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Research Article

Clinical Use of Liquid-Based Comprehensive Genomic Profiling in Gastrointestinal Stromal Tumors

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ABSTRACT

Treatment for gastrointestinal stromal tumor (GIST) focuses on tyrosine kinase inhibitors, the selection of which depends on specific mutations. We sought to determine the clinical use of liquid biopsy in advanced GIST. Liquid (n = 181) (FoundationOne Liquid CDx) and tissue (n = 2198) (FoundationOne and FoundationOne CDx) comprehensive genomic profiling of GIST were evaluated. The presence of circulating tumor DNA in liquid was determined via tumor fraction (TF), with an elevated TF defined as TF $\geq$ 1%. Liquid comprehensive genomic profiling revealed 30% (54/181) of samples had an elevated TF, among which the prevalence of *KIT* and *PDGFRA* alterations were 89% (48/54) and 2% (1/54), respectively. In patient-matched tissue/liquid samples (n = 49), the positive percent agreement of driver alterations in liquid with an elevated TF relative to tissue was 100%. Fifty-five percent (42/77) of liquid samples with a *KIT* driver mutation had a co-occurring imatinib-resistant alteration; a minority of cases harbored non-*KIT* mechanisms of resistance such as *FGFR2* fusions and *BRAF* or *EGFR* alterations. The relative prevalence of imatinib resistance *KIT* exon 13 and 17 mutations was enriched in liquid compared with tissue. Finally, in the liquid cohort, 2.2%, 1.7%, and 1.1% of patients were predicted to harbor germline *KIT*, *SDHx*, or *NF1* mutations, respectively. In conclusion, known driver and tyrosine kinase inhibitor–resistant mutations were identified in liquid biopsies of patients with GIST with high concordance to tissue in the presence of an elevated TF. Liquid biopsy may be valuable in the molecular classification and medical management of GIST.

Introduction

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal cancer of the digestive tract, affecting 10 to 15 patients per million worldwide annually and having a 5-year survival rate of 82%.[1] Mutations in the *KIT* proto-oncogene are driver events in approximately 70% to 80% of GISTs, whereas another 5% to 10% of GIST harbor driver *PDGFRA* alterations. Besides *KIT* and *PDGFRA*, less-frequent traditional driver events include alterations in the RAS-MAPK pathway, including *BRAF* and *NF1* or *SDHA/B/C/D* alterations.[2-4] More recently, rare fusions have also been identified, such as in *NTRK* (*NTRK3::ETV6*) and *FGFR1*

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(*FGFR1::HOOK3* and *FGFR1::TACC1*), in what were previously considered "wild-type" GIST. The standard of care focuses largely on surgical resection and tyrosine kinase inhibitors (TKI), the selection of which is based on specific mutations.

Oncogenic *KIT* genomic mutations lead to constitutive activation of the KIT receptor tyrosine kinase, thereby promoting cell proliferation and inhibiting apoptosis through downstream effector RAS/RAF/MAPK, PI3K/Akt/mTOR, and Src pathways. Most of the activating *KIT* mutations occur in exon 11, whereas exon 9 mutations are common in GISTs of the small or large bowel.[5,6] In contrast, *PDGFRA* mutations occur mainly in exons 12, 14, and 18, with exon 18 D842V point mutation being the most frequent.[5]

When localized, surgery is the only curative option for GIST. High-risk patients require postoperative imatinib treatment for at least 3 years, if well tolerated.[7,8] In the metastatic setting, imatinib is the first-line treatment; however, secondary mutations in *KIT* and *PDGFRA* may cause imatinib resistance.[9] Alternatively, avapritinib is specifically designed to target *PDGFRA* exon 18 D842V mutations, showing high efficacy with over 90% overall response rate and 70% duration of response at 1 year.[10] Secondary mutations in *KIT* exons 17, 13, and 14 lead to imatinib resistance in the second-line setting. In addition, Food and Drug Administration–approved sunitinib and regorafenib are used in the second- and third-line settings, respectively, whereas ripretinib is effective in the fourth line, particularly against secondary exon 13 mutations. Avapritinib is indicated as front-line or later therapy for GIST with *PDGFRA* exon 18 D842V mutations and possibly for GIST with other *PDGFRA* exon 18 mutations.[11] Wild-type GISTs may also respond to TKIs owing to low-frequency mutations. The optimal therapy for other molecular subtypes such as SDH-deficient, *NTRK* fusions, *BRAF* V600E mutations, and *NF1* mutations remains poorly defined.[12]

Prior studies have also identified negative molecular prognostic markers. In *KIT*-mutated GISTs, small deletions in exon 11, especially involving codons 557 and 558, have been shown to be associated with poorer prognosis compared with exon 11 point mutations.[13,14] *RB1*, a tumor suppressor and cell cycle inhibitor, mutations have been linked with morphologically high-risk features and clinically malignant behavior.[15] A messenger RNA expression study of 38 GISTs revealed that low expression of *CDKN2A* and high expression of *RB1* and *TP53* were associated with aggressive clinical behavior and unfavorable prognosis.[16,17] Although *TP53* mutations are rare in GISTs, overexpression of p53 is linked to high-risk GISTs and poor outcomes.[15,16,18-20] *MTAP* homozygous deletion was also found to be predictive of reduced progression-free survival, independent of high-risk status, Ki-67 index, and tumor location.[21] Last, the *SETD2* gene, which encodes a histone modifier, may be altered in up to 11% of high-risk GISTs but not in the low-risk subgroups, with *SETD2*-altered gastric GISTs exhibiting shorter relapse-free survival.[22]

Most of the *KIT*- or *PDGFRA*-mutated GISTs are sporadic tumors; however, GIST-prone syndromes occur (ie, Carney-Stratakis syndrome, Carney triad, and familial GIST syndrome), mostly depending on heritable GIST predisposing germline alterations, such as those in the SDH complex subunit genes. Familial GIST syndrome may also be associated with germline mutations in *KIT* or *PDGFRA* genes,[23] although prevalence of germline *KIT* mutations in patients with advanced GIST is unknown. In contrast, most patients with wild-type *KIT*/*PDGFRA* GIST harbor a germline pathogenic variant in other GIST-associated genes (*SDHA*, *SDHB*, *SDHC*, and *NF1*).[24] Despite germline associations with GIST driver genes, there are no standard guidelines for germline testing in patients with GIST.

