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Phase I Study of Rapid Alternation of Sunitinib and Regorafenib for the Treatment of Tyrosine Kinase Inhibitor Refractory Gastrointestinal Stromal Tumors

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Abstract

Purpose: Polyclonal emergence of KIT secondary mutations is a main mechanism of imatinib progression in gastrointestinal stromal tumor (GIST). Approved KIT inhibitors sunitinib and regorafenib have complementary activity against KIT resistance mutations. Preclinical evidence suggests that rapid alternation of sunitinib and regorafenib broadens the spectrum of imatinib-resistant subclones targeted.

Patients and Methods: Phase Ib study investigating continuous treatment with cycles of sunitinib (3 days) followed by regorafenib (4 days) in patients with tyrosine kinase inhibitor (TKI)-refractory GIST. A 3+3 dosing schema was utilized to determine the recommended phase II dose (RP2D). Plasma samples were analyzed for pharmacokinetics and circulating tumor DNA (ctDNA) studies using targeted error correction sequencing (TEC-seq) and droplet digital PCR (ddPCR).

Results: Of the 14 patients enrolled, 2 experienced dose-limiting toxicities at dose level 2 (asymptomatic grade 3 hypophosphatemia). Sunitinib 37.5 mg/day and regorafenib 120 mg/day was the RP2D. Treatment was well-tolerated and no unexpected toxicities resulted from the combination. Stable disease was the best response in 4 patients, and median progression-free survival was 1.9 months. Combined assessment of ctDNA with TEC-seq and ddPCR detected plasma mutations in 11 of 12 patients (92%). ctDNA studies showed that KIT secondary mutations remain the main mechanism of resistance in TKI-refractory GIST, revealing effective suppression of KIT-mutant subpopulations in patients benefiting from the combination.

Conclusions: Sunitinib and regorafenib combination is feasible and tolerable. Rapid alternation of TKIs with complementary activity might be effective when combining drugs with favorable pharmacokinetics, potentially allowing active doses while minimizing adverse events. Serial monitoring with ctDNA may guide treatment in patients with GIST.

Introduction

Mutational activation of KIT or PDGFRA receptor tyrosine kinases is the crucial transforming event in approximately 90% gastrointestinal stromal tumors (GIST; refs. 1, 2), and therapeutic inhibition of KIT/PDGFRA oncogenic signaling with first-line imatinib substantially improves outcomes in most patients (3). However, metastatic patients with initial clinical benefit will eventually progress, normally after a median of 20–24 months. Resistance to imatinib occurs in 80%–90% of patients with GIST with the expansion of heterogeneous subclones harboring different KIT secondary mutations (4, 5). Therefore, strategies aiming to target KIT activation after imatinib failure remain useful in GIST. However, response duration is relatively brief, 4–6 months, irrespective of the standard second- (sunitinib; ref. 6) and third-line (regorafenib; ref. 7) agent used. Other nonapproved tyrosine kinase inhibitors (TKI) with KIT inhibitory activity have shown similar clinical activity in phase II and III trials (8).

We have determined that each TKI with KIT-inhibitory activity has a drug-specific activity profile against a subset of the KIT secondary mutational spectrum, which constitutes the molecular basis for the modest clinical benefit observed after the onset of imatinib resistance (9). Remarkably, sunitinib and regorafenib have complementary activities against imatinib-resistant KIT

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Translational Relevance

Polyclonal emergence of KIT secondary mutations is the main driver of tumor progression in imatinib-resistant gastrointestinal stromal tumor (GIST), and rapid alternation of tyrosine kinase inhibitors (TKI) with complementary activity against resistant subpopulations is a feasible and innovative treatment strategy to overcome tumor heterogeneity in cancer. This strategy allows achieving active doses while minimizing overlapping toxic effects, although pharmacokinetic data show that drug exposure is critical to effectively target specific resistant subpopulations, and therefore drugs with short times to reach active concentrations in plasma are more likely to benefit from this approach. ctDNA monitoring using complementary technologies detect plasma mutations in most patients with advanced GIST, reflects the course of disease, and shows that secondary mutations in KIT remain the main driver in TKI-refractory GIST.

