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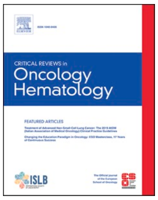

Plasma over tissue? Liquid biopsy in the management of gastrointestinal stromal tumors (GISTs)

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ABSTRACT

Gastrointestinal stromal tumors (GISTs) are widely regarded as a paradigm of precision oncology, primarily due to their well-characterized molecular drivers and the availability of effective targeted therapies. Thus, GISTs represent an ideal candidate for personalized approaches leveraging liquid biopsy. Liquid biopsy allows the detection of tumor-derived biomarkers containing genomic information in body fluids, mainly blood: circulating tumor cells, circulating free DNA and RNA, circulating tumor DNA, tumor-derived extracellular vesicles, exosomes and tumor-educated platelets. Moreover this technique offers significant advantages compared to tissue biopsy, including reduced invasiveness, faster turnaround times, lower costs and, consequently, repeatability. All these features contribute to making liquid biopsy ideal for several clinical applications, such as real-time monitoring of treatment efficacy and onset of resistance mutations. Accumulating evidence over the years has supported the application of liquid biopsy in GIST patients, culminating in its inclusion in large clinical trials. Therefore, the aim of this review is to critically evaluate the advantages and limitations of liquid biopsy technologies in the context of GIST research to highlight current challenges and guide improvements in future studies.

1. Introduction

Tissue biopsy remains the gold standard for tumor diagnosis and molecular characterization, providing critical information for treatment selection and prognostication. However, its invasive nature and limitations in feasibility pose significant challenges (Fernández-Lázaro et al., 2020; Ma et al., 2024; Siravegna et al., 2017). Furthermore, nucleic acids extracted from formalin-fixed paraffin-embedded (FFPE) samples may undergo degradation, compromising sequencing quality and reliability of molecular analyses (Caputo et al., 2023). Tissue biopsy is also not well-suited for longitudinal monitoring of treatment response or for capturing the evolving tumor heterogeneity (Fernández-Lázaro et al., 2020). Indeed, sampling a single lesion may not reflect intratumoral heterogeneity, where different regions of the same tumor harbor distinct molecular profiles, or intermetastatic heterogeneity, referring to molecular variations among different metastases within the same patient (Diaz and Bardelli, 2014). Consequently, lacking the full mutational spectrum can lead to suboptimal treatment decisions. This is where liquid biopsy comes into play, offering a promising, complementary approach.

2. Liquid biopsy

Liquid biopsy is a non-invasive technique enabling the detection of tumor-derived biomarkers in body fluids, primarily blood, exploiting the high cancer cell turnover rate (Caputo et al., 2023; Fernández-Lázaro et al., 2020; Ma et al., 2024; Siravegna et al., 2017). Several analytes can be assessed, including circulating tumor cells (CTCs), circulating free

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DNA and RNA (cfDNA, cfRNA), circulating tumor DNA (ctDNA), tumor-derived extracellular vesicles (EVs), exosomes, and tumor-educated platelets (TEPs) (Ma et al., 2024). These components often harbor clinically relevant genomic information, such as point mutations, gene fusions, and copy number alterations, representative of the tumor molecular landscape (Fig. 1).

ctDNA has emerged as particularly informative, offering both quantitative and qualitative insights. Total cfDNA levels often correlate with tumor burden, especially in advanced disease, while ctDNA somatic mutations can guide targeted therapy (Siravegna et al., 2017). Additionally, ctDNA methylation patterns can reflect tumor-specific epigenetic alterations, such as CpG island hypermethylation in tumor suppressor genes (Siravegna et al., 2017).

Liquid biopsy offers significant advantages over tissue biopsy, including reduced invasiveness, faster turnaround times and the ability to be performed repeatedly, becoming ideal for several clinical applications like real-time monitoring of treatment efficacy and onset of resistance mutations (Fernández-Lázaro et al., 2020). ctDNA is also emerging as a powerful tool for minimal residual disease (MRD) detection, enabling early identification of relapse risk after surgery or systemic therapy.

Conversely, while the CTC enumeration has been explored as a prognostic marker, its clinical reliability remains limited due to variability across tumor types and detection technologies (Siravegna et al., 2017). In parallel, distinguishing very low-frequency ctDNA from background cfDNA remains a technical hurdle, particularly in early-stage disease or MRD contexts (Diaz and Bardelli, 2014).

