analyses.
†One patient included in the primary tumor genotype analysis had a primary exon 11 mutation and secondary exon 13 and 17 mutations in separate lesions and was excluded from secondary tumor genotype analysis.
‡Clinical benefit is defined as response or stable disease for ≥ 6 months according to Response Evaluation Criteria in Solid Tumors.
§Arrows separate primary and secondary genotype results (eg, *KIT* 11 → 11 is a primary *KIT* exon 11 mutation with no secondary mutation detected; *KIT* 11 → 11 + [13 or 14] is a primary *KIT* exon 11 mutation + secondary *KIT* exon 13 or 14 mutations).
‖P = .002 compared with primary *KIT* exon 11 mutation.
¶P = .08 compared with primary *KIT* exon 11 mutation.

months; 95% CI, 8.8 to 19.6; Fig 1B). OS did not differ significantly between patients with exon 9 mutations or a wild-type genotype.

Secondary Tumor Genotype and Efficacy

A total of 109 post-imatinib biopsy specimens were available from 67 patients, and secondary *KIT* mutations were identified in 33 patients (Appendix Table A1). Consistent with prior reports, the mutation distribution was nonrandom, and clusters occurred in exons 13 and 14 that encode the drug/ATP binding pocket of the receptor and exon 17 that encodes the kinase activation loop (Fig 2A). The most commonly identified secondary mutation was V654A in exon 13. Two tumors had secondary *KIT* exon 18 mutations. One patient had different secondary mutations (exon 13 V654A and exon 17 D816H) in different lesions. Secondary kinase mutations were significantly more common in GISTs with primary *KIT* exon 11 mutations than in those with exon 9 mutations (73% *v* 19%; $P = .0003$). Of the four samples with primary *PDGFRA* mutations, one had a secondary mutation in exon 18 (primary mutation in exon 12), two lacked secondary mutations (both had primary exon 18 D842V mutations), and the fourth lacked a post-imatinib sample. No secondary mutations were found in the eight post-imatinib samples that lacked primary *KIT* or *PDGFRA* mutations.

Among all patients with *KIT* mutations, the median PFS with sunitinib was significantly longer for the 18 patients who had secondary *KIT* exon 13 or 14 mutations (7.8 months; 95% CI, 4.5 to 10.1) than for the 13 patients who had exon 17 or 18 mutations (2.3 months; 95% CI, 1.0 to 5.1; $P = .0157$; Fig 2B). Likewise, median OS was numerically longer in the former than the latter group (13.0 months [95% CI, 8.9 to 22.4] *v* 4.0 months [95% CI, 2.2 to 19.6]; $P = .160$; Fig 2C), and clinical benefit rates were higher (61% *v* 15%; $P = .011$; Table 2). Nearly identical results were obtained when only patients with primary exon 11 mutations were considered. For patients with primary exon 11 mutations, there were no significant differences in PFS or OS between those patients with or without secondary mutations.

In Vitro Measures of Activity With Specific Mutants

Sunitinib potently inhibited the activity of ligand-activated wild-type KIT, and the KIT exon 11 V560D and exon 9 AY insertion mutants: 50% inhibitory concentration (IC_{50}) values were less than 100 nmol/L for all three kinases (Table 3; Fig 3A). By comparison, the corresponding IC_{50} values for imatinib were approximately 1,000 nmol/L for wild-type KIT, 100 nmol/L for the V560D mutant, and 1,000 nmol/L for the exon 9 AY mutant. Sunitinib also potently inhibited the phosphorylation of KIT double mutants, in which the second mutation occurred in the drug/ATP binding site of the receptor, such as V560D + V654A (exons 11 + 13) and V560D + T670I (exons 11 + 14). These double mutants were resistant to inhibition by imatinib in vitro. Conversely, KIT double mutants, in which the second mutation occurred in the activation loop (V560D + D816H, V560D + D820G, V560D + N822K, and V560D + Y823D), were resistant to inhibition by sunitinib or imatinib, with sunitinib IC_{50} values of 1,000 nmol/L or higher. Notably, the V560D + A829P double mutant had an imatinib IC_{50} that was only two- to three-fold higher than that of V560D alone. In contrast, V560D + A829P was resistant to sunitinib at doses of up to 1,000 nmol/L. The rarity of A829P as a secondary mutation could be caused by its relatively preserved imatinib sensitivity. Similar results to those obtained when exon 11 V560D was used as the primary mutation were obtained when the exon 9 AY insertion was used instead (Table 3; Fig 3A).