oncoproteins. Namely, sunitinib inhibits mutations in the ATP-binding pocket, whereas regorafenib primarily inhibits mutations in the activation loop, the two regions of the KIT kinase domain in which resistance mutations typically cluster. Therefore, this evidence supports combination of TKIs with complementary activity to suppress heterogeneous cross-resistant subpopulations in imatinib-resistant GIST. To improve tolerability while reaching effective doses, we established the preclinical rationale for an innovative therapeutic combination strategy based on rapid alternation of sunitinib (3 days) with regorafenib (4 days; ref. 9).

In this phase I study we report, for the first time in cancer, safety and efficacy from a therapeutic combination based on rapid alternation of TKIs with complementary activity against resistant subpopulations. As part of the planned biomarker analysis, circulating tumor DNA (ctDNA) assessment was used to determine the spectrum of resistance in patients with TKI-refractory GIST, and to evaluate clonal dynamics in GIST with clinical benefit from the rapid alternation combination approach.

Patients and Methods

Patient population

The study enrolled patients aged ≥ 18 years with histologically confirmed diagnosis of metastatic and/or unresectable GIST with prior failure to at least imatinib, sunitinib, and regorafenib (fourth-line and beyond). Patients had documented progressive disease, Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, RECIST 1.1 measurable disease, and normal organ function (full eligibility criteria in Supplementary Data). Primary KIT/PDGFRA tumor tissue genotype by Sanger sequencing was known in all recruited patients. The study was approved by the institutional review board and was conducted in compliance with guidelines for Good Clinical Practice. Patients provided written informed consent before study participation.

Study design and treatment

This was a phase I/II, single-center, open-label, Simon two-stage, dose escalation, and dose expansion study to evaluate the safety and the preliminary efficacy of short cycles of sunitinib alternated with regorafenib in TKI-refractory GIST.

Treatment consisted of 3 days of once daily sunitinib followed by 4 days of once daily regorafenib, continuously in 28-day cycles. The starting dose level was sunitinib 37.5 mg/day and regorafenib 120 mg/day. Doses were escalated following a classical 3+3 design up to sunitinib 50 mg/day and regorafenib 160 mg/day or until MTD and recommended phase II dose (RP2D) was determined (further details in Supplementary Data). Once 12 subjects were enrolled, we evaluated an interim efficacy analysis following the operating characteristics of a Simon two-stage design. With a null hypothesis of 10% and alternative hypothesis if 30%, we had 80% power with 4% type 1 alpha. We needed at least 2 responses within the first 12 patients to continue enrollment through the second stage.

The primary objective of the study was to evaluate the safety and tolerability for sunitinib alternating with regorafenib in patients with unresectable and/or metastatic GIST with prior failure to at least all established therapies approved in GIST. Pharmacokinetics and efficacy assessments were evaluated as secondary objectives. ctDNA evaluation to determine the resistance profile in advanced GIST and the role of serial monitoring was an exploratory endpoint.

Pharmacokinetic assessments

Pharmacokinetic blood samples were collected predose in all patients for sunitinib and sunitinib metabolite SU012662 on days 1, 15, 18, and 22; and for regorafenib and its metabolites (M2/5), predose and on days 4, 8, 11, 15, 18, and 22 (Supplementary Table S1).

Efficacy assessments

Tumor assessments by CT scan were performed at baseline and every 8 weeks thereafter. Tumor response was evaluated according to RECIST 1.1.

ctDNA evaluation

Plasma was collected and stored at −80°C on cycle 1 days 1 (time 0) and 15, day 1 on subsequent cycles, and at the end of treatment. Cell-free DNA (cfDNA) was extracted by QIAamp Circulating Nucleic Acid Kit (Qiagen).

