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Circulating tumor DNA analysis of the phase III VOYAGER trial: *KIT* mutational landscape and outcomes in patients with advanced gastrointestinal stromal tumor treated with avapritinib or regorafenib

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Abstract

Background: The current treatment paradigm of imatinib-resistant metastatic gastrointestinal stromal tumor (GIST) does not incorporate *KIT*/*PDGFRA* genotypes in therapeutic drug sequencing, except for *PDGFRA* exon 18-mutant GIST that are indicated for avapritinib treatment. Here, ctDNA sequencing was used to analyze plasma samples prospectively collected in the phase III VOYAGER trial to understand how the *KIT*/*PDGFRA* mutational landscape contributes to tyrosine-kinase inhibitor (TKI) resistance and to determine its clinical validity and utility.

Patients and Methods: VOYAGER (*N*=476) compared avapritinib with regorafenib in patients with KIT/PDGFRA-mutant GIST previously treated with imatinib and 1 or 2 additional TKIs (NCT03465722). *KIT/PDGFRA* ctDNA mutation profiling of plasma samples at baseline and end-of-treatment was assessed with 74-gene Guardant360® CDx. Molecular subgroups were determined and correlated with outcomes.

Results: 386/476 patients with KIT/PDGFRA-mutant tumors underwent baseline (pre-trial treatment) ctDNA analysis; 196 received avapritinib, and 190 received regorafenib. *KIT* and *PDGFRA* mutations were detected in 75.1% and 5.4%, respectively. *KIT* resistance mutations were found in the activation loop (A-loop; 80.4%) and ATP-binding pocket (ATP-BP; 40.8%); 23.4% had both. An average of 2.6 *KIT* mutations were detected per patient; 17.2% showed 414 different *KIT* resistance mutations. Of all pathogenic *KIT* variants, 28.0% were novel, including alterations in exons/codons previously unreported. *PDGFRA* mutations showed similar patterns. ctDNA-detected *KIT* ATP-BP mutations negatively prognosticated avapritinib activity, with a median progression-free survival (mPFS) of 1.9 versus 5.6 months for regorafenib. mPFS for regorafenib did not vary regardless of the presence or absence of ATP-BP/A-loop mutants and was greater than mPFS with avapritinib in this population. Secondary *KIT* ATP-BP pocket mutation variants, particularly V654A, were enriched upon disease progression with avapritinib.

Conclusions: ctDNA sequencing efficiently detects *KIT/PDGFRA* mutations and prognosticates outcomes in patients with TKI-resistant GIST treated with avapritinib. ctDNA analysis can be used to monitor disease progression and provide more personalized treatment.

Keywords

INTRODUCTION

Detection and monitoring of cancer-related mutations in circulating tumor DNA (ctDNA) theoretically allows capture of the spatial and temporal evolution of tumors across therapeutic interventions.[1] Although mutations are ubiquitous in all cancers, neoplasms driven by targetable genomic alterations may benefit the most from implementation of

ctDNA assessments to determine the mutational status of the underlying disease at the time of treatment. In this sense, gastrointestinal stromal tumor (GIST), the most common sarcoma histological subtype, constitutes a paradigm to address the clinical impact of ctDNA determination.[2–4]

The majority of GISTs are driven by gain-of-function mutations in genes encoding KIT (~70%) or PDGFRA (~15%) receptor tyrosine kinases.[5–7] Accordingly, targeted inhibition of these receptors with first-line imatinib achieves major responses and durable clinical benefit.[7–9] KIT oncogenic signaling remains the primary oncogenic driver after imatinib failure, as emergence of secondary mutations in *KIT* is the primary cause of imatinib resistance in ~90% of KIT-mutant GISTs.[4,10] Previous tumor tissue-based series showed these mutations cluster in the ATP-binding pocket (ATP-BP; encoded by exons 13 and 14) and the activation loop (A-loop; encoded by exons 17 and 18).[10–13] Approved tyrosine kinase inhibitors (TKIs) for the treatment of imatinib-resistant GIST are sunitinib (second line), regorafenib (third line), and ripretinib (fourth line), each displaying an inhibitory spectrum against certain secondary *KIT* mutations.[14–16] Of note, several studies support that *KIT* primary and secondary genotype correlates with the activity of some or all of these TKIs.[10,11,13,17,18] Similarly, avapritinib, a type I TKI approved for the treatment of PDGFRA D842V-mutant GIST, specifically targets the kinase A-loop, and resistant subpopulations emerge through secondary mutations in the ATP-BP.[19,20] This, in turn, confirms the relevance of KIT and PDGFRA signaling throughout GIST progression and reinforces the variable activity of anti-GIST TKIs between GIST-specific molecular subsets.

Despite these data, the current treatment paradigm of imatinib-resistant metastatic GIST does not consider the presence of specific *KIT*/*PDGFRA* mutations to drive therapeutic choices due to limited clinical data validating these approaches, and patients continue to be treated sequentially following the regulatory approval order of the different available TKIs. Several studies have attempted to provide evidence to support use of ctDNA determination to guide therapeutic decisions.[3] A smaller, retrospective study of 243 patients with GIST developed a receiver-operating characteristic sensitivity curve for ctDNA testing and found patient outcomes superior to historical controls when management incorporates ctDNA testing.[4] However, the rarity of this disease together with the heterogeneity of technologies used in previous studies have limited the understanding of GIST progression and the development of ctDNA analysis technology as a clinical tool. Herein, we evaluated prospectively collected ctDNA molecular data from the phase III VOYAGER trial[21] to understand the evolving landscape of TKI progression and expand on the potential clinical utility of ctDNA determination in patients with advanced GIST who experienced progression after 2 or 3 lines of treatment.