Currently, a main challenge in GISTs is the acquisition of TKI resistance owing to secondary mutations as result of the selection pressure exerted by TKIs.[25] Although testing of solid tissue may be the gold standard to detect driver and secondary resistance mutations, liquid testing with peripheral blood has emerged as a viable alternative to tissue. Extensive parallel sequencing to analyze circulating tumor DNA (ctDNA) has enabled quick detection of molecular alterations in different cancers, providing a less-invasive method for comprehensive genomic profiling (CGP) compared with solid tumor tissue. Although prior studies have reported the potential usefulness of liquid biopsy in GIST, large real-world studies examining the clinical use of liquid biopsy in the context of both ctDNA tumor fraction (TF) (a measure of ctDNA content) and concordance with tissue from same patients are scant.

In the current study, we explore the role, clinical use, and feasibility of CGP via liquid biopsy in patients with advanced GIST, especially considering its potential therapeutic implications in precision oncology in the setting of an elevated ctDNA TF. Given the emergence of secondary mutations in the natural progression of the disease, understanding these changes is crucial. The feasibility of this approach may provide a wealth of information without the need for invasive rebiopsies, thereby offering insights into circulating, active clones. Liquid biopsy has the potential to aid our understanding and management of GISTs, aligning with the evolving landscape of personalized medicine.

Materials and Methods

Gastrointestinal Stromal Tumor Cohorts From Foundation Medicine

A retrospective search at Foundation Medicine Inc (FMI), a Clinical Laboratory Improvement Amendments– and College of American Pathologists–certified molecular laboratory, was performed for cases with the submitting diagnosis of GIST during the course of clinical care. A total of 181 GIST liquid biopsy specimens were included that were assayed by FMI via FoundationOne Liquid CDx (F1LCDx) up to October 2023. A cohort of 49 paired solid tissue and liquid samples from the same patients was also evaluated to determine the positive percent agreement (PPA) between liquid and solid tissue testing. For comparison with liquid, a total of 2198 GIST solid tissue specimens were analyzed that were assayed by FMI via either FoundationOne or FoundationOne CDx through 2022. F1LCDx and FoundationOne CDx are FMI's Food and Drug Administration–approved, next-generation sequencing (NGS)-based CGP assays based on hybridization capture technology for the detection of substitutions, short insertion and deletion alterations, copy-number alterations, and select rearrangements in 324 genes, and of genomic signatures, including microsatellite instability and tumor mutational burden.[26,27] FMI's ctDNA TF was quantified with an algorithm that incorporates aneuploidy, variant allele frequency (VAF), and canonical alterations detected on F1LCDx.[28] A *KIT* alteration was predicted to be germline in liquid biopsies when VAF was approximately 50% with a concurrent low TF (<1%). Clinicopathological data including patient age were extracted from the accompanying pathology reports or associated clinical records when available.

Gastrointestinal Stromal Tumor Cohort From Geneva University Hospital

Clinical data from 43 patients diagnosed with GIST were retrieved from the Geneva University Hospital database for the period of January 2018 to April 2023. Inclusion criteria included a patient age of >18 years and molecular testing. Three patients had a PCR-based molecular test; 16 patients had NGS of 50 genes; 13

patients had NGS of 100 genes; 10 patients had NGS of 400 genes; and 1 patient had NGS of 500 genes. Statistical analysis was performed with IBM SPSS v29.01 software (IBM). The study was approved by internal ethics committees.

Results

Liquid Biopsy Comprehensive Genomic Profiling of Gastrointestinal Stromal Tumors

A cohort of 181 patients diagnosed with GIST and receiving liquid biopsy–based CGP via F1LCDx was used to determine the liquid mutational landscape of GISTs. TF, a measure of ctDNA content, was elevated (TF $\geq$ 1%) in 30% of samples. In the overall cohort regardless of TF levels, alteration frequencies of known GIST drivers included 43% (*KIT*); 2.8% (*PDGFRA*); 9% (*NF1*); 2.3% (*SDHx*); 3% (*BRAF*); and 3.9% (*KRAS/NRAS*) (Supplementary Fig. S1). Fifty-five percent (42 out of 77) of samples with a *KIT* driver mutation had a co-occurring imatinib-resistant *KIT* alteration. Alteration frequencies of known prognostic markers included 11% (*TP53*); 2.2% (*RB1*); and 1.7% (*SETD2*) (Supplementary Fig. S1).

We further stratified the liquid biopsy cohort based on elevated ctDNA TF levels ($\geq$1%). Via this subanalysis, in liquid biopsies with ctDNA TF $\geq$ 1%, the frequency of *KIT* was 89%, whereas the alteration frequencies of other drivers and prognostic markers were as follows: 1.9% (*PDGFRA*); 11% (*NF1*); 6% (*BRAF*); 4% (*KRAS*); 11% (*TP53*); 6% (*RB1*); and 4% (*SETD2*) (Fig. 1). The occurrences in the latter 3 genes (*TP53*, *RB1*, and *SETD2*) were secondary alterations that have been associated with poor prognosis as canonical driver alterations were also present in most of those tumors. With an elevated TF, 3 out of 54 or 5.5% of cases lacked a canonical GIST driver and were considered "wild-type" GISTs. Direct comparison between liquid and tissue frequencies according to TF levels is shown in Figure 2. At elevated TF levels, liquid biopsies generally showed similar rates of driver alterations and "wild-type" GISTs as solid tissue testing. In addition, enrichment in alterations in *NF1* and *BRAF*, and in PI3K signaling components, such as *PTEN* and *PIK3CA,* was detected in liquid compared with tissue (Fig. 2). In contrast to cases with elevated TF, alteration frequencies for driver genes and prognostic markers in liquid biopsies with low TF (ie, TF <1%) are show in Supplementary Fig. S2.