Droplet digital PCR (ddPCR). ddPCR reagents were ordered from Bio-Rad. Primer/probe mix for KIT-resistant mutations was custom-made by Life Technologies and are described in Supplementary Table S2. The following KIT-resistant mutations were analyzed: V654A (exon 13), T670I (exon 14), exon 17, and A829P (exon 18). Cell lines GIST430/654, GIST-T1/670, GIST48, and GIST-T1/829 were used as positive controls; characteristics have been described previously (9). Genomic DNA of the cell lines were extracted by QIAamp DNA Mini Kit (Qiagen). PCR reactions (25 μL) that comprised ddPCR Supermix for Probes, custom-made TaqMan primer/probe mix, and appropriate DNA template were prepared in a 96-well PCR plate and transferred to the automated droplet generator. A second 96-well PCR plate which held the emulsified droplets were amplified by PCR on an S1000 thermal cycler. Cycling conditions were: 10 minutes at 95°C followed by 40 cycles of a two-step thermal profile of 30 seconds at 94°C denaturation and 60 seconds at specific annealing temperature (57°C for V654A, 55°C for T670I, 62°C for A829P, and 57°C for pan- ex17 mutations). The 96-well plate with amplified PCR products was then read by a QX200 Droplet Reader (Bio-Rad).

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TEC-Seq next-generation sequencing. cfDNA genomic libraries were prepared from 2.5 to 49.5 ng of cfDNA as described previously (10). Targeted capture was performed using a custom set of hybridization probes targeting 81 kb from 58 cancer-driver genes (Supplementary Table S3). TEC-seq libraries were sequenced using 100-bp paired-end runs on the Illumina HiSeq 2500. Primary processing of next-generation sequence data for analyses of sequence alterations in cfDNA samples was performed as described previously (10). Candidate somatic mutations, consisting of point mutations, small insertions, and deletions were identified using VariantDx (Personal Genome Diagnostics) across the targeted regions of interest (11). For the scope of this study, an alteration was considered a candidate somatic mutation in *KIT* when at least two distinct paired reads contained the mutation in the plasma and the number of distinct paired reads containing a particular mutation in the plasma was at least 0.05% of the total distinct read pairs. As tissue was not available for comparative sequencing analyses, we excluded from our ctDNA dynamics approach all detected variants that could be related to clonal hematopoietic expansion as described previously (12), including alterations in *DNMT3A*, *FGFR3*, *GNAS*, or *TP53*.

Results

Patient characteristics

A total of 14 patients with TKI-refractory, metastatic GIST were enrolled into the study between August 2014 and September 2016. One patient showed rapid symptomatic deterioration after signing the informed consent and never received study drugs. A second patient rapidly deteriorated 10 days after treatment initiation due to clinical progression. The remaining 12 patients completed at least one cycle (28 days) of sunitinib alternating with regorafenib. Baseline demographics and disease characteristics are summarized in Table 1.

Drug exposure and safety

Thirteen of 14 patients were evaluable for safety and dose-limiting toxicity (DLT). Patients in the initial dose level completed the first treatment cycle without experiencing DLTs. Two DLTs were evidenced in 5 patients recruited in the second dose level, which consisted on sunitinib 37.5 mg/day for 3 days followed by regorafenib 160 mg/day for 4 days. Both patients showed refractory asymptomatic grade 3 hypophosphatemia despite intravenous replacement. Four more patients added in the first dose level did not show DLTs, and therefore the RP2D was sunitinib 37.5 mg/day for 3 days followed by regorafenib 120 mg/day for 4 days.

All the 13 evaluable patients experienced treatment-related adverse events (AE) of any grade, although the majority were grade 1–2 in severity. Most common treatment-related AEs are listed in Table 2 and were fatigue (92%), weight loss (62%), palmar-plantar erythrodysesthesia syndrome (PPES; 54%), diarrhea (54%), anorexia (38%), hoarseness (38%), hypertension (38%), and mucositis (23%). Four patients experienced grade 3 AEs, including PPES ($n = 2$) and hypertension ($n = 2$), all of them clinically manageable. No toxicity could be specifically attributed to one of both drugs. Seven patients (58%) required dose delay/modification, and 1 patient (8%) definitive treatment interruption and removal from the study due to recurrent G2 rash despite dose reduction and optimal supportive dermatologic support, which occurred outside the DLT window.