PATIENTS AND METHODS

Patients

Main inclusion and exclusion criteria were as follows: eligible patients were aged 18 years or older, had histologically confirmed unresectable/metastatic GIST, and were previously treated with imatinib and up to 2 additional TKIs. Patients were not eligible if previously

treated with either avapritinib, regorafenib, or 4 or more different TKIs. Complete inclusion/ exclusion criteria can be found in Kang et al.[21]

Study design

The open-label, randomized (1:1), multicenter phase III VOYAGER trial compared oral avapritinib (300 mg QD in continuous 28-day treatment cycles) versus regorafenib (160 mg QD for 21 days every 28 days [3 weeks on, 1 week off]) in patients with GIST previously treated with imatinib and a maximum of 2 other additional TKIs (NCT03465722). Random assignment was stratified by third- and fourth-line TKI treatment, geographic region, and *PDGFRA* D842V status measured by ctDNA. Crossover from regorafenib to avapritinib was allowed for patients with centrally confirmed radiologic disease progression. Further design and main clinical outcome details were published previously.[21]

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The Protocol was approved by the institutional review board or independent ethics committee at each study center. All patients provided written informed consent for data collection supporting these analyses.

Sample collection

Plasma samples were obtained from patients with advanced GIST enrolled in VOYAGER at baseline prior to initiation of either avapritinib or regorafenib. Additionally, end-of-treatment plasma samples were collected from patients progressing on avapritinib.

Sequencing and data analysis

Tumor mutation profiling of plasma at baseline and end-of-treatment was assessed with 74-gene Guardant360® CDx sequencing technology. Following the recommendations of the manufacturer, only 2 samples failed quality control evaluations and were consequently discarded from downstream analyses. *KIT/PDGFRA*-detected variants were annotated using Ensembl's Variant Effect Predictor tool, restricting the prioritization to RefSeq canonical transcripts (KIT: NM_000222.3; PDGFRA: NM_006206.6). Nonsynonymous variants were considered pathogenic when meeting at least 3 of the following criteria in order of priority: (1) ClinVar significance is available and (likely) pathogenic, (2) at least 3 predictors (SIFT, PolyPhen-2) or meta-predictors (BayesDel, ClinPred, REVEL) report the variant as pathogenic/damaging, (3) any domain of clinical-biological relevance is affected, (4) the variant is not frequent in the general population (minor allele frequency <1%), and (5) the locus is conserved in mammals.

Statistical analysis

The Kaplan-Meier method was used to estimate progression-free survival (PFS) probabilities. The Cox regression model was used to assess hazard ratios and 95% confidence intervals (CI). To assess statistical associations, Chi-square (discrete variables) and Wilcoxon rank-sum tests (continuous variables) were used for univariate statistical analyses. Logistic regressions were used for multivariate analyses. Descriptive statistics were used to summarize cohort data. The cutoff date for these analyses was March 9, 2020.

RESULTS

Baseline characteristics and ctDNA detection

Baseline ctDNA analysis was performed in 386/476 patients (81.1%) enrolled in the trial, 196 in the avapritinib arm and 190 in the regorafenib arm. A total of 327 (84.7%) patients received avapritinib or regorafenib in the third line, and 59 (15.3%) in the fourth line. Demographics and baseline characteristics did not differ between treatment arms in the subgroup analyzed with ctDNA compared to the overall population (Supplementary Table S1).

All patients had KIT- or PDGFRA-mutant GIST by inclusion criteria. ctDNA mutations in any gene were found in 333/386 patients (86.3%) and showed similar distribution across treatment arms (Figure 1). Of the patients with any ctDNA mutations identified, at least 1 *KIT* or *PDGFRA* pathogenic variant was detected in the plasma of 250/333 (75.1%) and 18/333 (5.4%) patients, respectively. Median ctDNA variant allele frequency was 0.55% (range, 0.02–86.47%) for all ctDNA mutations, and 1.08% (range, 0.03–86.47%) and 1.38% (range, 0.11–20.32%) for *KIT* and *PDGFRA* mutations, respectively. Clinical variables correlating with the presence of *KIT* or *PDGFRA* mutations in plasma in the multivariate analysis were colorectal and gastric primary tumor locations, presence of liver metastases, and the sum of diameters from target lesions (Supplementary Table S2).

KIT mutational landscape in imatinib-resistant GIST

A total of 641 *KIT* pathogenic variants, 179 of which were unique, were detected in the subset of 250 patients with at least 1 *KIT* mutation detected by ctDNA testing prior to treatment on the VOYAGER study. In this population, *KIT* primary and secondary mutations were found in 94.0% and 73.6% of patients, respectively. Both *KIT* primary and secondary mutations were found simultaneously in 68.0% of patients, while 26.4% had only *KIT* primary variants in the absence of any *KIT* resistance mutation, and 6.0% had at least 1 *KIT* resistance mutation without detecting the primary. The majority of these genetic alterations were missense (45.3%), followed by in-frame deletions (39.1%), complex indels (7.8%), and insertions (5.0%). Overall, the distribution of these proportions was similar between treatment arms (Supplementary Table S3).

Primary and secondary *KIT* mutations are distributed across hotspot regions of the kinase, although these data largely originate from scattered tumor tissue series prior to the advent of modern next-generation sequencing.[10,11,13,17,18] The ctDNA profile of *KIT* primary mutations largely paralleled that from the early series, with 78.8% of alterations in *KIT* exon 11, 19.9% in exon 9, and 1.3% in exon 13 (Supplementary Table S3). Notably, the frequency of codons affected across *KIT* exon 11 follows the same distribution of historical tissue-based analysis, which supports the reliability of ctDNA sequencing in GIST (Figure 2). *KIT* secondary mutations in the A-loop—encoded by exons 17 and 18—were more frequent (80.4%) than in the ATP-BP (40.8%)—encoded by exons 13 and 14. Additionally, 23.4% of patients with *KIT* secondary mutations had both ATP-BP and A-loop mutations. Although some specific codons were more commonly found (i.e. D820, N822, V654, Y823) (Figure 2), ctDNA analysis demonstrated for the first time a widespread distribution of secondary

mutations across multiple *KIT* codons and exons (Figure 2; Supplementary Figure S1A). The frequency of all amino acid changes can be seen in Supplementary Table S3.