For comparison with liquid, landscape analysis of 2198 GIST tissue cases that were previously submitted for CGP during the course of routine clinical care is shown in Supplementary Figure S3. Tissue CGP of GIST revealed the following prevalence of driver *KIT* (77%), *PDGFRA* (8%), *NF1* (6%), *SDHx* (3%), and *BRAF* (1%) alterations (Supplementary Fig. S3). In all, 7% of cases had no reportable known pathogenic alterations in the canonical GIST genes (wild-type GIST), whereas 2% of cases had a mutation in more than 1 driver. Alteration frequencies of known poor prognostic markers included 29% (*CDKN2A*); 9% (*RB1*); 6% (*TP53*); and 4% (*SETD2*) (Supplementary Fig. S3). Majority of tumors were microsatellite stable (98.3%) with low tumor mutational burden, < 10 mutations/Mb (99.5%).

Analysis of Paired Liquid and Tissue Samples From Same Patients

A cohort of 49 paired tissue and liquid samples from same patients was available to evaluate PPA of the key GIST driver genes between liquid and tissue samples. In the tissue biopsy for these 49 patients, main tumor drivers included *KIT* (45/49; 92%), *PDGFRA* (3/49; 6%), and *BRAF* (1/49; 2%). When TF was low (<1%), liquid biopsy detected a main driver alteration in 11 of 35 specimens for a PPA of 31% (Table 1). In contrast, liquid biopsies detected driver mutations in 14 of 14 patients that were identified in tissue when liquid TF was $\geq$1% with a PPA of 100% (Table 1). Furthermore, in patients with an elevated TF ($\geq$1%), 11 of 14 patients (78.6%) harbored an imatinib-resistant *KIT* mutation, and in 7 out of these 11 patients (63.6%), imatinib-resistant *KIT* mutations were present in the liquid sample only and not in the paired tissue sample.

Analysis of KIT Mutations Detected Using Liquid Biopsy (F1LCDx)

We next sought to assess the distribution of *KIT* variants across different exons that are associated with either driver events (exons 9 and 11) or imatinib resistance (exons 13, 14, 17, and 18). The distribution and types of *KIT* alterations that were detected in liquid specimens across different *KIT* exons are shown in Figure 3. In the overall cohort, the relative prevalence of *KIT* exon 9 alterations was comparable in tissue and liquid specimens. In contrast, tissue specimens showed a proportionally higher frequency of exon 11 alterations ($P = .028$), whereas liquid specimens showed higher prevalence of secondary TKI-resistant alterations, which was as follows: significant enrichment of exon 13 and 17 alterations ($P < .001$ and $P < .006$), as well as higher prevalence of exon 14 and 18 alterations. In liquid samples, 55% of *KIT* alterations were missense substitutions and 42% were inframe short variant insertions/deletions, whereas 3% were splice site mutations.

In the liquid cohort, *KIT* mutations were predicted to be germline in 2.2% (4 of 181) of patients based on a VAF of ~50% and a low TF (<1%) (Table 2). Mean patient age ranged from 47 to 76 years. Three patients were male, and 1 patient was a female. Half (2/4) of the predicted *KIT* germline mutations were located in exon 11 (patients with *KIT* D579del), whereas one-fourth was located in exon 13 (*KIT* K642E) and the other one-fourth was located in exon 17 (*KIT* D820G, an imatinib resistance mutation). Clinical history was available for 1 patient with a predicted *KIT* K642E germline mutation. This was a 47-year-old male patient at the time of diagnosis with a history of multiple small intestine primary GISTs, hyperplasia of myenteric plexus, and pigmented skin lesions, including dysplastic skin nevi. For these reasons, a *KIT* germline mutation and a familial syndrome were clinically suspected. The liquid biopsy was obtained after a GIST recurrence, ~15 years following the initial resection. A paired tissue sample from the small intestine obtained 4 years prior to the liquid biopsy was also available for this patient (Fig. 4), which showed a concordant *KIT* K642E mutation at 58% VAF. Finally, in the liquid cohort, 1.7% and 1.1% of patients were predicted to harbor germline *SDHx* or *NF1* mutations, respectively (Table 2).

Analysis of Geneva University Hospital Tissue Cohort

Out of the 43 patients with GIST who underwent local tissue molecular analysis, and based on the Armed Forces Institute of Pathology classification, most patients (51.3%) were at high risk, 17.9% were at moderate risk, 23.1% were at low risk, and 7.7% were at very low risk. Fifty-one percent of the cases were metastatic, whereas 49% were localized. Among the localized group, 56% underwent adjuvant imatinib treatment for an average of 22.3 months (range, 1-47 months), and 9% received neoadjuvant imatinib for an average of 4.9 months (range, 1-9 months).