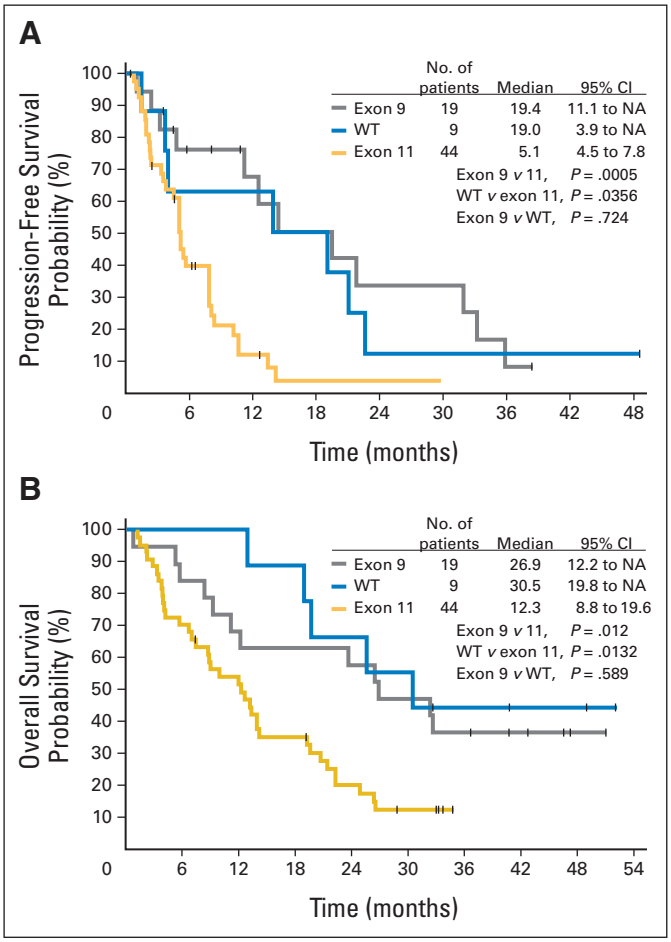

Fig 1. Impact of primary (pre-imatinib) *KIT* genotype on efficacy of sunitinib treatment. (A) Progression-free survival. (B) Overall survival. NA, not yet attained; WT, wild type.