Notably, the determination of ctDNA in this large cohort of patients with GIST in the third- or fourth-line setting after disease progression on imatinib and sunitinib allowed identification of 50 novel pathogenic *KIT* variants, 28.0% of the total identified. Although half of them were deletions, insertions, or indels affecting *KIT* exon 11, a total of 14 *KIT* variants were missense substitutions affecting a wide range of exons. Interestingly, secondary in-frame deletions (*N*=6) and in-frame insertions (*N*=2) were identified for the first time in *KIT* exon 17 (Supplementary Table S4).

PDGFRA mutational landscape in imatinib-resistant GIST

Similar mutational patterns were observed across the 18 PDGFRA-mutant GISTs as determined by ctDNA. Half of the patients had *PDGFRA* mutations in the A-loop, with the most frequent being the D842V substitution (27.8%), which agrees with known frequencies (Supplementary Figure S1B; Supplementary Figure S2; Supplementary Table S5). However, ctDNA analysis also detected pathogenic *PDGFRA* mutations in several other exons beyond the expected 12, 14, and 18. Furthermore, ctDNA analyses provided insight on resistance mutations, which remains largely unexplored. Only 3 patients showed secondary mutations in *PDGFRA* (Supplementary Figure S2; Supplementary Table S5). As occurred with *KIT*, 7 novel pathogenic *PDGFRA* variants were found by ctDNA determination (Supplementary Table S4).

Heterogeneity of KIT and PDGFRA resistance mutations

The spectrum of secondary resistance mutations in GIST that can be identified by ctDNA is currently unclear, as it has been only investigated in few studies with small numbers of patients using a wide range of technologies with varying sensitivities. Herein, among KIT-mutant tumors, multiple *KIT* mutations were commonly detected with an average of 2.6 mutations per patient. The majority of patients had 1–3 *KIT* variants detected, although 17.2% of the patients had substantial heterogeneity, with 4–14 different *KIT* mutations detected in plasma (Figure 3). There were no statistically significant differences between patient distributions in the avapritinib and regorafenib arms, based on the number of *KIT* mutations. Only 3/18 PDGFRA-mutant GISTs had more than 1 variant detected in plasma, 2 patients with 2 each, and 1 patient with 5 (Supplementary Figure S2B). The extent of *KIT* mutational heterogeneity did not correlate with any of the baseline clinicopathological features (Supplementary Table S2).

KIT ctDNA determination correlates with clinical efficacy in avapritinib- and regorafenib-treated patients with GIST

Current treatment of metastatic KIT-mutant GIST relies on sequential administration of TKIs solely based on their regulatory approval order, without considering the evolving GIST mutational landscape. Therefore, we investigated whether ctDNA analysis has a prognostic role in this setting. Of the 386 patients with a baseline ctDNA test performed, 352 had baseline measurable target lesions. In this subset of patients, median PFS (mPFS) for

avapritinib and regorafenib was 3.8 and 5.6 months, respectively, which did not differ from the total population (Supplementary Figure S3).

The detection of *KIT* secondary mutations in the ATP-BP was a negative prognostic factor of avapritinib clinical efficacy, with a mPFS of 1.9 months (Figure 4A). Indeed, the absence of ATP-BP mutations in plasma correlated with avapritinib activity in patients, with a mPFS of 5.6 months (Supplementary Figure S4A). In comparison, regorafenib efficacy in patients with GIST harboring ATP-BP mutations detected in the ctDNA was significantly better than that of avapritinib, with a mPFS of 5.7 months (*P*<0.001; Figure 4A). The antitumor activity of regorafenib did not differ regardless of the presence or absence of *KIT* ATP-BP resistance mutations in plasma (5.7 vs 5.6 months, *P*=0.829; Supplementary Figure S4B). Also, any dimensional shrinkage per RECIST in patients with ATP-BP mutations was more frequently observed in patients treated with regorafenib (65.7% vs 25.8%, *P*=0.002).

The detection of KIT A-loop mutants in plasma was prognostic of better mPFS with regorafenib compared to avapritinib (6.3 vs 3.9 months, *P*=0.011; Figure 4B). However, in the absence of ATP-BP mutations, the presence of a KIT A-loop mutation alone in ctDNA was neither prognostic of clinical efficacy with avapritinib nor regorafenib, likely indicating better avapritinib activity in the absence of ATP-BP mutants (Supplementary Figure S4C–D). Finally, the simultaneous presence of ATP-BP and A-loop mutations in plasma was also a negative prognostic factor of avapritinib versus regorafenib efficacy (1.9 vs 5.7 months, *P*=0.004; Figure 4C). Accordingly, PFS curves in this subset of patients tended to overlap with outcomes from patients with GIST harboring only ATP-BP mutations, confirming their relevance to determine avapritinib/regorafenib treatment outcomes in patients with GIST (Supplementary Figure S4E–F).

The number and heterogeneity of mutations found in plasma can be associated with outcomes. We first studied *KIT*-positive versus *KIT*-negative ctDNA populations, since *KIT* is the clonal driver mutation, and all patients had KIT- or PDGFRA-mutant GIST by inclusion criteria. From the 386 patients who underwent ctDNA testing, *KIT* mutations were not detected in 136 (avapritinib, *N*=72; regorafenib, *N*=64). The 18 patients with PDGFRA-mutant GIST were excluded from this analysis. Surprisingly, avapritinib and regorafenib displayed different behavior (*P*=0.0008, Figure 4D): while patients with *KIT*-positive ctDNA had a shorter mPFS with avapritinib (3.7 vs 5.7 months), regorafenib activity was not affected by the detection of *KIT* variants in plasma (5.5 vs 5.7 months). The heterogeneity of *KIT* mutations detected in plasma was further evaluated. However, increased heterogeneity (>3 mutations) did not impact mPFS in either treatment arm (Supplementary Figure S4G–H). Together, the detection of *KIT* mutations in ctDNA correlates with outcomes in patients with metastatic GIST. Particularly, the determination of specific regions mutated across the *KIT* gene is relevant to determine the sensitivity or resistance of specific TKIs before treatment initiation.