In terms of primary driver mutations, 28 harbored *KIT* mutations, with 25 being *KIT* exon 11 mutations (comprising 5 indels, 7

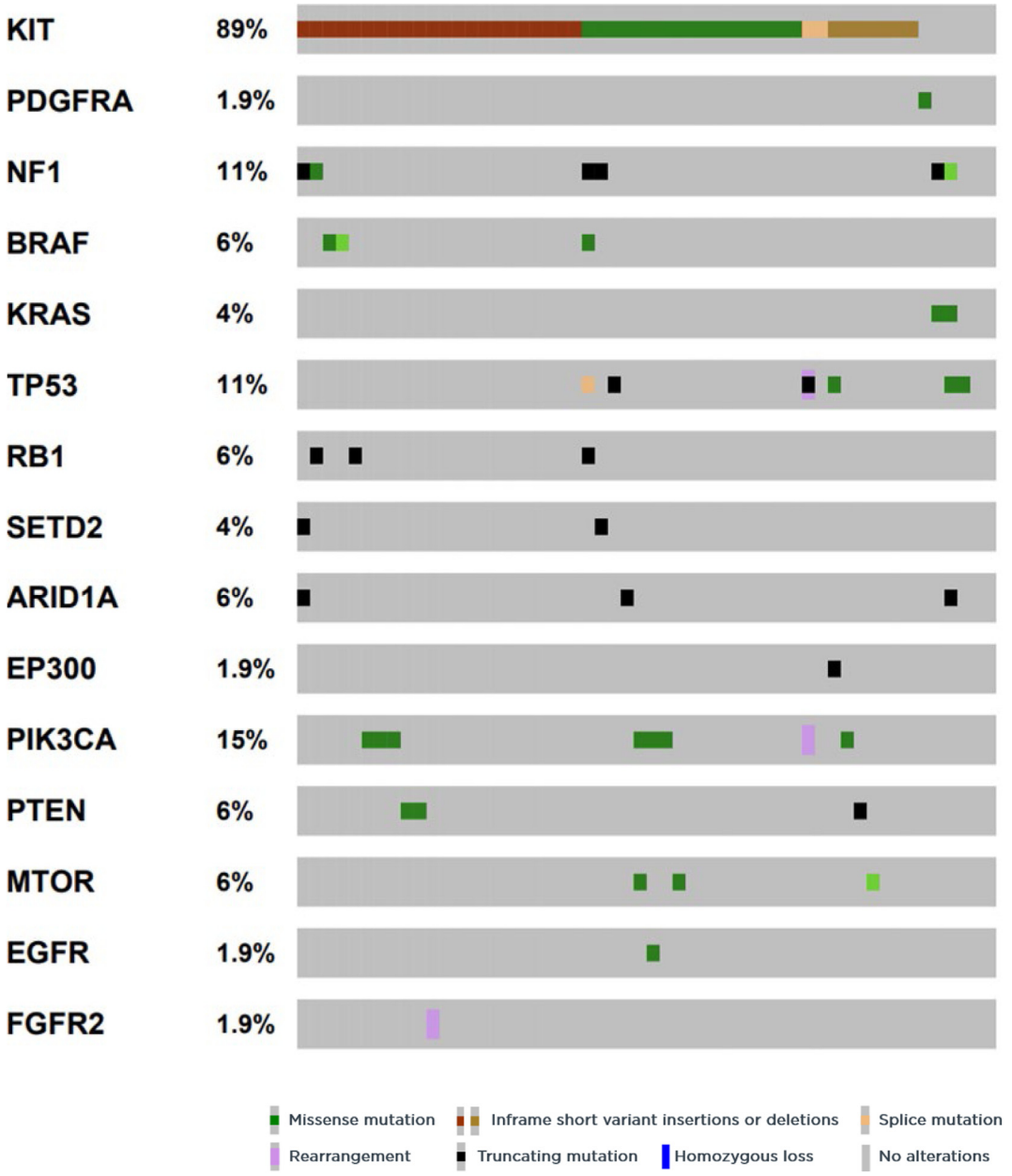

Figure 1.
Alteration frequencies of known drivers and prognostic markers in the GIST liquid biopsy cohort, as stratified based on an elevated TF (TF $\geq$ 1%; n = 54). GIST, gastrointestinal stromal tumor; TF, tumor fraction.

missense mutations, and 13 deletions) and 3 being exon 13 mutations. There were 2 tumors with *PDGFRA* mutations (1 with *PDGFRA* D842V and 1 with D842_N848delinsVDV), 1 with an *SDHB* mutation, and 2 with *NF1* mutations. Notably, 1 patient had a rare *KIT* exon 13 N655K primary mutation linked to familial disease but lacked any personal or family syndromic history (Supplementary Fig. S4A, C). As anticipated, the *SDH*-mutant GIST was located in the stomach. The remaining cohort without a known driver consisted of 7 quadruple-negative GISTs, of which 3 harbored *CDKN2A and CDKN2B* loss, and single cases harbored *FGFR3*, *SRSF2*, and *CSF3R* mutations (Supplementary Fig. S4A). Secondary mutations included 2 cases each with *RB1* and *TP53*, known to be associated with negative prognosis. In addition, there were 2 secondary *NF1* mutations identified. A patient with the primary *KIT* exon 13 N655K mutation developed *NF1* and *RB1* mutations after recurrence and imatinib treatment, as confirmed based on a new biopsy (Supplementary Fig. S4B).

All *KIT* mutations in exon 17 (totaling 4) appeared post-imatinib first-line treatment. Interestingly, an *FGFR2::NRBF2* fusion was also identified post-imatinib treatment via RNA sequencing, suggestive of a *KIT*-independent mechanism of imatinib resistance. This patient had progressed after 17 years of imatinib treatment, and identification of an *FGFR2::NRBF2* fusion allowed a potential new line of pemigatinib treatment after standard therapies had been exhausted. Other mutations of uncertain significance included *MRE11*, *TET2*, *FANCA*, *CREBBP*, and *ATR*.