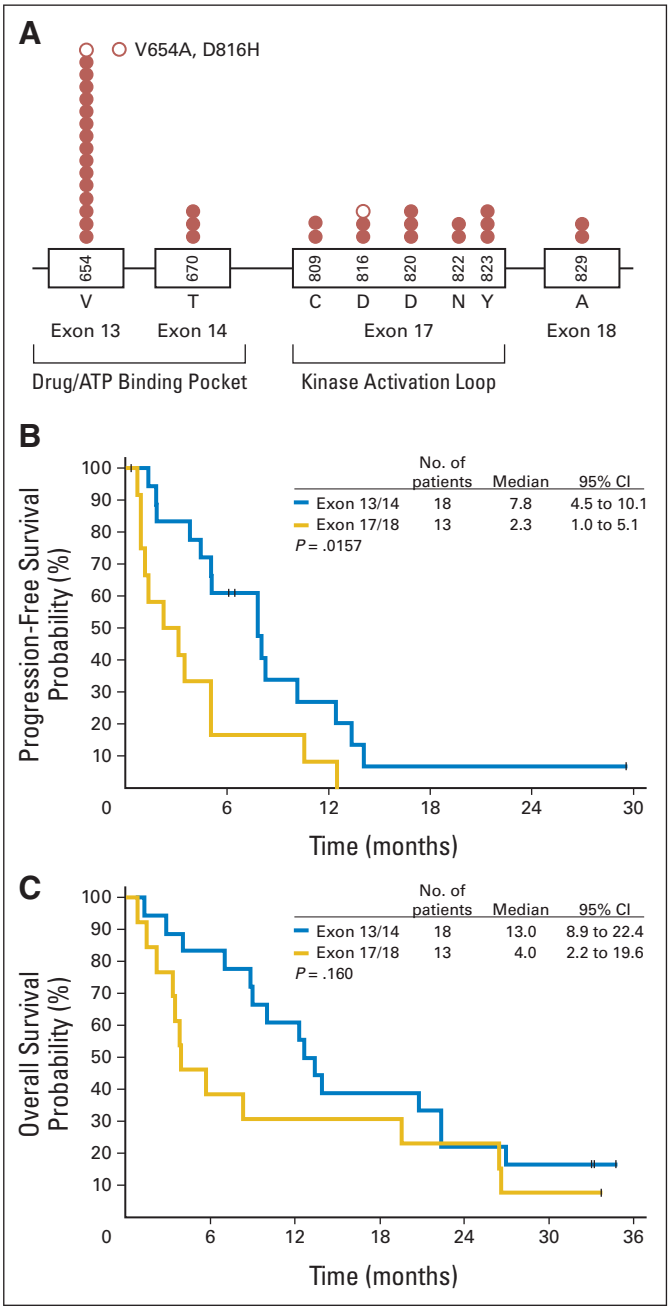

Fig 2. (A) Distribution and frequency of unique secondary (post-imatinib) *KIT* mutations (per patient) in this study. One patient had different mutations in different biopsy specimens: a V654A mutation in one lesion, a D816H mutation in another (○). Impact of secondary *KIT* genotype on (B) progression-free survival and (C) overall survival with sunitinib.

To confirm these findings, we tested the relative potency of imatinib or sunitinib at inhibiting KIT kinase activity in GIST cell lines obtained from imatinib-resistant tumors (Fig 3B). The GIST48 cell line is homozygous for a primary *KIT* exon 11 V560D mutation and is heterozygous for a secondary exon 17 D820A mutation.[17] Concentrations of imatinib greater than 1,000 nmol/L were insufficient to completely inhibit KIT activation in this cell line. (This concentration is 10-fold higher than that necessary to block KIT exon 11-mutant isoforms in GIST cell lines in other studies.[24,31,32]) Sunitinib was less potent than imatinib at inhibiting KIT autophosphorylation in GIST48 cells. Notably, low doses (100 nmol/L) of either imatinib or sunitinib had a partial inhibitory effect on KIT phosphorylation, presumably because of inhibition of a minority population of V560D homodimers. The GIST430 cell line is heterozygous for a *KIT* exon 11 deletion mutation and an exon 13 V654A substitution (both on the same allele).[17] Sunitinib had significantly greater potency than imatinib for inhibition of KIT autophosphorylation in GIST430 cells (IC_{50}, 1,000 nmol/L for imatinib $v < 100$ nmol/L for sunitinib).

We also tested the potency of sunitinib at inhibiting the phosphorylation of wild-type PDGFRA or the V561D point mutant: the IC_{50} values were less than 100 nmol/L for both (Table 3; Fig 3C). V561D, located in the receptor juxtamembrane domain encoded by exon 12, is a relatively common primary *PDGFRA* mutation in patients with GIST.[1] Conversely, D842V, which is the most common *PDGFRA* mutation in GISTs, which resides in the activation loop encoded by exon 18, and which confers imatinib resistance both as a primary or a secondary mutation,[1] conferred resistance to sunitinib in these in vitro experiments (Table 3; Fig 3C). In the clinical study, D842V was detected as a primary mutation in two patients and as a secondary mutation in one patient.

Table 3. In Vitro Effects of Sunitinib and Imatinib on Autophosphorylation of KIT and PDGFRA Mutants Expressed in Chinese Hamster Ovary Cells