Individual ctDNA mutations in KIT and PDGFRA determine TKI sensitivity and resistance in metastatic GIST

Preclinical studies suggested that individual primary and secondary mutations in *KIT* and *PDGFRA* can predict the efficacy of TKIs either approved or having shown activity in

GIST. However, little supportive correlative science is available so far beyond the first-line setting.[10,11,13,17,18] Here, individual *KIT* secondary ctDNA mutations were not associated overall withTKI response/progression. This can be explained by the heterogeneity of *KIT* secondary mutations with different sensitivity profiles and the predominance of disease stabilization as best response outcomes. However, the presence of baseline *KIT* exon 13 V654A mutations in plasma, together with other less common substitutions in the ATP-BP (T670, N680), tended to correlate against avapritinib activity, with only 1 patient showing a RECIST partial response compared to the 4 partial responses achieved by regorafenib (Figure 5A). The proportion of partial responses with avapritinib or regorafenib did not differ substantially regardless of the individual secondary mutation in the most common codons across the A-loop (exons 17 and 18), although alterations found in the last part of the A-loop (Y823, A829) were arguably better surrogate markers of activity for regorafenib compared with avapritinib (Figure 5A).

The specific exonic or amino acid involvement of primary mutations in *KIT* and *PDGFRA* can predict the clinical efficacy of first- and second-line imatinib and sunitinib regardless of the status of the secondary mutations. Herein, avapritinib appeared to have similar efficacy regardless of the location of primary mutations in *KIT*, displaying a mPFS of 3.7 and 3.5 months, respectively, against *KIT* exon 11 and exon 9 mutants. However, the detection of primary *KIT* exon 11 mutations in the plasma of patients treated with regorafenib showed a nonsignificant trend ($P=0.067$) towards an improved mPFS in comparison with those patients harboring a primary *KIT* exon 9 mutation (7.9 vs 3.7 months, Figure 5B and 5C). Likewise, ctDNA detection of a primary *PDGFRA* exon 18 D842V mutation demonstrated improved clinical efficacy with avapritinib, as the mPFS was not reached relative to 4.5 months with regorafenib ($P=0.035$; Figure 5D). This, in turn, agrees with prior data confirming the high activity of avapritinib in this molecular subgroup of GIST as well as the little clinical efficacy of other TKIs approved for treatment of metastatic GIST.[19,22]

Serial ctDNA determinations detect the emergence of resistant subpopulations

To further understand the sensitivity/resistance profile of avapritinib, plasma samples from 41 patients with GIST were collected at the time of disease progression, and 40 could be analyzed. Compared to baseline, there was a significant increase in the detection of *KIT* secondary mutations ($P=0.022$) at the time of disease progression. Although this increase was largely polyclonal, *KIT* ATP-BP pocket variants were found highly enriched ($P=0.001$; Figure 6A), particularly V654A. Subclonal dynamics of a representative case shows that, in the molecular context of *KIT* mutational heterogeneity, there is an allele frequency increase in a resistant subpopulation harboring the *KIT* exon 13 V654A substitution that parallels the primary clonal *KIT* exon 9 mutation. Conversely, subpopulations with secondary mutations in the A-loop remained inhibited at the time of avapritinib progression (Figure 6B). Together, ctDNA analyses at the time of disease progression are useful to determine the resistant subclones responsible for TKI resistance, confirm the accuracy of baseline ctDNA determination in prognosticating treatment outcomes, are helpful in selecting next-line therapy, and are overall consistent with the known mechanism of action for avapritinib specifically targeting the A-loop of *KIT* and *PDGFRA*.

DISCUSSION

GIST constitutes an excellent model to evaluate implementation of ctDNA as a routine clinical tool to guide cancer treatment decisions. Supporting this view is the fact that oncogenic activation of *KIT* or *PDGFRA* is the essential driver for GIST development and maintenance. Unlike most mutant KIT/PDGFRA-driven tumors, the main mechanism of resistance in GIST is the polyclonal emergence of cis-allele, secondary mutations in the primary oncogenic driver, either *KIT* (~90% of patients) or *PDGFRA*, and preclinical studies consistently show that TKIs used in GIST treatment display drug-specific activity against subsets of the *KIT/PDGFRA* mutational spectrum.[10,11,13,17,18,20] However, only a limited number of studies have analyzed mechanisms of tumor progression after first-line imatinib, and they did not establish the clinical role of ctDNA determination in therapeutic decision-making.[3] Thus, this study evaluates, for the first time, the clinical validity and potential clinical utility of ctDNA determination in a large cohort of patients with metastatic GIST with plasma samples collected prospectively in the VOYAGER trial.

The detection rate of *KIT* and *PDGFRA* mutations in ctDNA was 69%, with an average allele frequency of ~1%. Although these third- or fourth-line patients typically have bulky disease, ctDNA shedding in GIST is comparatively lower than other cancer types,[4] which appears common for many sarcomas.[23–25] Regardless of these tumor-related limitations, primary mutations in known exonic regions of *KIT* and *PDGFRA* were found at similar proportions as described previously. This also relates to the profile of codons affected within *KIT* exon 11, which often includes complex insertions, deletions, or indels that could be detected in plasma.[7] Furthermore, baseline ctDNA analysis from the VOYAGER trial uncovered a shift in resistance patterns after 2–3 lines of treatment. Namely, *KIT* secondary mutations in the A-loop were predominant at the expense of a decrease in clones harboring ATP-BP mutations—known to be the most common at the onset of imatinib failure[13]—which is consistent with the selective pressure exerted by second-line sunitinib, which has potency against ATP-BP mutant clones. Another relevant aspect was an arguably low level of complex heterogeneity, with 82% of the patients displaying only 1–3 *KIT* variants. Although it is likely that some mutations exist below our threshold of detection, accurate prognosis of outcomes may increae the possibility that undetected clones are not clinically meaningful, even when present. Finally, this ctDNA analysis also determined a greater spectrum of *KIT* pathogenic variants compared with previous studies, including exons and codons previously not known to be affected. Together, this analysis of the mutational spectrum of acquired resistance as demonstrated by ctDNA reinforces the critical role of *KIT* oncogenic signaling and provides the basis for future drug development in GIST after progression to various lines of treatment.