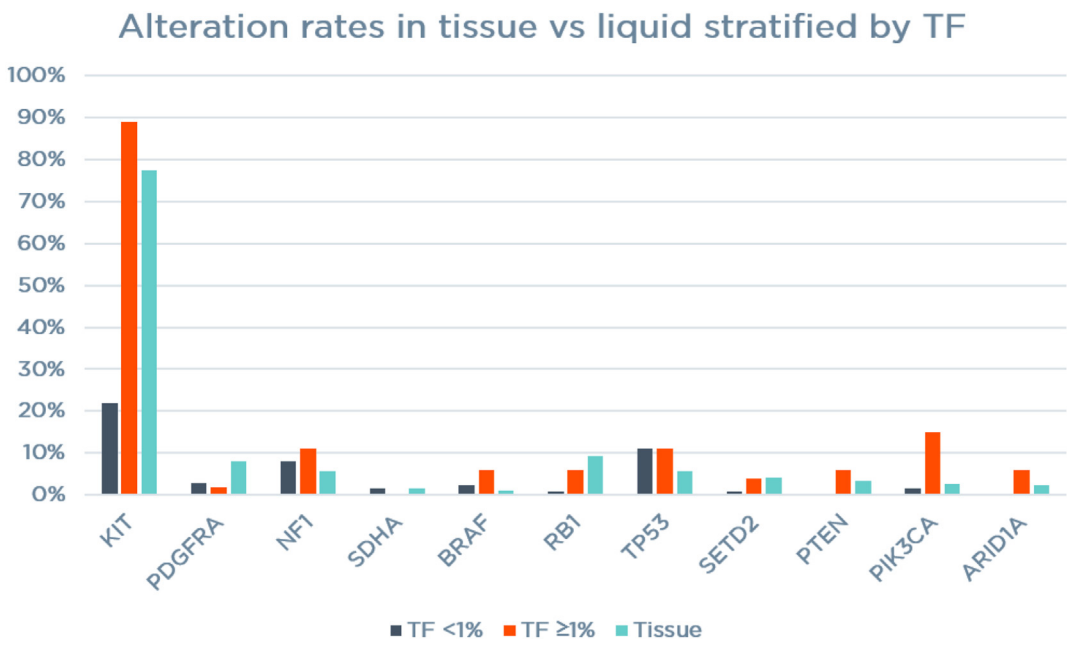

Figure 2.
Alteration frequencies of known drivers and prognostic markers in the gastrointestinal stromal tumor liquid biopsy cohort (n = 181), as stratified based on TF, and compared with frequencies in the tissue biopsy cohort (n = 2198). TF, tumor fraction.

Among the quadruple-negative, wild-type group (7 in total), broader analysis confirmed 5 as wild-type. One had *FANCA and CREBBP* mutations of undetermined significance, and 1 had a *KIT* exon 11 p.Trp557del mutation. Four out of the 7 were classified as "high risk" based on the Armed Forces Institute of Pathology classification, all having spindle cell (5 cases) or mixed morphology (2 cases) and were nongastric.

To validate the significance of our findings in the solid tissue biopsy samples, we conducted a comparative analysis with data from the publicly available MSK-IMPACT GIST data set, which was obtained on July 9, 2023, from the cbioPortal database (N = 499 GIST samples available). Comparisons were made among solid tissue samples from FMI, solid samples from the MSK-IMPACT cohort, and solid samples from the Geneva cohort. *P* value was calculated using the Fisher exact test comparing data from the MSK-IMPACT cohort and the FMI cohort. The Geneva cohort was not compared owing to its smaller sample size. The differences observed between the 2 groups included the prognostic markers CDKN2A ($P = .0016$), RB1 ($P < .00001$), SDHA ($P = .0003$), MUTYH ($P = .0016$), LRP1B ($P = .0002$), and TERT ($P = .0052$) (Supplementary Fig. S5).

In an exploratory study, we analyzed patients with a co-mutation (8 in total). They had a median overall survival time of 47.75 months (range, 7-138 months). In contrast, those without a co-mutation (27 in total) had a better median overall survival time of 86.3 months (range, 13-555 months), with a *P* value of .9.

Table 1
PPA of main GIST driver genes in paired liquid and tissue samples from same patient and stratified based on TF

TF estimate	PPA
TF < 1%	11/35 (31%)
TF ≥ 1%	14/14 (100%)

GIST, gastrointestinal stromal tumor; PPA, positive percent agreement; TF, tumor fraction.

Discussion

In this study, we demonstrate that liquid biopsy can be a valuable tool in the management of GISTs by providing insights into their molecular characteristics, including alterations indicative of imatinib resistance. Liquid biopsy is an emerging and minimally invasive technique that can detect driver, prognostic, and TKI resistance mutations in GIST by analyzing ctDNA in the bloodstream via a simple blood draw. This may allow for the identification of specific mutations that can guide treatment decisions. Liquid biopsy can be particularly beneficial for patients who may not have a current, post-therapy surgical biopsy readily available. Therefore, liquid biopsy may enable oncologists to select the most appropriate targeted therapy for patients with GIST, based on their specific genetic profile. This personalized approach may improve treatment outcomes and minimize unnecessary side effects. Overall, liquid biopsy may be valuable in the molecular classification of GIST during the medical management of patients with high-risk, advanced, or metastatic disease.

Prior studies have previously established a proof of concept for ctDNA as a surrogate for tissue molecular testing, and they have identified its potential and limitations in detecting primary and secondary mutations. For instance, a 2016 study used ctDNA to detect *KIT* and *PDGFRA* mutations in localized GIST, although challenges related to sensitivity and DNA quality highlighted the need for methodologic advancements.[29] In addition, another study demonstrated the genetic landscape of GIST via liquid biopsy, but a smaller gene panel was used with no measurement of ctDNA or TF content.[30] In 2021, Johansson et al[31] successfully identified primary mutations in 9 of 32 patients and 22 of 161 plasma samples using simple, multiplexed, PCR-based barcoding of DNA for sensitive mutation detection using sequencing (SiMSen-Seq) but noted that detections occurred mainly during disease progression.[31] Similarly, an exploratory study involving 8 patients with GIST in 2022 found significant limitations in detecting ctDNA in low-risk localized GIST, emphasizing the