	Mutation				Treatment			
	1		2		Sunitinib		Imatinib	
Mutant Construct	Exon	Function	Exon	Function	Approximate IC_{50} (nmol/L)	S/R	Approximate IC_{50} (nmol/L)	S/R
KIT								
Ligand-activated WT	—	—	—	—	< 100	S	1,000	R
V560D	11	JM	—	—	< 100	S	100	S
V560D + V654A	11	JM	13	ATP BP	< 100	S	2,500	R
V560D + T670I	11	JM	14	ATP BP	< 50	S	> 5,000	R
V560D + D816H	11	JM	17	AL	≥ 1,000	R	5,000	R
V560D + D820G	11	JM	17	AL	≥ 1,000	R	1,000	R
V560D + N822K	11	JM	17	AL	> 1,000	R	2,000	R
V560D + Y823D	11	JM	17	AL	> 1,000	R	> 5,000	R
V560D + A829P	11	JM	18	Extended AL	> 1,000	R	200	I
Exon 9 AY	9	DM	—	—	< 100	S	1,000	R
Exon 9 AY + V654A	9	DM	13	ATP BP	100	S	3,000	R
Exon 9 + D816H	9	DM	17	AL	500	R	3,000	R
PDGFRA								
WT	—	—	—	—	< 100	S	< 100	S
V561D	12	JM	—	—	< 100	S	< 100	S
D842V	18	AL	—	—	> 1,000	R	2,500	R
V561D + D842V	12	JM	18	AL	> 1,000	R	2,500	R

Abbreviations: PDGFRA, platelet-derived growth factor receptor α; S, sensitive; R, resistant; WT, wild type; JM, juxtamembrane region; ATP BP, adenosine triphosphate binding pocket; AL, activation loop; I, intermediate; DM, dimerization.

DISCUSSION

These results extend previously reported findings from this study that showed a correlation between sunitinib activity and GIST kinase genotype in patients who have metastatic/unresectable GIST and have experienced imatinib failure.[33] Data on the relative responsiveness of different molecular subgroups of imatinib-resistant GIST may help to optimize treatment of patients with GIST and may help to better understand the basis of sunitinib activity in these patients. Such studies may also advance understanding of the mechanisms of resistance and may facilitate development of strategies to circumvent it.

The analyses reported here assessed the effect of tumor kinase genotype on sunitinib activity by using clinical study data complemented by in vitro cellular assays. Although sunitinib demonstrated clinical activity against GISTs of the three most common primary genotypes, both datasets indicated that primary and secondary mutations in the pathogenic kinase strongly influence sunitinib activity. Both the clinical benefit and the objective response rates with sunitinib were higher in patients with primary *KIT* exon 9 mutations than with exon 11 mutations (clinical benefit rates: 58% *v* 34%; objective response rates: 37% *v* 5%; $P = .002$). Similarly, PFS and OS were significantly longer in patients with primary *KIT* exon 9 mutations or a wild-type genotype than in those with *KIT* exon 11 mutations. These results are the converse of those reported for imatinib, in which objective response rates were higher and PFS and OS were longer in patients with GIST who harbored exon 11 mutations than in those who had exon 9 mutations or a wild-type genotype.[3,14,15] Notably, the potency of sunitinib against wild-type and exon 9-mutant KIT was superior to that of imatinib in vitro, whereas both drugs exhibited similar potency against KIT exon 11 mutant kinases. A possible explanation is that these mutational sites have different structural effects on KIT, with different consequences for interaction with the two TKIs. Indeed, exon 9 mutations were recently reported to have structural consequences similar to ligand-mediated receptor dimerization.[34] This mechanism of kinase activation appears distinct from that caused by mutation of the intracellular juxtamembrane domain encoded by exon 11.[35] Others have also observed the impact of mutational site on TKI potency in vitro: by using an isogenic BaF3 model, the imatinib IC_{50} in cells that expressed exon 9 mutations was found to be approximately eight-fold higher than that obtained in cells that expressed the exon 11 V559D mutation.[36] These results suggest that the greater clinical benefit seen for sunitinib-treated patients with exon 9-mutant or wild-type imatinib-resistant GISTs may be related to the greater potency of sunitinib against these kinases. They also suggest that genotypically defined subsets of patients may experience different clinical outcomes when treated with first-line imatinib than with sunitinib. Sunitinib is currently approved only as second-line therapy for GIST, but studies are being planned to evaluate its efficacy and safety as first-line treatment. On the other hand, sunitinib has yet to be tested in imatinib-naïve patients, and the majority of patients in this study with primary *KIT* exon 11 mutations had acquired secondary *KIT* mutations that confer imatinib resistance. Studies in imatinib-naïve patients will be required to definitely assess the effect of a primary exon 11 mutation alone on sunitinib activity in vivo.