Table 1. Patient and tumor characteristics

Characteristics	
Number of patients	14
Median age, years (range)	64 (42–78)
Gender	
Male	8 (57%)
Female	6 (43%)
ECOG PS status	
0	9 (64%)
1	5 (36%)
Metastatic sites	
Peritoneum	7 (50%)
Liver	8 (57%)
Lung	2 (14%)
Prior lines of treatment	
Imatinib	14 (100%)
Sunitinib	14 (100%)
Regorafenib	14 (100%)
Pazopanib	3 (21%)
TKI rechallenge	2 (14%)
Sorafenib	2 (14%)
Ponatinib	1 (7%)
Rapamycin	1 (7%)
Doxorubicin	1 (7%)
Primary mutation status	
KIT exon 11	8 (57%)
KIT exon 9	5 (36%)
KIT/PDGFRA WT	1 (7%)

Abbreviations: ECOG PS: Eastern Cooperative Oncology Group performance status; WT, wild-type.

Antitumor activity

The 13 patients evaluable for toxicity were evaluable for radiographic response. No objective responses were observed by RECIST v1.1. criteria. Stable disease (SD) was observed in 4 patients. The clinical benefit rate at 8 weeks was 31% ($n = 4$). The median progression-free survival (mPFS) was 1.9 months [95% confidence interval (CI), 1.4–3.6 months]. A total of 3 patients died on trial or during follow-up. The median overall survival was 10.8 months (95% CI, 5.9–∞). The 6-month overall survival rate was 62% (95% CI, 7%–91%). Four patients (29%) continued on treatment after progression for a median of 3.6 months (range, 3.6–7.3 months) due to clinical benefit, as subjectively assessed by the investigator.

Pharmacokinetics

Trough levels of sunitinib and regorafenib were obtained across several timepoints (Supplementary Table S1). Including all patients and timepoints, the maximum concentration and steady state of sunitinib and regorafenib were below reported threshold target inhibition concentrations (Table 3) due to scarce accumulation of sunitinib, regorafenib, and their metabolites during the on/off drug periods (Supplementary Fig. S1; refs. 13, 14). Patients with SD had slightly higher median drug exposure to sunitinib (4.99 ng/mL) and regorafenib (1,879.17 ng/mL) than those progressing at the first reevaluation (3.99 ng/mL and 991.43 ng/mL, respectively). Median sunitinib and regorafenib concentrations at the time of the two DLTs (1.47 ng/mL and 1,198.35 ng/mL, respectively) did not differ from median drug concentration across all the timepoints per drug.

ctDNA evaluation

ctDNA was prospectively assessed by two complementary technologies, TEC-seq and ddPCR, to investigate the mechanisms

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Table 2. Study treatment–related toxicities occurring in ≥10% of patients