3. Gastrointestinal stromal tumors

Gastrointestinal stromal tumors (GISTs) are widely regarded as a paradigm of precision oncology, primarily due to their well-characterized molecular drivers and the availability of effective targeted therapies; thus, they represent an ideal candidate for liquid biopsy (Antonescu, 2011). Nearly 80 % of sporadic GISTs harbor mutually exclusive *KIT* or *PDGFRA* activating mutations, which are their key oncogenic drivers (Fig. 2) (Corless et al., 2011).

Approximately 70–75 % of GISTs harbor primary *KIT* mutations, most frequently in exon 11 followed by exon 9. Less commonly, exons 13 and 17 are involved, affecting the ATP-binding pocket and activation loop, respectively. *PDGFRA* mutations, present in 5–10 % of cases, mostly affect exons 18, 14 or 12 (Heinrich et al., 2003b). These alterations result in constitutive, ligand-independent activation of downstream signaling pathways, including the MAPK and PI3K/AKT cascades, driving tumorigenesis and influencing sensitivity to tyrosine kinase inhibitors (TKIs) (Demetri et al., 2002; Heinrich et al., 2020, 2008, 2003a). Most GIST patients experience disease progression within ~20 months of treatment initiation, primarily due to the emergence of secondary resistance mutations (Heinrich et al., 2006). These events typically arise in *KIT* exons 13, 14, and 17, resulting in structural changes that impair TKI binding. Importantly, these secondary alterations are often polyclonal, meaning that different metastatic lesions within the same patient may harbor distinct events (Liegl et al., 2008). This molecular heterogeneity complicates treatment and underlies the limited efficacy of single-agent TKIs beyond the first line. To address this, second- and third-line therapies such as sunitinib and regorafenib have been developed, each against specific secondary mutations (Demetri et al., 2013b). More recently, ripretinib, a broad-spectrum switch-control TKI, has shown efficacy in overcoming multiple resistance mechanisms and is approved for use as later-line option (Blay et al., 2020).

Given the GIST molecular complexity and dynamic evolution, liquid biopsy may serve as a powerful adjunct to traditional tissue-based diagnostics, holding promise across several clinical applications, which are the focus of the present review (Table 1).

4. Clinical relevance

4.1. Primary KIT/PDGFRA mutation detection

The initial exploration of liquid biopsy in GISTs began with the pivotal analysis conducted in the GRID trial. ctDNA was analyzed using BEAMing technology, which combines digital PCR (dPCR) with flow cytometry to identify somatic mutations. The primary aim of the research was to assess the correlation between plasma-derived and tissue-based mutational status and to explore the association of these findings with clinical outcomes. The study reported an 84 % concordance rate for the detection of primary *KIT* mutations between tissue and plasma, providing early evidence that ctDNA could potentially serve as a non-invasive surrogate for tumor genotyping, especially in advanced or metastatic settings where repeated tissue biopsies are often unfeasible (Demetri et al., 2013a).

Subsequently, Maier *et al.* demonstrated the feasibility of detecting mutant ctDNA in GISTs using allele-specific PCR assays (Maier et al.,

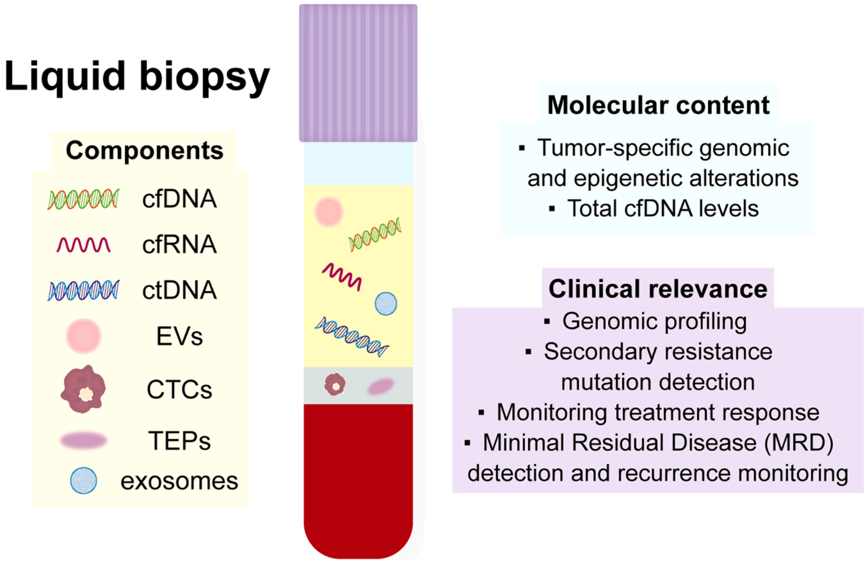

Fig. 1. Liquid biopsy overview.