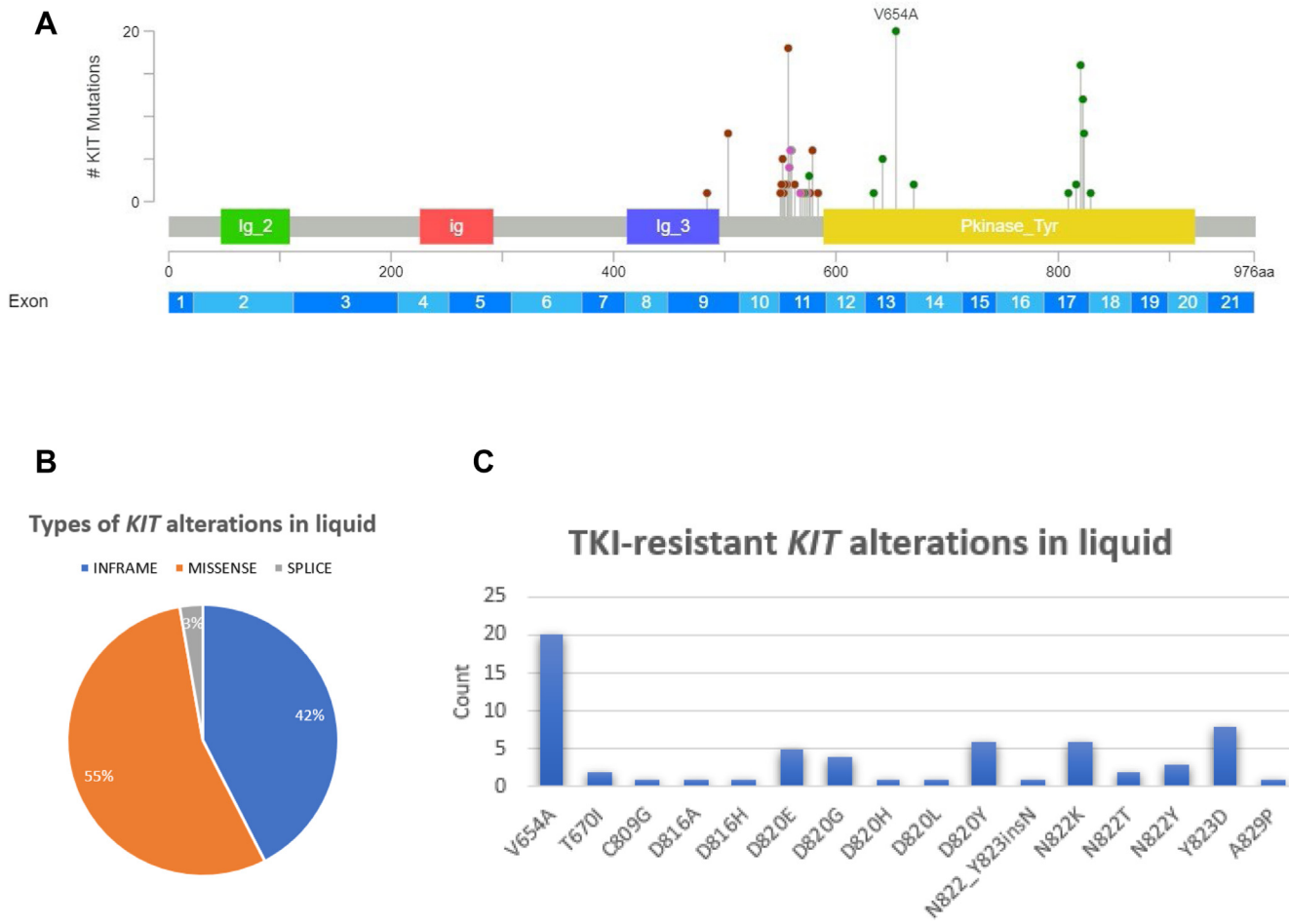

Figure 3.
(A) Distribution, (B) types, and (C) TKI-resistant *KIT* variants in liquid specimens. TKI, tyrosine kinase inhibitor.

importance of high-sensitivity assays, the need for correlation with disease burden, and ctDNA content.[32] More recently in 2024, another exploratory study involving 6 patients with *KIT*-mutated GIST identified a correlation between ctDNA levels and tumor volume in metastatic GIST, showing that ctDNA levels decreased with effective TKI therapy and re-emerged with disease progression.[33] Furthermore, innovations in high-sensitivity assays and workflows have addressed challenges in mutation detection, particularly in low-tumor-burden settings. Falkenhorst et al[34] highlighted discrepancies in mutation detection between automated and manual DNA extraction methods, emphasizing the potential of droplet digital PCR and hybrid capture NGS for high-sensitivity ctDNA analysis while cautioning against false positives from NGS panels optimized for tissue. Another study, using a limited, targeted 29-gene panel, described the ability of ctDNA to track TKI resistance mutations and their temporal evolution during successive TKI treatment lines.[35]

In parallel, clinical trials have integrated ctDNA to uncover resistance mechanisms and stratify patients for targeted therapies. For instance, ctDNA analysis was incorporated into a phase II study of ponatinib, showing concordance between plasma and tumor tissue mutations and identifying secondary *KIT* exon 17/18 mutations during resistance development.[36] In the VOYAGER trial (NCT03465722), it was confirmed that *KIT* activation loop mutations predominated in later treatment lines, demonstrating ctDNA's use in tracking resistance and guiding treatment.[37] In the INVICTUS trial (NCT03353753), the efficacy of ripretinib was corroborated in patients with *KIT* activation loop mutations, which were detected by tissue and liquid biopsies.[38] Via a 74-gene panel liquid biopsy, analysis of the INTRIGUE (NCT03673501) trial showed that ATP-binding pocket *KIT* mutations favored sunitinib response, whereas activation loop mutations were associated with better ripretinib outcomes.[39] Finally, a preliminary liquid biopsy study from the NAVIGATOR trial (NCT02508532) for *PDGFRA*-mutant GIST revealed that 63% of patients had detectable mutant ctDNA, and *PDGFRA* mutations conferring resistance to TKI

Table 2
Patients with GIST with alterations of predicted germline origin that were detected on liquid biopsy

Age (y)	Sex	Alteration	% Reads	TF
47	M	*KIT* K642E	48	Low
76	F	*KIT* D579del	48	Low
68	M	*KIT* D579del	49	Low
55	M	*KIT* D820G	50	Low
43	F	*SDHA* S530fs*17	46	Low
79	M	*SDHB* R27*	52	Low
33	F	*SDHC* R133*	47	Low
63	F	*NF1* C1016fs*4	49	Low
73	F	*NF1* L1153fs*4	48	Low

F, female; GIST, gastrointestinal stromal tumor; M, male; TF, tumor fraction.