	Cohort 1 ($n = 8$)[a]		Cohort 2 ($n = 5$)[b]		Total ($N = 13$)
	Grade 1/2 *n* (%)	Grade 3/4 *n* (%)	Grade 1/2 *n* (%)	Grade 3/4 *n* (%)	*N* (%)
Fatigue	8 (100%)	0 (0%)	4 (80%)	0 (0%)	12 (92%)
Weight loss	4 (50%)	0 (0%)	4 (80%)	0 (0%)	8 (62%)
PPES	2 (25%)	2 (25%)	3 (60%)	0 (0%)	7 (54%)
Diarrhea	4 (50%)	0 (0%)	3 (60%)	0 (0%)	7 (54%)
Anorexia	1 (13%)	0 (0%)	4 (80%)	0 (0%)	5 (38%)
Hoarseness	3 (38%)	0 (0%)	2 (40%)	0 (0%)	5 (38%)
Hypertension	1 (13%)	1 (13%)	2 (40%)	1 (20%)	5 (38%)
Oral mucositis	1 (13%)	0 (0%)	2 (40%)	0 (0%)	3 (23%)
Chills	1 (13%)	0 (0%)	1 (20%)	0 (0%)	2 (15%)
Skin rash	0 (0%)	0 (0%)	2 (40%)	0 (0%)	2 (15%)
Abdominal pain	2 (25%)	0 (0%)	0 (0%)	0 (0%)	2 (15%)
Constipation	1 (13%)	0 (0%)	1 (20%)	0 (0%)	2 (15%)
Flatulence	2 (25%)	0 (0%)	0 (0%)	0 (0%)	2 (15%)
Nausea	1 (13%)	0 (0%)	1 (20%)	0 (0%)	2 (15%)
Hypophosphatemia	0 (0%)	0 (0%)	0 (0%)	2 (30%)	2 (15%)
Myalgia	1 (13%)	0 (0%)	1 (20%)	0 (0%)	2 (15%)
Musculoskeletal - other	0 (0%)	0 (0%)	2 (40%)	0 (0%)	2 (15%)

[a]Cohort 1 dosing: sunitinib 37.5 mg daily (3 days) and regorafenib 120 mg daily (4 days).
[b]Cohort 2 dosing: sunitinib 37.5 mg daily (3 days) and regorafenib 160 mg daily (4 days).

Table 3. Cycle 1 pharmacokinetic results for sunitinib and regorafenib

Drug	Measure	Mean	Median	*P*
Sunitinib	AUC	95.3	97.7	0.72
	C_{max}	7.1	6.3	0.05
	T_{max}	22	22	—
Regorafenib	AUC	33,047.7	31,722.1	0.69
	C_{max}	3,178.1	3,459.6	0.69
	T_{max}	23.0	22.0	0.05

responsible of resistance in heavily pretreated GIST patients, and to determine whether ctDNA monitoring can be a potential biomarker during treatment. Primary and secondary mutations in KIT were detected at any timepoint, respectively, in 8 of 9 (89%) and 7 of 9 (78%) patients with TEC-seq, while ddPCR identified secondary mutations in hotspot regions of KIT in 11 of 12 patients (92%; Table 4). Together, both technologies detected KIT-resistant mutations at any timepoint in all but 1 patient. Median mutant allele frequency (MAF%) for KIT secondary mutations at baseline draw ranged from 0.09% to 16.4% by TEC-seq (median 1.3%) and 1.7%–19.9% (median 5.3%) by ddPCR. As noted in Table 4, the majority of KIT secondary mutations identified were in the KIT activation loop. Remarkably, there was limited heterogeneity of mechanisms of resistance, with a median of one resistant mutation per patient (range 0–2). Mutations involving genes other than KIT were present in 8 of 10 patients (80%; Table 5), although a hotspot mutation in *NRAS* in 1 patient was the only known alteration potentially related to TKI resistance.

Clonal dynamics was further studied by TEC-seq in the 3 KIT-mutant patients achieving treatment benefit. In all cases, an early decrease in KIT primary and/or secondary mutations was observed during cycle 1 of treatment, followed by prolonged suppression until clone reemergence at the time of disease