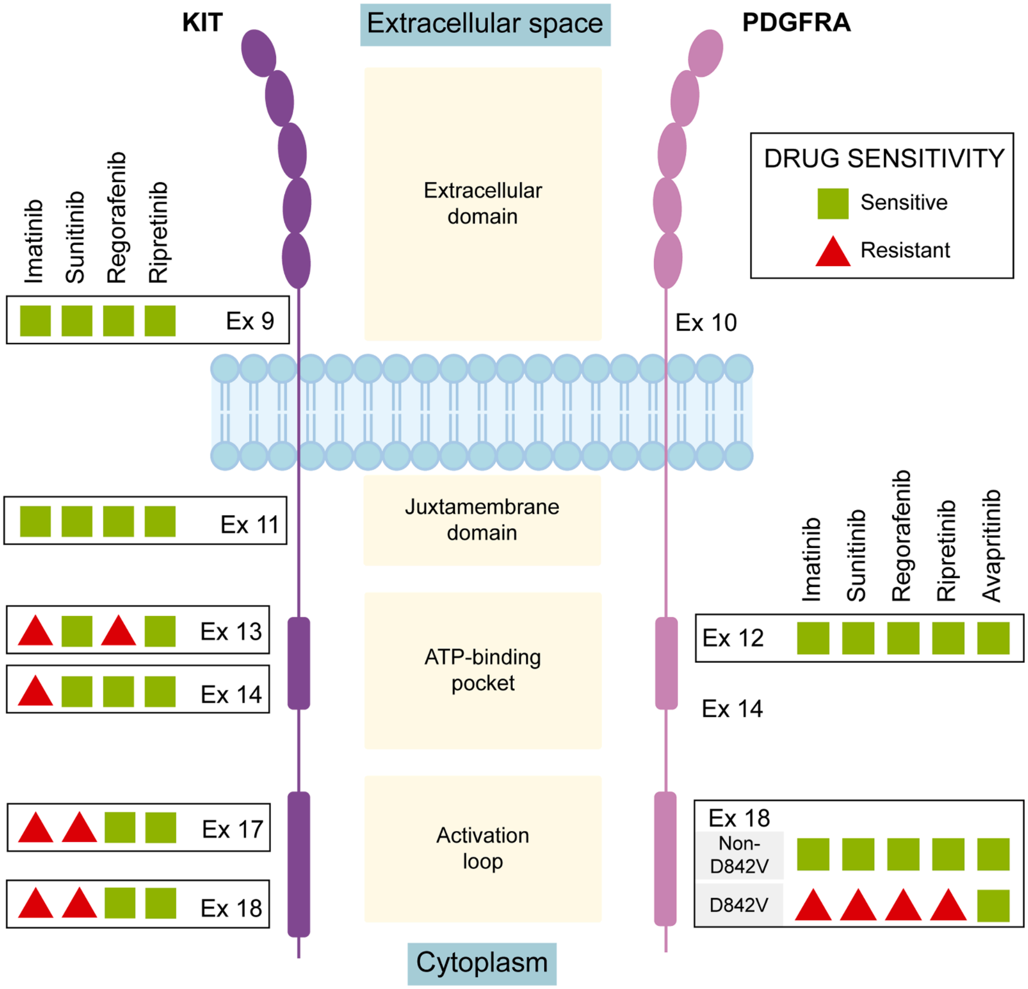

Fig. 2. *KIT/PDGFRA* mutations involved in drug sensitivity and resistance.

2013). Their study showed a 39 % overall concordance for primary mutations between plasma and tissue. They also highlighted technical challenges in detecting low-abundance ctDNA, particularly in patients with minimal disease burden or complete remission, suggesting that ctDNA detection is more reliable in patients with active disease (Maier et al., 2013).

In a phase II study evaluating dovitinib in TKI-refractory GIST patients, Yoo *et al.* identified primary kinase mutations in only 17 % of cases, since the BEAMing assay employed was mainly optimized for exon 11 primary mutations. However, the detected mutations matched tumor tissue analyses with 100 % concordance (Yoo et al., 2014).