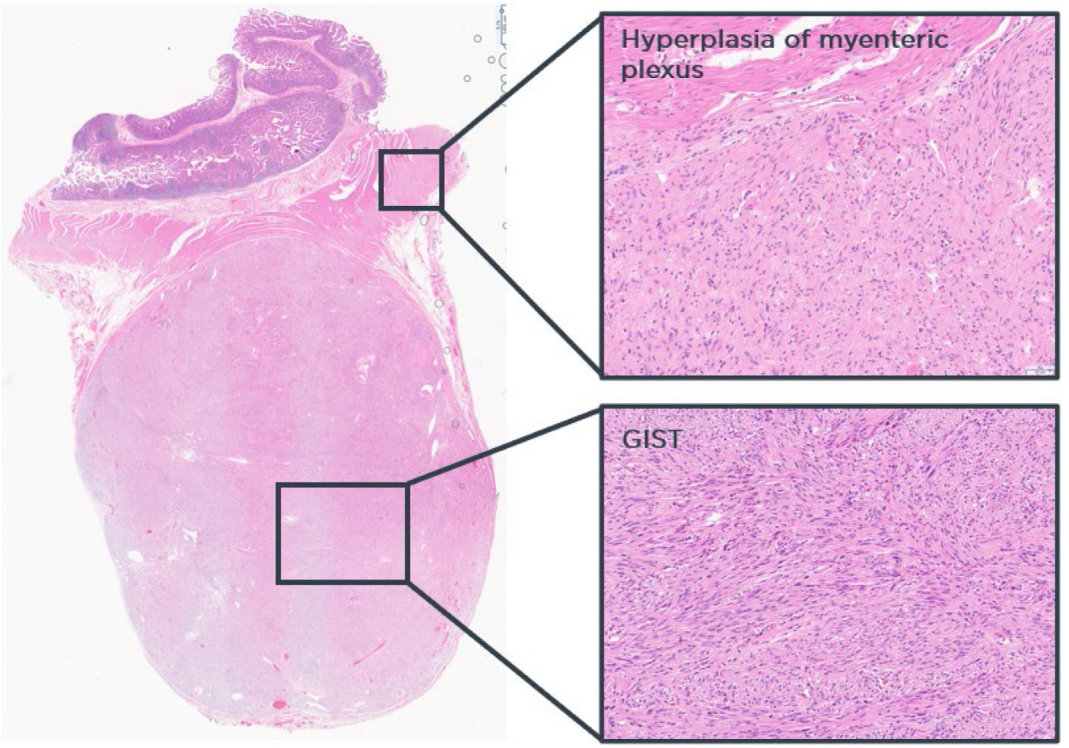

Figure 4.
A tissue sample (hematoxylin and eosin) from a patient with GIST, clinical suspicion of a *KIT* germline mutation and a paired liquid sample with a predicted *KIT* K642E germline mutation.

therapy were identifiable in plasma, underscoring the feasibility of liquid biopsy in this subset of patients.[40]

Our study builds on these foundational and clinical investigations by analyzing real-world, 2198 GIST tissue samples and 181 liquid biopsies, including 49 matched solid-liquid pairs from same patients with a broad liquid biopsy panel that analyzes 324 genes as well as TF as a measure of ctDNA content. Using an expanded NGS panel and detection of elevated ctDNA TF levels, we provided an unprecedented breadth of mutation coverage compared with earlier studies. This comprehensive approach allowed us to detect a higher frequency of resistance mutations in ctDNA than in tissue, highlighting the enrichment of actionable mutations in liquid biopsies in the advanced setting. In addition, our study uncovered rare mutations in quadruple-negative GIST, identifying therapeutic targets that would have been overlooked with narrower assays and rare non-*KIT*, non-*PDGFRA*, and TKI-resistant alterations. By validating ctDNA tissue concordance and revealing novel insights into GIST biology, our work bridges the gap between research and clinical implementation. It reaffirms the role of liquid as a noninvasive tool for understanding resistance, guiding treatment, and expanding the molecular characterization of this heterogeneous disease and highlights a role for ctDNA TF for the correct interpretation of liquid biopsy results for patients with GIST.

GISTs are currently treated with 4 standard lines of therapy when they harbor a targetable *KIT* mutation and with 1 standard line when they have *PDGFRA* mutations. Only a limited set of mutations have been identified to be directly linked with primary or secondary resistance to imatinib. In fact, resistance to imatinib is primarily attributed to mutations in *KIT*, *PDGFRA*, *BRAF*, and *SDH* genes. Other "non-traditional" genes have not been extensively studied; however, recent studies suggest *KIT*-independent mechanisms of resistance including tyrosine kinase switch,[41] overexpression of FAK, *IGF1R* amplification, *BRAF* mutations, or *FGFR1/2/3* alterations.[42] Expanding the range of oncogenes that are routinely checked during imatinib treatment could help discover new biomarkers for early detection of treatment inefficacy. In addition, examining a broader set of genes might be particularly beneficial for wild-type GIST to possibly identify actionable genomic alterations.