This study also demonstrates that ctDNA assessment might predict TKI activity in metastatic GIST in the third- and fourth-line setting. Thus, although the VOYAGER trial did not yield significant mPFS differences between avapritinib and regorafenib, ctDNA determination revealed the detection of ATP-BP mutations in plasma was a strong negative prognostic marker of avapritinib activity. Conversely, patients with GIST harboring A-loop mutations in the absence of ATP-BP achieved equal benefit from avapritinib and regorafenib. This, in turn, is consistent with the known mechanism of action of avapritinib,

its superior efficacy in patients with tumors harboring the *PDGFRA* D842V mutation, and the enrichment of *KIT* exon 13 V654A subpopulations observed at the time of progression. Surprisingly, regorafenib appeared to have similar activity irrespective of the type of *KIT* secondary mutation, while prior preclinical studies set V654A as a potential liability.[13] It is conceivable the multikinase nature of regorafenib allows blockage of several other signaling intermediates downstream of KIT relevant for its oncogenic function and mediation of GIST cell growth, thus compensating for incomplete upstream KIT inhibition.

In summary, we have shown ctDNA sequencing reliably detects *KIT/PDGFRA* mutations and correlates with outcomes in patients with metastatic, TKI-resistant GIST treated with avapritinib or regorafenib. Therefore, consideration of ctDNA to monitor disease progression and provide more personalized treatment options in patients with GIST should continue to be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- This study comprehensively documents the landscape of *KIT* and *PDGFRA* mutations in metastatic, imatinib-resistant GIST

- The selective pressure exerted with prior lines promotes a shift toward increased resistant subpopulations in the *KIT* A-loop

- Individual mutations in *KIT*/*PDGFRA* determine TKI sensitivity and resistance in metastatic GIST

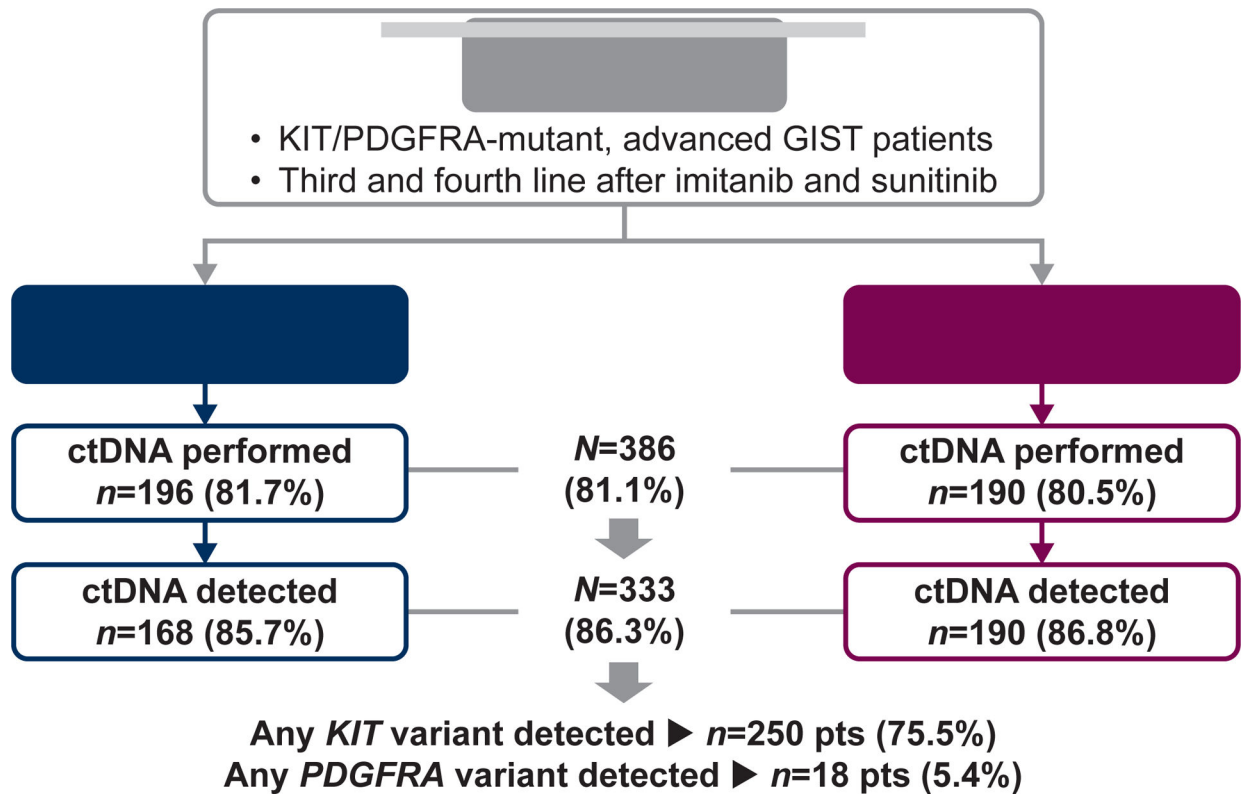- ctDNA-detected *KIT/PDGFRA* mutations in imatinib-resistant GIST prognosticate third- or fourth-line TKI treatment outcomes