Bauer *et al.* utilized a custom-designed targeted deep sequencing panel on plasma samples from 22 metastatic GISTs. Their analysis detected primary mutations in 41 % of cases, all of which were consistent with those identified in the corresponding tissues (Bauer et al., 2015).

Further support came from Kang *et al.* who demonstrated a 100 % concordance between plasma and tumor DNA using next-generation sequencing (NGS) in 3 aggressive *KIT*-mutant GISTs, reinforcing the robustness of ctDNA detection methods even at a low allele frequency (1.58 %) (Kang et al., 2015). Boonstra *et al.* developed a highly sensitive digital droplet PCR (ddPCR) assay targeting multiple *KIT* exon 11 mutations. Across 22 plasma samples from GIST patients, this assay achieved a 93 % detection rate in metastatic cases but showed limited utility in localized disease, where ctDNA was only detected in a single patient, reaffirming the strong correlation between ctDNA levels and tumor burden (Boonstra et al., 2018).

Namlos *et al.* applied a targeted NGS panel in a treatment-naïve cohort of 44 GISTs and in 6 TKI-treated cases, identifying *KIT* and *PDGFRA* mutations in 36 % and 50 % of cases, respectively. Among treatment-naïve GISTs, the detection rate varied significantly depending on the mutation type: *KIT* indels were found in 57 % of plasma samples, while single-nucleotide variants (SNVs) were detected in only 21 %. *PDGFRA* deletions were identified in one of three plasma samples, but no SNVs were detected in plasma. Thus, ctDNA detection was markedly higher in metastatic or higher-risk patients, and within localized cases ctDNA positivity was associated with larger tumors (Namløs et al., 2018).

Similarly, Xu *et al.* reported a higher detection rate of ctDNA mutations (56.3 %) in 32 advanced GISTs using high-throughput sequencing compared to older technologies like BEAMing and PCR. The overall concordance between plasma and tissue DNA reached 71.9 %, with primary tumor size positively correlating with concordance rates and ctDNA detection (Xu et al., 2018).

Jilg *et al.* evaluated the use of long-range PCR (L-PCR) and dPCR in 25 GISTs with active disease, achieving detection rates of 64 % and 80 %, respectively. L-PCR focuses on the amplification of longer DNA fragments compared to the standard PCR, while dPCR works by splitting a DNA sample into several PCR reactions, enabling absolute quantification. When combining both methods, ctDNA was detected in 92 % of cases, emphasizing the value of multimodal approaches to enhance sensitivity (Jilg et al., 2019). Once again, according to the dPCR results, ctDNA levels increased with tumor size.

In a large-scale study, Arshad *et al.* assessed ctDNA testing alongside

Table 1
Liquid biopsy in GIST studies.