Table 4. KIT primary and secondary mutations detected by TEC-seq and ddPCR

		TEC-Seq		ddPCR
Case ID	Tumor tissue genotype	KIT prim. mut.	KIT sec. mut.	KIT sec. mut.
1	KIT/PDGFRA WT	ND	ND	ND
2	KIT Ex 11 K558_G565delinsR	KIT Ex 11 K558_G565delinsR	KIT Ex 17 N822K	KIT Ex 17
3	KIT ex 9 Y503_F504insAY	KIT Ex 9 Y503_F504insAY	KIT Ex 13 V654A	KIT Ex 13 V654A KIT Ex 17
4	KIT ex 11 N564_Y578del	NA	NA	ND
5	KIT ex 9 Y503_F504insAY	KIT Ex 9 Y503_F504insAY KIT ex 17 N822K	KIT Ex 17 N822K	KIT Ex 17
6	KIT ex 9 A502_Y503dup	KIT Ex 9 A502_Y503dup	KIT Ex 17 D820E KIT Ex 18 A829P	KIT Ex 17 KIT Ex 18 A829P
7	KIT ex 11 D579del	KIT Ex 11 D579del	ND	KIT Ex 17
8	KIT ex 9 A502-Y503dup	ND	KIT Ex 17 D820G	KIT Ex 17
9	KIT ex 11 W557_K558del	KIT Ex 11 W557_K558del	ND	KIT Ex 17
10	KIT ex 11 P551del	KIT Ex 11 P551del	KIT Ex 17 D816H	KIT Ex 17
11	KIT ex 11 W557_K558>Q	NA	NA	KIT Ex 17
12	KIT ex 11 V559D KIT ex 13 V654A	KIT Ex 11 V559D	KIT Ex 13 V654A	KIT Ex 13 V654A KIT Ex 17
13	KIT ex 11 L576P	NA	NA	KIT Ex 13 V654A KIT Ex 17
14	KIT ex 9 A502-Y503dup	NA	NA	NA

Abbreviations: Ex, exon; NA, not assessed; ND, not detected; Prim. mut., primary mutation; Sec. mut., secondary mutation.

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Table 5. Other alterations detected in ctDNA

Gene symbol	Amino acid (protein)	Mutation type	Consequence	Mutant allele fraction	Hot-spot alteration
ABL1	H214Y	Substitution	Nonsynonymous coding	0.43%	No
PDGFRA	R500*	Substitution	Nonsense	3.17%	No
AKT1	T34S	Substitution	Nonsynonymous coding	0.29%	No
EGFR	F712L	Substitution	Nonsynonymous coding	0.30%	No
MYC	Y89F	Substitution	Nonsynonymous coding	0.31%	No
PTEN	V9G	Substitution	Nonsynonymous coding	0.78%	No
ERBB4	Q1181H	Substitution	Nonsynonymous coding	0.17%	No
AR	Y365N	Substitution	Nonsynonymous coding	0.39%	No
HNF1A	R200W	Substitution	Nonsynonymous coding	0.93%	No
NRAS	G12D	Substitution	Nonsynonymous coding	9.15%	Yes
NRAS	G12D	Substitution	Nonsynonymous coding	8.03%	Yes
RB1	R451S	Substitution	Nonsynonymous coding	1.13%	No
NRAS	G12D	Substitution	Nonsynonymous coding	5.23%	Yes
NRAS	G12D	Substitution	Nonsynonymous coding	3.75%	Yes
MYC	C186S	Substitution	Nonsynonymous coding	0.24%	No
STK11	N266Y	Substitution	Nonsynonymous coding	0.24%	No

progression, thus predicting the course of the disease (Fig. 1A–C). In contrast, this pattern was not observed in a patient with GIST with rapid progression (Fig. 1D).

Discussion

Polyclonal resistance in GIST entails that a single drug is insufficient to target all resistant KIT variants that drive tumor progression, thus leading to modest clinical benefit irrespective of the second- or third-line used (4, 6, 7). Combination of targeted therapies will potentially augment the magnitude and/or duration of response. However, enhanced toxicities are often seen in such combinations, thereby limiting the use of effective doses (15). We have recently shown that sunitinib and regorafenib share a complementary pattern against resistant mutations in KIT, and therefore combination of both drugs would widen the spectrum of secondary-resistant clones effectively targeted (9). We subsequently established the preclinical rationale for a therapeutic regimen involving rapid alternation of sunitinib (3 days) followed by regorafenib (4 days), aiming to reach effective doses while improving tolerability (9).