This study also showed that secondary kinase mutations were significantly more common in GISTs with primary *KIT* exon 11 than exon 9 mutations and that they did not occur in GISTs with a wild-type genotype, which is consistent with previous reports that secondary kinase mutations are common in GISTs that exhibit secondary imatinib resistance but not in those that exhibit primary resistance.[16,17] Moreover, the frequency of secondary mutations is likely to

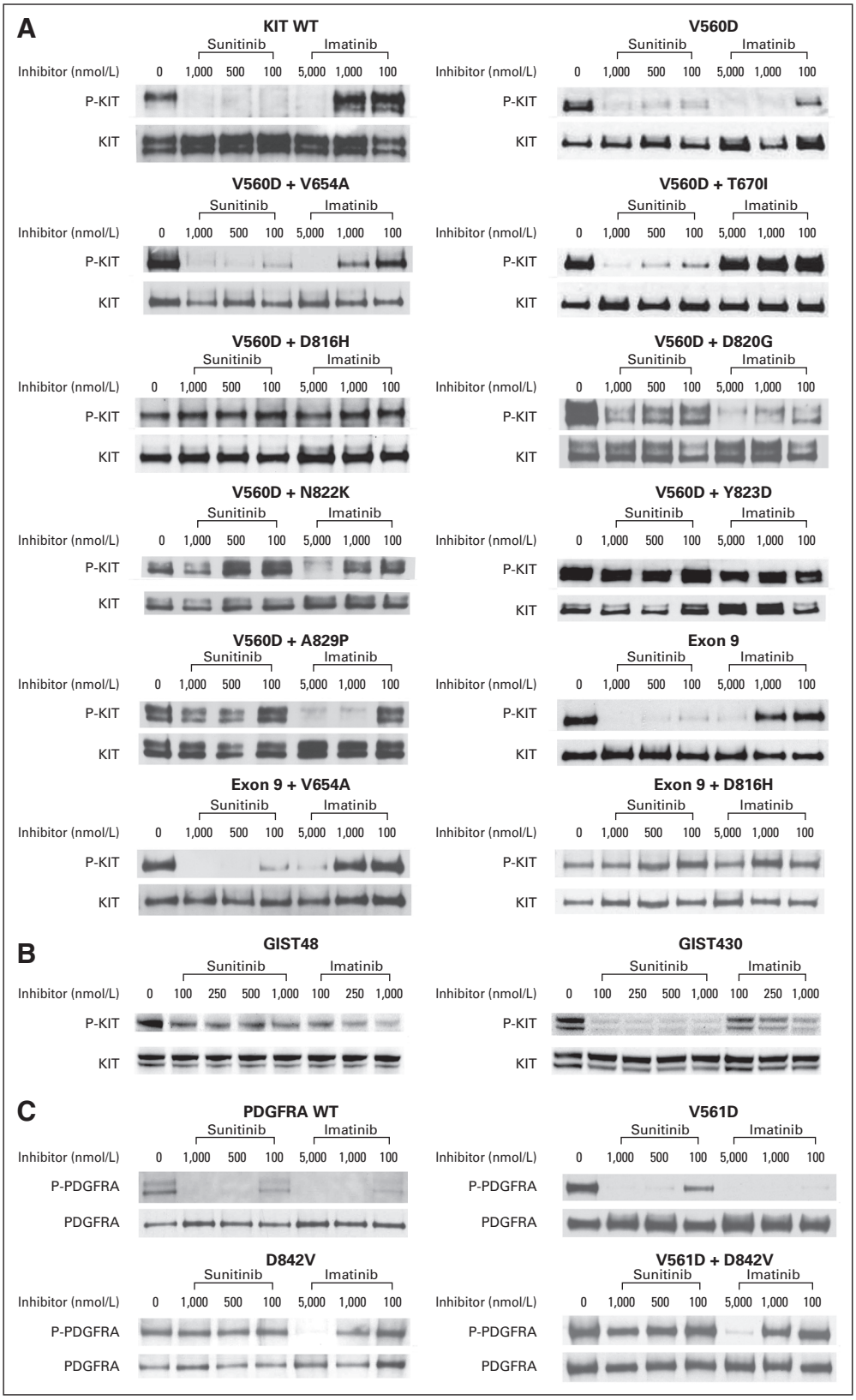

Fig 3. Effects of sunitinib and imatinib on autophosphorylation of (A) wild-type KIT and KIT mutants transiently expressed in Chinese hamster ovary cells; (B) KIT mutants expressed by gastrointestinal stromal tumor cell lines; or (C) platelet-derived growth factor receptor α mutants transiently expressed in Chinese hamster ovary cells. Wild-type, but not mutant, receptors were ligand-activated. P-KIT, phosphorylated KIT; WT, wild type.