STUDY	N° PLASMA SAMPLES	ASSAY	ANALYZED GENES	STUDY ENDPOINTS	CLINICAL IMPLICATIONS
Demetri et al. (2013a)	163	BEAMing	*KIT, PDGFRA, BRAF, KRAS*	Detection of primary and secondary mutations in plasma	Demonstration of ctDNA feasibility for molecular profiling in advanced GISTs
Maier et al. (2013)	291	Allele-specific PCR	*KIT, PDGFRA*	Detection of primary and secondary mutations; longitudinal ctDNA analysis	ctDNA dynamics as a biomarker of treatment response
Yoo et al. (2014)	30	BEAMing	*KIT, PDGFRA, BRAF*	Plasma detection of kinase mutations in TKI-refractory disease	Exploratory association between mutational status and clinical outcome
Bauer et al. (2015)	30	NGS	Custom panel including *KIT, TP53, RAS/RAF*-family	Comprehensive plasma mutational profiling	Evidence of spatial and temporal tumor heterogeneity
Kang et al. (2015)	3	NGS	*KIT, PDGFRA, BRAF*	Proof-of-concept plasma mutation detection	Feasibility of NGS-based liquid biopsy in GISTs
Boonstra et al. (2018)	47	ddPCR	*KIT*	High-sensitivity detection of primary *KIT* mutations	Early pharmacodynamic monitoring of treatment response
Namløs et al. (2018)	44	NGS	Panels including cancer-related genes	Detection of driver mutations and ctDNA quantification	ctDNA levels as a surrogate of tumor burden
Xu et al. (2018)	32	NGS	416 cancer-related genes	Broad plasma mutational profiling	Concordance between plasma and tissue genotyping
Jilg et al. (2019)	156	Allele-specific PCR + ddPCR + NGS	*BRAF, CDKN2A, CTNNB1, EGFR, HER2/ERBB2, HRAS, KEAP1, KIT, KRAS, MET, NFE2L2, NRAS, PDGFRA, PIK3CA, PTEN, SDHA, SMAD4, TP53*	Mutation detection, treatment monitoring, MRD/relapse assessment	Potential role of ctDNA in disease surveillance
Arshad et al. (2020)	279	NGS	74 genes	Detection of primary and secondary mutations across disease stages	Large-scale clinical validation of ctDNA testing
Serrano et al. (2020)	37	ddPCR + NGS	60 genes	Longitudinal mutational profiling	Monitoring of resistance evolution
Olivera-Salazar et al. (2024)	15	ddPCR	*PDGFRA, SEPT9*	Plasma detection of *PDGFRA* mutations	Limited sensitivity in localized disease; exploratory epigenetic signals
Mechahougui et al. (2025)	181	NGS	324 genes	Comprehensive genomic profiling	Identification of actionable and rare variants using liquid biopsy
Kang et al. (2016)	25	NGS	*KIT, PDGFRA*	Detection of secondary resistance mutations	Support for ctDNA-guided resistance assessment
Wada et al. (2016)	10	NGS	*KIT*	Detection of resistance mutations during therapy	Correlation with treatment response
Serrano et al. (2019)	28	ddPCR + NGS	58 genes	Serial resistance mutation analysis	Anticipation of disease progression
George et al. (2022)	105	BEAMing	*KIT*	Detection of multiple secondary *KIT* mutations	Assessment of clonal heterogeneity
Rassner et al. (2023)	11	ddPCR + NGS	*BRAF, CDKN2A, CTNNB1, EGFR, HER2/ERBB2, HRAS, KEAP1, KIT, KRAS, MET, NFE2L2, NRAS, PDGFRA, PIK3CA, PTEN, SDHA, SMAD4, TP53*	Ultra-sensitive detection of low-frequency mutations	Early identification of emerging resistance
Bleckman et al. (2024)	94	ddPCR	*KIT*	Longitudinal ctDNA quantification	Correlation with tumor volume and long-term response

NGS analysis of FFPE tumor tissues across 243 GISTs. ctDNA analysis showed a specificity and positive predictive value (PPV) of 100 %, even though sensitivity remained at 56 %. Sensitivity was particularly low (0 %) in localized or low-burden disease and in patients responding to treatment, whereas it was higher in those with metastatic, high-burden, or progressing disease. Indeed, there was no concordance between the ctDNA and FFPE analyses in low-burden advanced cancers (Arshad et al., 2020).

Serrano *et al.* reported an overall sensitivity of 28.6 % for detecting tumor mutations in cfDNA using NGS in 37 plasma samples from 18 patients, with high concordance in confirmatory ddPCR analyses. Detection rates were lower in localized disease but increased to 38.5 % in metastatic patients and during progression after multiple treatment lines, supporting ctDNA dynamic nature as a real-time biomarker reflecting tumor evolution (Serrano et al., 2020). By contrast, in primary tumors, tumor size and mitotic count did not influence ctDNA levels.

In a prospective pilot study by Olivera-Salazar *et al.*, the *PDGFRA* D842V mutation was easily detected by ddPCR in GIST tissue samples but remained undetectable in plasma, despite attempts to increase sensitivity by using fresh plasma, larger volumes, and DNA concentration techniques (Olivera-Salazar et al., 2024).

Recently, Mechahougui *et al.* also investigated the utility of liquid biopsy in GIST comprehensive genomic profiling (Mechahougui et al., 2025). ctDNA was detectable in 30 % of liquid samples ($n = 181$), with a tumor fraction ≥ 1 %. Within this ctDNA-positive group, 89 % harbored *KIT* mutations and 2 % had *PDGFRA* mutations. Importantly, in 49 matched tissue–liquid pairs with elevated ctDNA, there was 100 % concordance for driver mutations, confirming the assay feasibility for accurate GIST genotyping. Furthermore, ~2 % of cases were found to carry potential germline variants, including alterations in *KIT*, *SDHx*, and *NF1*, reflecting the ability of liquid biopsy to uncover also rare hereditary variants (Mechahougui et al., 2025).