Figure 1.
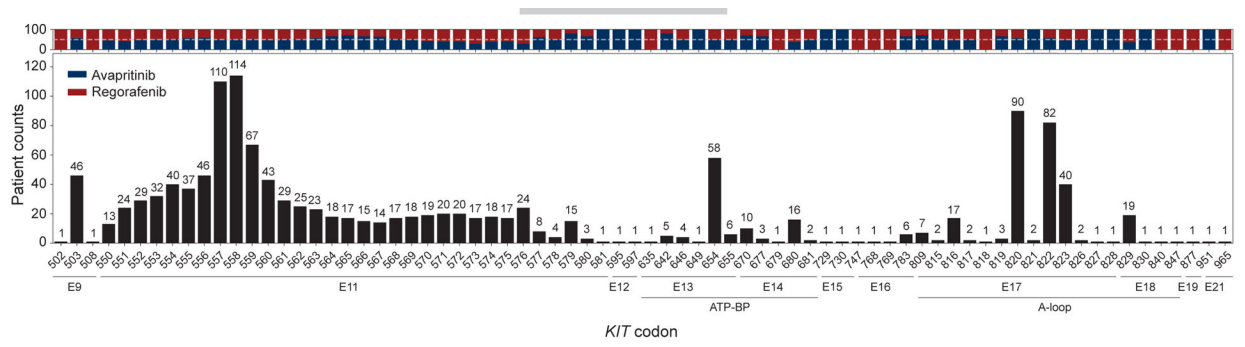Flow chart of ctDNA analyses performed and mutational findings.

Figure 2. *KIT* affected exons and codons.
The distribution of codons affected by any KIT variant was similar between treatment arms, which is better observed in those with higher frequency.
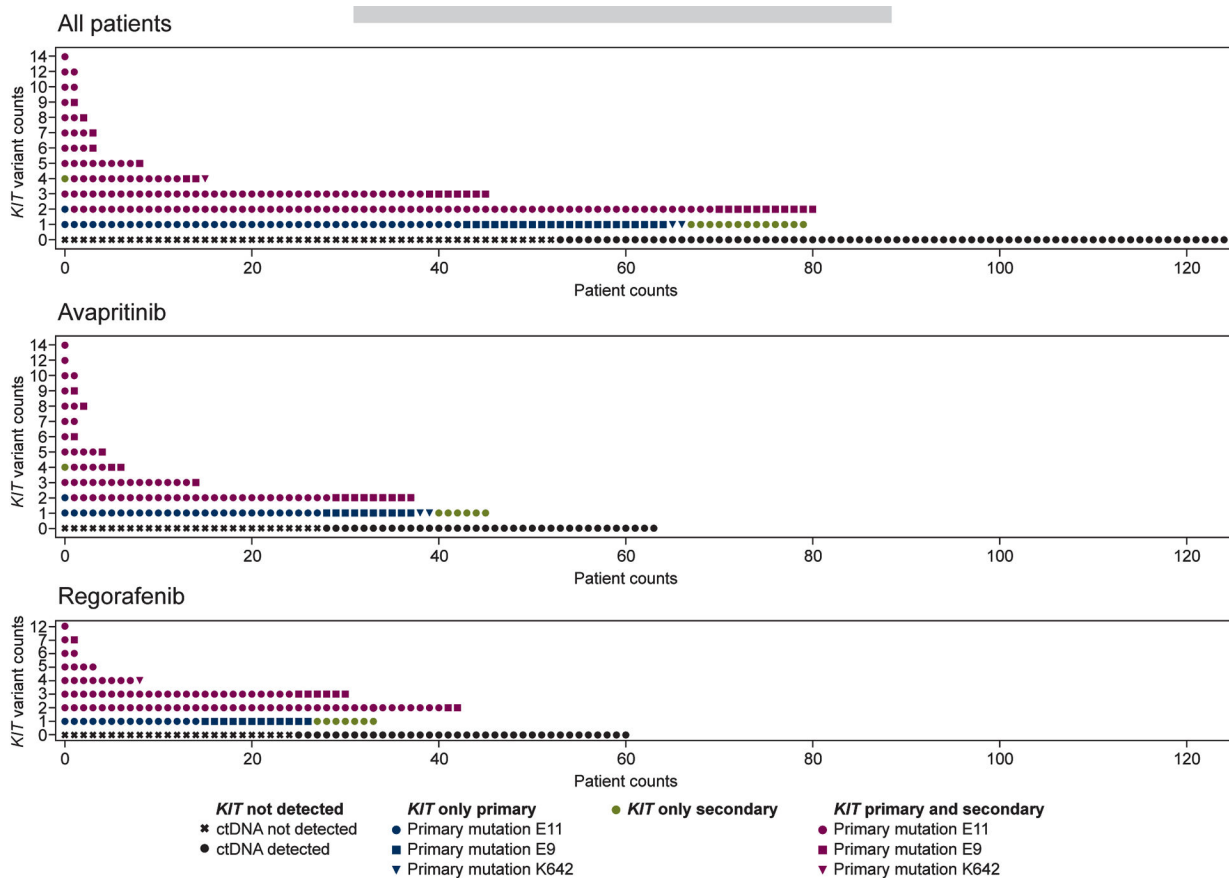
Figure 3. *KIT* mutational heterogeneity.
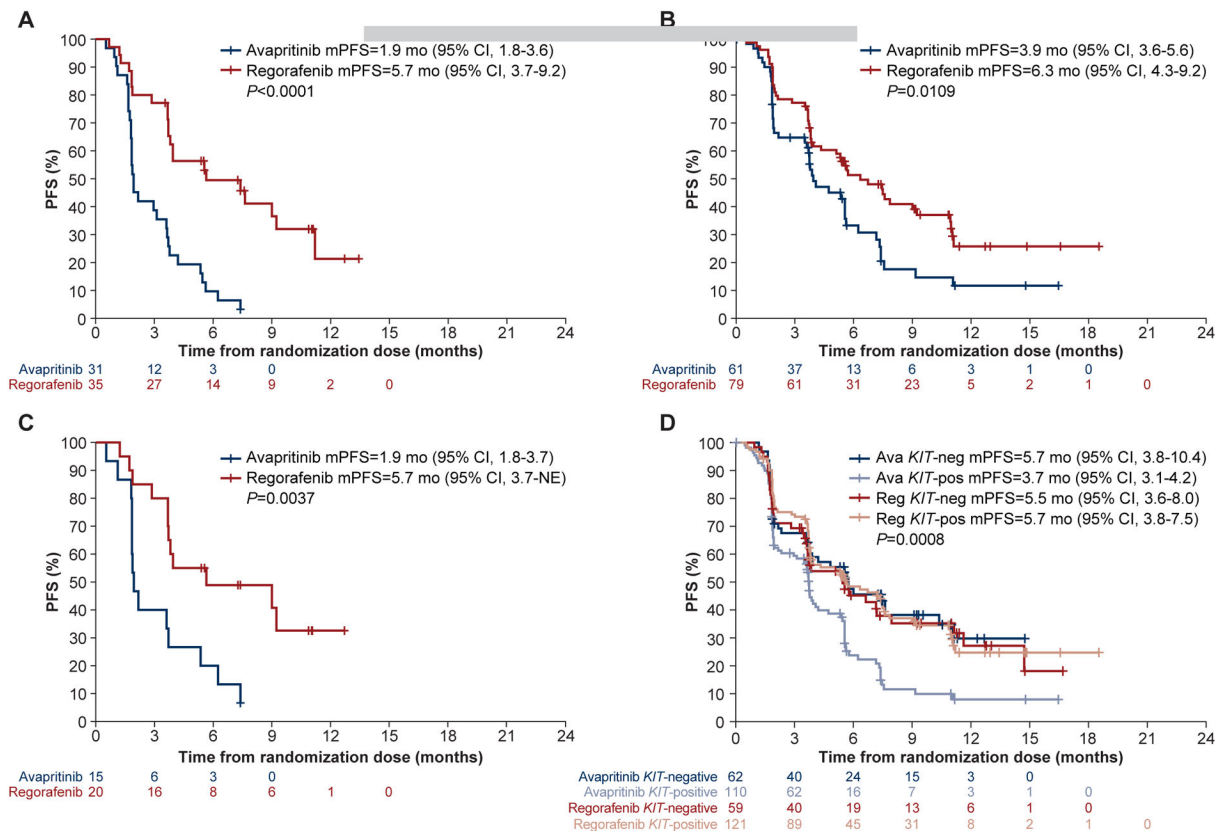Multiple *KIT* mutations were commonly detected among KIT-mutant tumors.