have been underestimated in this analysis, because only one patient in our analysis was found to have different secondary mutations in different lesions, and intra- and interlesion heterogeneity of secondary mutations in GISTs has been documented by others.[20,25] Only a limited number of small-needle biopsy specimens were available per patient in our study (mean, 1.4 biopsy specimens per patient; range, 0-3). In particular, it is probable that further sampling would have revealed secondary mutations in those tumors with primary *KIT* exon 11 mutations that appeared to lack them. Because exon 11 mutants are strongly inhibited by imatinib, secondary resistance is more likely to require the selection and subsequent expansion of clones expressing a second, resistance-conferring mutation than GISTs with exon 9 mutations or a wild-type genotype, which are more likely to be intrinsically resistant to imatinib. Consistent with this, the median duration of prior imatinib treatment for patients who had primary exon 11 mutations was 22.8 months, compared with 12.5 and 10.5 months for patients who had exon 9 mutations or a wild-type genotype, respectively (Table 2). However, it is worth noting that, although the duration of imatinib treatment was a significant prognostic factor for PFS and OS in a univariate analysis, it was not a significant factor in a multivariate analysis (data not shown). Although multivariate analyses performed on such a small sample must be interpreted with caution, they confirmed that primary and secondary *KIT* genotype were significant prognostic factors for PFS and were marginally significant prognostic factors for OS.

Consistent with previous studies,[16,18-28] secondary *KIT* mutations in patients with imatinib-resistant GIST enrolled on the current study tended to cluster in exons 13 and 14, which encode the drug/ATP binding pocket of the receptor, or in exon 17, which encodes the kinase activation loop. Of note, our in vitro studies showed that sunitinib potently inhibited the kinase activity of KIT receptors that contained secondary mutations in the drug/ATP binding pocket and that are resistant to imatinib, such as V654A (exon 13) and T670I (exon 14). These secondary mutations were coexpressed with a common primary mutation (V560D), which recreated the situation often observed in GISTs that exhibit secondary imatinib resistance. Previous ex vivo studies have also shown that sunitinib inhibits imatinib-resistant KIT receptors that contain mutations in the drug/ATP binding pocket.[29,30] However, the in vitro studies performed here also showed that sunitinib was relatively ineffective at inhibiting KIT receptors that contained secondary mutations localized to the activation loop. Consistent with these in vitro findings, PFS and OS were longer and the clinical benefit rate was higher for patients in the clinical trial who had secondary *KIT* exon 13 or 14 (ie, ATP-binding-pocket) mutations than those with secondary *KIT* exon 17 or 18 (ie, activation-loop) mutations.

The results of this study provide one explanation for the activity of sunitinib in patients with imatinib-refractory GIST that has been seen in this and other trials.[13] However, antiangiogenic effects of sunitinib treatment also may contribute to its effectiveness. In addition to KIT and PDGFRA activity, sunitinib also selectively inhibits PDGFRB and all three isotypes of VEGFR, whereas imatinib inhibits PDGFRB but not VEGFRs. Studies in animal models indicate that dual inhibition of PDGFR and VEGFR produces greater antiangiogenic effects than inhibition of only one or the other,[37-39] which suggests that sunitinib may produce greater antiangiogenic effects than imatinib and that these effects may contribute to its activity against imatinib-refractory GISTs.

Of note is our observation that secondary KIT mutants that involve the activation loop are insensitive to both sunitinib and imatinib. Given that different tumor clones in one individual may acquire imatinib resistance because of different secondary mutations, including those involving the KIT activation loop,[20,25] not all imatinib-resistant tumors may respond well to sunitinib therapy. Conversely, some GISTs with secondary *KIT* activation-loop mutations may still be susceptible to sunitinib because of its potent antiangiogenic effects. Additional research of this issue is warranted.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Appendix

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