4.2. Secondary resistance mutation detection

The role of ctDNA in detecting secondary resistance mutations in GISTs has been extensively explored in multiple studies, providing valuable insights into tumor heterogeneity and the mechanisms of acquired resistance to TKIs.

Demetri *et al.* demonstrated that secondary *KIT* mutations were detected in 47 % of plasma samples, whereas matched tissue biopsies only identified these mutations in 12 % of cases. This substantial difference underscored the ability of liquid biopsy to capture the GIST molecular heterogeneity and to detect emerging resistance mutations that may not be present in limited or single-site tissue biopsies (Demetri et al., 2013a).

Similarly, Maier *et al.* showed that secondary mutations can be detected much earlier in plasma than in tissue. They identified secondary resistance mutations such as *KIT* exon 11 V654A and exon 17 D820Y in plasma samples up to two years before these mutations became detectable in tissue biopsies, reinforcing ctDNA potential of monitoring early resistance and timely guiding therapeutic interventions (Maier et al., 2013).

Yoo *et al.* reported the detection of secondary kinase mutations in 41 % of patients, with most *KIT* mutations involving the activation loop domain, while those affecting the binding pocket characterized only one patient. Importantly, the presence of these secondary mutations was significantly associated with worse overall survival (OS), emphasizing the prognostic implications of ctDNA profiling (Yoo et al., 2014).

Moreover, Bauer *et al.* identified resistance mutations in 86 % of patients, predominantly in the *KIT* exon 17. However, resistance events identified in tumor biopsies were not always concordant with plasma findings, highlighting the GIST spatial and temporal heterogeneity. Additionally, their analysis revealed *TP53* and *RAS/RAF* mutations in 77 % and 59 % of patients, respectively, albeit at low levels, suggesting that multiple resistance mechanisms may coexist, contributing to the complexity of acquired resistance (Bauer et al., 2015).

Kang *et al.* reported the detection of additional ctDNA mutations via NGS, including *PDGFRA* exon 18 D842V and *KIT* exon 17 S821F alterations in a patient originally harboring primary *KIT* exon 9 duplication and in one case displaying *KIT* exon 11 deletion, respectively (Kang et al., 2015). Both patients were characterized by dismal response to TKIs, being the *PDGFRA* secondary event associated with both imatinib and sunitinib resistance. Additionally, the *KIT* secondary mutation was identified just before a relapse. All these findings reinforced the role of liquid biopsy as a valuable tool to guide treatment decisions and to detect disease progression. The research group later expanded their work analyzing presurgical plasma samples from 25 patients with localized GIST through NGS, achieving a 72 % concordance for *KIT* primary mutations between plasma and tissue. Interestingly, while point mutations were detected correctly, indels were called as single-base variants in plasma samples. Thus, ctDNA utility in mutational profiling prior to surgical intervention was further supported, even when the disease was still confined; however, the need to combine NGS with PCR techniques to identify long indels was also highlighted (Kang et al., 2016).

Wada *et al.* corroborated these findings detecting secondary *KIT* mutations using allele-specific PCR and deep sequencing in four patients undergoing resection of imatinib-resistant lesions. Despite the limited sample size, their work provided additional validation that liquid biopsy could reliably identify resistance mutations, even in challenging post-surgical or refractory settings (Wada et al., 2016).

Jilg *et al.* confirmed NGS utility in detecting resistance mutations in all four patients, indicating ctDNA as a complementary tool to tissue biopsies in monitoring resistance evolution (Jilg et al., 2019).

In the phase I study by Serrano *et al.*, which evaluated rapid alternation of sunitinib and regorafenib in pretreated, TKI-refractory GISTs, ctDNA profiling was performed by targeted error correction sequencing (TEC-seq), an extremely sensitive method to detect alterations through parallel sequencing. Subsequently, primary and secondary *KIT* mutations were identified in 89 % and 78 % of patients, respectively. ddPCR showed an even higher sensitivity for detecting secondary mutations (92 %). Interestingly, despite comprehensive profiling, the study noted limited heterogeneity in resistance mechanisms, with a median of one resistance mutation per patient. Alterations in genes excluding *KIT* were observed in 80 % of patients, but only an *NRAS* hotspot mutation was potentially related to TKI resistance, reflecting a predominance of *KIT*-driven resistance mechanisms in this population (Serrano et al., 2019).