Figure 4. *KIT* ATP-BP and A-loop mutations in ctDNA are prognostic of TKI activity in GIST.
Regorafenib had greater efficacy than avapritinib in patients with an ATP-BP mutation
(A), a *KIT* A-loop mutation (B), and those with both ATP-BP and A-loop mutations (C).
(D) Avapritinib improved PFS in the absence of a *KIT* mutation, while regorafenib was
relatively unaffected. Ava, avapritinib; CI, confidence interval; mPFS, median progression-
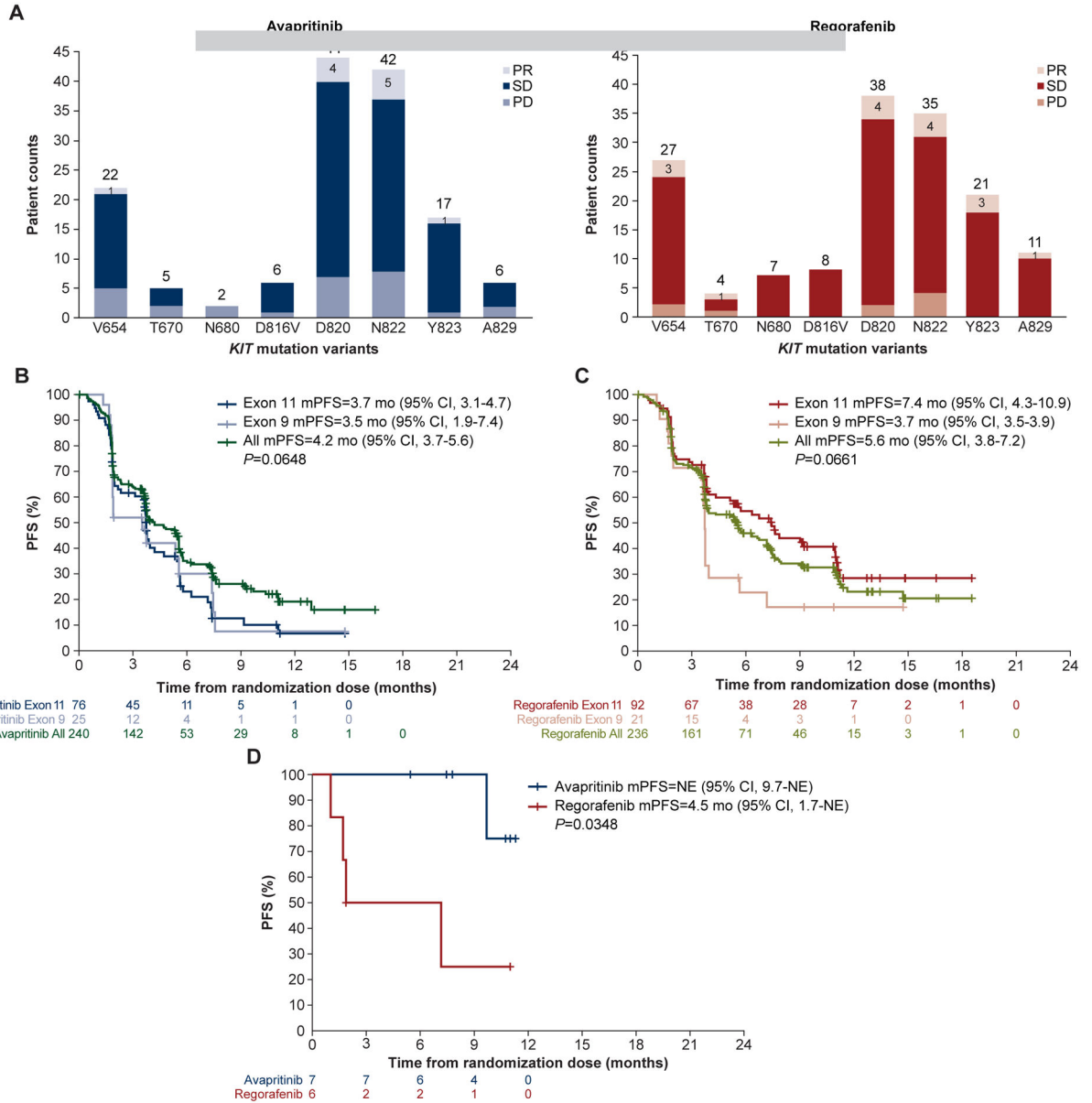free survival; NE, not estimable; neg, negative; pos, positive; Reg, regorafenib.