The primary endpoint of this study was to determine the safety of the combination. Treatment was tolerable across the first dose level (sunitinib 37.5 mg/day and regorafenib 120 mg/day). The two DLTs occurred in the second dose level (sunitinib 37.5 mg/day and regorafenib 160 mg/day) in form of grade 3 hypophosphatemia. Toxicities were common but manageable, being most of them grade 1 and 2. Adverse events lied within the expected profile for each TKI, with no new or enhanced toxicities resulting from the novel combination strategy. Therefore, rapid alternation

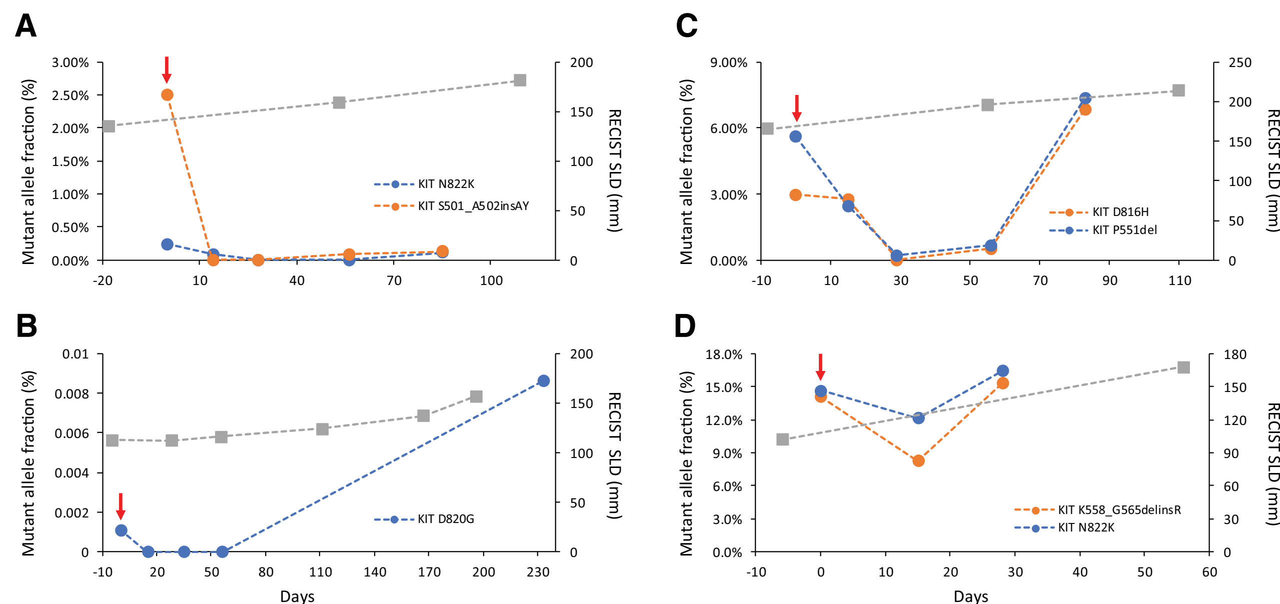

Figure 1.
Dynamic changes of ctDNA in patients with SD (**A–C**) and progressive disease (**D**) receiving sunitinib and regorafenib. Mutant allele fractions of KIT clones identified in ctDNA through the TEC-Seq approach are shown for each timepoint analyzed with the treatment initiation day depicted with the red arrow. RECIST 1.1 sum of longest diameters (SLD; gray boxes) are depicted in the right axis.

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of sunitinib and regorafenib appears to be feasible and tolerable; however, the low plasma drug concentrations suggest that clinically meaningful drug exposure was not achieved.