In this study, we provide evidence that liquid biopsies may identify *KIT*-related imatinib resistance mutations in exons 13 and 17 as well as non-*KIT*–related mutations such as in *FGFR*2, *BRAF*, *NF1*, *KRAS*, and *EGFR* in the FMI cohort. These acquired resistance mutations were not in the primary diagnostic biopsy and were enriched in liquid biopsies with elevated TF levels, supporting a potential role in TKI resistance. In addition, enrichment of activating alterations in *PTEN* and *PIK3CA* in liquid biopsies with an elevated TF, which is downstream of receptor tyrosine kinases, suggests that alterations in the PI3K signaling pathway may also be a nontraditional mechanism of TKI resistance in GISTs. Our findings support the previous proposal of mTOR inhibition alone or in combination with MEK inhibition as a salvage strategy for a subset of advanced TKI-resistant GIST.[43]

In the Geneva cohort, similarly to the FMI cohort, a separate *FGFR2::NRBF2* fusion was also identified in a patient after imatinib treatment. *FGFR2* fusions are known to be pathogenic in intrahepatic cholangiocarcinoma, which respond to FGFR-specific inhibitors. Although FGFR signaling has been implicated in imatinib resistance in GIST, that particular *FGFR2* fusion had never been previously described as a resistance mechanism in GIST.[44] These rare resistance mechanisms[25,45,46] may be underestimated in current practice given the difficulties to rebiopsy, and they may

suggest new treatment possibilities that would target unique non-*KIT* resistance mutations.

Currently, treatment is not adjusted based on the appearance of these secondary mutations, and the treatment lines are pursued sequentially, regardless of the emergence of secondary mutations. This scenario exposes patients to potentially toxic and costly treatments without any potential benefit. A major limitation to the global implementation of personalized care for patients with advanced GIST is the difficulty of rebiopsying the primary tumor and/or metastases at each progression. In addition, many patients with advanced GIST have heterogenous mechanisms of resistance in different lesions,[43,47,48] potentially undermining the role of treatment planning based on the results from the biopsy of an individual lesion. In some areas of oncology, such as lung tumors, it has become the standard practice to perform liquid biopsy to identify resistance mutations, and bispecific antibodies such as amivantamab, which targets both the initial driver mutation (*EGFR*) and the resistance mutation (*MET*), are approved in this setting.[49]

In the context of a rare tumor type, this study evaluates a large number of GIST tissue and liquid CGP samples, including 49 paired samples from the same patients. Known driver and TKI-resistant mutations of both somatic and germline origin were identified in the peripheral blood ctDNA of patients with GIST. Our results suggest that, in the presence of elevated TF levels (ie, $\geq$1%), liquid biopsies are able to robustly identify driver and resistance alterations in GIST. In contrast, in the presence of a low TF (<1%), reflex to tissue testing should be considered if the liquid biopsy results are not consistent with the current clinical situation (eg, no resistance mutations in a patient with clinical TKI resistance). Prior research has shown that for patients with other tumor types (eg, non−small cell lung cancer, colorectal cancer, breast cancer, and prostate cancer), PPA for oncogenic drives detected in tissue is expected to be greater than 95% when an elevated TF level is detected. Of note, Rolfo et al[28] reported lower specificity for known resistance mutations such as *ESR1* mutations in breast cancer, which is consistent with our finding that the imatinib resistance mutations are commonly detected in liquid biopsy even when not observed in tissue biopsy. In addition, 2.2% of patients in the liquid cohort were predicted to harbor a germline *KIT* alteration, which was a higher rate than that for *SDHx* and *NF1*. If confirmed in an independent study, these results suggest that medical genetics counseling and potential germline testing for *KIT* should be routinely considered for all patients with *KIT*-mutant GIST. Paired liquid and tissue analysis from same patients revealed that liquid biopsy showed high PPA to tissue in identifying driver mutations in the presence of elevated TF levels and exhibited TKI resistance–specific alterations.

Finally, our study used a liquid biopsy with broad, 324-gene coverage for patients with GIST to demonstrate the use and feasibility of using a comprehensive panel to detect not only driver mutations but also resistance, prognostic, and rare mutations. This is particularly relevant for patients who develop secondary metastases after having undergone only limited genomic testing at diagnosis (typically restricted to *KIT* and *PDGFRA*). By expanding the scope of mutation detection, this study seeks to enhance the understanding of the genomic landscape of advanced GIST via liquid biopsy and its potential impact on patient management and outcomes, especially in the context of ctDNA TF detection. In conclusion, known driver and TKI-resistant mutations are identified in liquid biopsies of patients with GIST, with high concordance to tissue in the presence of elevated TF levels. Therefore, liquid biopsy may be valuable in the molecular classification of GIST during the medical management of advanced disease. Although liquid biopsy is a promising tool, it may not completely replace traditional tissue biopsies, especially in the context of a low TF. The choice between liquid biopsy and tissue testing should be based on the specific clinical scenario, intended use of tests, and the information needed for optimal patient care. Nevertheless, liquid biopsy has the potential to play a significant role in the diagnosis, treatment, and monitoring of GISTs by providing a minimally invasive and real-time approach to understanding the tumor's genomic characteristics.

Author Contributions

D.I.L. and J.H. conceived the study. J.H., D.I.L., and H.M. performed data analysis and visualization. H.M., L.H., J.H., S.S., H.T., R.M., J.K.K., R.S.P.H., J.A.E., E.M., M.C.H., T.K., and D.I.L. wrote and reviewed the manuscript.

Data Availability

All relevant data can be found on the manuscript or supplementary files.

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Declaration of Competing Interest

The authors declare no competing nonfinancial interests but the following competing financial interests: S.S., H.T., R.M., J.K.K., R.S.P.H., J.A.E., and D.I.L. are employed by Foundation Medicine Inc, a wholly owned subsidiary of Roche, and are stockholders of Roche.

Ethics Approval and Consent to Participate

Approval for this study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board, (protocol number: 20152817). The study was performed in accordance with the Declaration of Helsinki.

Supplementary Material

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