Figure 5. *KIT/PDGFRA* primary and secondary mutations determined in ctDNA in GIST are prognostic of TKI activity.

(A) *KIT* secondary mutations did not significantly affect the number of partial responses between treatments arms. Avapritinib efficacy was similar between primary *KIT* exon 9 and 11 mutations (B), whereas regorafenib trended to be more effective in patients with a primary exon 11 mutation (C). (D) Avapritinib had greater efficacy in patients with a primary *PDGFRA* exon 18 D842V mutation than regorafenib. CI, confidence interval; mPFS, median progression-free survival; NE, not estimable; PD, progressive disease; PR, partial response; SD, stable disease.
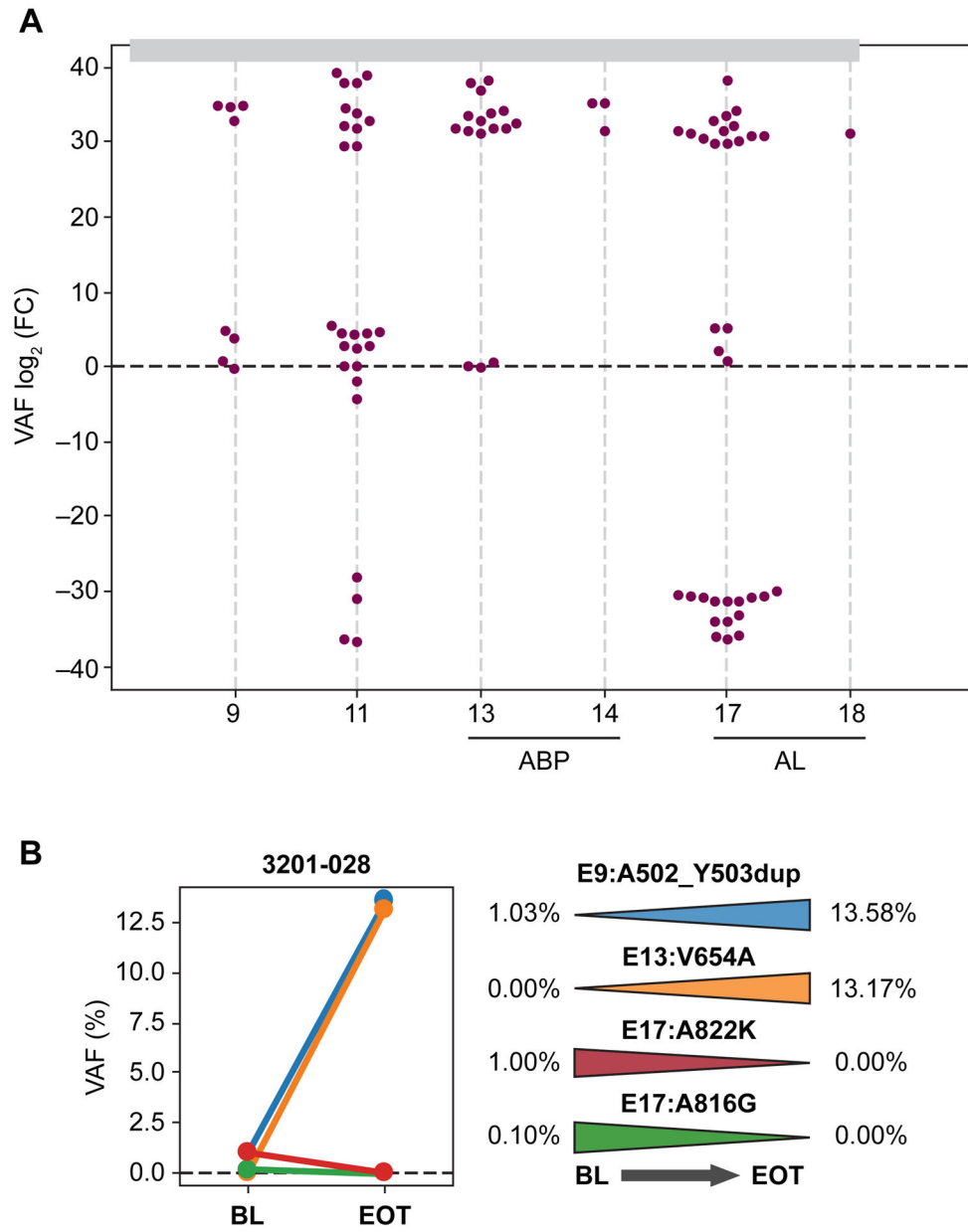
A

B

Figure 6. Serial *KIT* ctDNA determinations detect resistance.
(A) *KIT* secondary mutation detection significantly increased upon disease progression. (B) Representative subclonal evolution showing increased allele frequency in subpopulations with a *KIT* exon 13 V654A mutation, in parallel to primary clonal *KIT* exon 11 mutations. ABP, ATP-BP; AL, activation loop; BL, baseline; EOT, end-of-treatment; FC, fold change; VAF, variable allele frequency.