mPFS in this heavily pretreated population was 1.9 months, comparable with that of imatinib rechallenge (16) and superior to placebo in recent GIST phase III trials (6, 7, 16). In addition, 4 of 13 patients reached SD >8 weeks, and several patients remained on trial due to clinical benefit. Together, these data suggest partial KIT inhibition despite neither sunitinib nor regorafenib reached reported steady states (13, 14). Indeed, the 3 KIT-mutant patients achieving SD demonstrated a ctDNA dynamics indicative of early and sustained clonal suppression, including known KIT-resistant mutations. Therefore, drug concentrations obtained in plasma with this rapid alternation scheme likely reached the threshold of inhibition of imatinib-sensitive subpopulations, but were insufficient to suppress all imatinib-resistant clones due to the long time for both drugs (7–10 days) needed to obtain active plasma concentrations (13, 14). One single dose of imatinib is capable of extinguishing FDG-PET activity in 24 hours (17). However, reaching the steady state appears to be meaningful for sunitinib and regorafenib efficacy against clones with sensitive KIT-secondary mutants for each agent as monotherapy.

Current knowledge on resistance to KIT inhibition comes from studies mostly performed at the onset of imatinib failure (4, 5), and little is known after subsequent lines of treatment. We showed by both TEC-seq and ddPCR of plasma samples that KIT secondary mutations were significant drivers of progression in this heavily pretreated population, with a predominance for mutations in the activation loop of KIT, which is highly relevant for the design of future treatment strategies. Remarkably, we observed little heterogeneity of mechanisms of resistance, and mutants were at low allele frequencies despite the usual high tumor burden in this neoplasia. ctDNA monitoring using complementary technologies identified cfDNA mutations in all but 1 patient and predicted the course of the disease, thus holding the potential for treatment monitoring and therapy selection against emerging resistant subpopulations (9).

In conclusion, rapid drug alternation is an unprecedented strategy for TKI combination in cancer that might be effective when combining drugs with favorable pharmacokinetics, potentially allowing active doses while minimizing AEs. ctDNA studies demonstrate that KIT remains the critical driver in TKI-refractory GIST, and supports further studies for its implementation as a biomarker to guide treatment in patients with GIST.

Disclosure of Potential Conflicts of Interest

C. Serrano is an employee/paid consultant for Deciphera and Blueprint, reports receiving commercial research grants from Deciphera, Bayer, and Pfizer, reports receiving speakers bureau honoraria from Bayer and Blueprint, and reports receiving other remuneration from Pharmamar, Pfizer, Bayer, Novartis, and Lilly. A.J. Wagner is an employee/paid consultant for Daiichi-Sankyo, Eli Lilly, Nanosphere, Five Prime, Deciphera, and Novartis, and reports receiving commercial research grants from Plexxikon, Daiichi-Sankyo, Karyopharm, Aadi Bioscience, Eli Lilly, and Five Prime. G.D. Demetri is an employee/paid consultant for Bayer, Novartis, Pfizer, EMD-Serono, Sanofi, Janssen Oncology, Ignyta, Roche/Genentech, LOXO Oncology, AbbVie, Mirati Therapeutics, ICON PLC, Epizyme, PharmaMar, Daiichi-Sankyo, WIRB-Copernicus Group, Zio-Pharm, Polaris, Blueprint Medicines, Merrimack, Translate Bio, G1 Therapeutics, CARIS Life Sciences, and ERASCA, reports receiving commercial research grants from Bayer, Novartis, Pfizer, Janssen Oncology, Ignyta, Roche/Genentech, Loxo Oncology, AbbVie, Epizyme, Adaptimmune, GlaxoSmithKline, and Daiichi-Sankyo, holds ownership interest (including patents) in Blueprint Medicines, Merrimack, Translate Bio, CARIS Life Sciences, and ERASCA, and is listed on a patent for imatinib in GIST. V.E. Velculescu is an employee/paid consultant for Personal Genome Diagnostics and Takeda, and holds ownership interest (including patents) in Personal Genome Diagnostics. C.P. Paweletz reports receiving speakers bureau honoraria from Bio-Rad, and is an unpaid consultant/advisory board member for Dropworks and Xsphera Biosciences. S. George is an employee/paid consultant for Blueprint Medicines, Deciphera Pharmaceuticals, Bayer, AstraZeneca, Eli Lilly, and Exelixis, and reports receiving other remuneration from Bayer. No potential conflicts of interest were disclosed by the other authors.

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