Arshad *et al.* extended these observations analyzing patients who progressed on treatment and identifying 3, 8 and 3 cases with *KIT* exon 13, 17 and combined 13–17 mutations, respectively. Notably, these events informed treatment decisions in this cohort, supporting ctDNA-guided therapy selection in advanced GISTs (Arshad et al., 2020).

In another study by Serrano *et al.*, three distinct *KIT* activating loop mutations were detected in two patients by plasma NGS. However, in patients where ctDNA analysis failed to detect resistance mutations, tissue biopsies still identified multiple imatinib-resistance alterations, suggesting that the absence of plasma detectable mutations does not fully exclude resistant clones, particularly in patients with limited disease progression or low tumor burden. Additionally, three patients with progressing lesions exhibited no resistance mutations in either plasma or tumor biopsies, indicating possible non-genomic resistance mechanisms (Serrano et al., 2020).

In the phase II study by George *et al.*, the ctDNA BEAMing analysis revealed extensive tumor heterogeneity, with at least two and up to eight unique secondary *KIT* mutations detected in 35 % of patients, and in 54 % of those harboring primary *KIT* exon 11 mutations. This underscored the complexity of GIST resistance evolution and the ctDNA value in capturing this diversity (George et al., 2022).

Rassner *et al.* reported the development of an ultra-sensitive ddPCR assay capable of detecting low-abundance, resistance-mediating *KIT* mutations in cfDNA with exceptional precision. This advanced ddPCR approach surpassed traditional sequencing methods in sensitivity, enabling the detection of emergent resistance mutations earlier and at lower levels, which could be critical for timely treatment adjustments (Rassner et al., 2023). Ultimately, ctDNA levels mirrored the clinical course: the identification of a new mutation (*KIT* exon 17 Y823D) or a higher detection of *KIT* exon 13 V654A corresponded to disease progression.

Lastly, the study by Mechahougui *et al.* revealed that, among 77 liquid samples with *KIT* driver mutations, 55 % also harbored imatinib-resistance *KIT* alterations (Mechahougui et al., 2025). Additionally, rare resistance mechanisms, such as *FGFR2* fusions and *BRAF/EGFR* mutations, were identified. Importantly, mutations conferring imatinib resistance in *KIT* exons 13 and 17 were more frequently detected in plasma than in matched tissue samples. Overall, these findings highlighted the power of liquid-based comprehensive genomic profiling to uncover resistance mutations and provided actionable information to guide personalized treatment decisions in GISTs.

4.3. Monitoring treatment response

Several studies have also demonstrated the potential of ctDNA as a dynamic biomarker to monitor treatment response in GIST patients.

Maier *et al.* explored the correlation between tumor-derived cfDNA levels and treatment response. Patients experiencing progressive disease or relapse exhibited increased mutant cfDNA levels, while patients responding to TKIs demonstrated a decline or complete clearance of mutant cfDNA from plasma, supporting the role of ctDNA as a tumor-specific biomarker reflecting treatment efficacy in real time (Maier et al., 2013).

Similarly, Yoo *et al.* assessed the relationship between serum ctDNA mutations and treatment response. Remarkably, although no significant correlation was observed between ctDNA mutation status and response, 24-week disease control or metabolic response rate, all four patients who achieved durable disease control at 24 weeks lacked detectable secondary mutations. In contrast, all patients harboring secondary mutations experienced disease progression within 24 weeks, suggesting that secondary mutations may predict poor response to dovitinib in TKI-refractory settings (Yoo et al., 2014). Interestingly, the OS was significantly better in cases without secondary mutations (p-value=0.047).

The ctDNA kinetics during treatment were further highlighted in a case report by Wada *et al.*, where serial monitoring of cfDNA concentration and MAF was conducted in 4 patients. ctDNA levels remained undetectable prior to clinical recurrence and increased as imatinib-resistant lesions progressed. Notably, upon initiation of sunitinib treatment, ctDNA levels declined concomitantly with radiological response, suggesting ctDNA as an early indicator of treatment response and progression (Wada et al., 2016).

Boonstra *et al.* investigated ctDNA dynamics in a cohort of 11 metastatic GISTs receiving TKIs, using a sensitive ddPCR assay targeting *KIT* exon 11 mutations. Across all patients, ctDNA levels decreased below baseline levels within six weeks after treatment initiation or shortly thereafter, which aligned with the radiological assessments showing tumor response in 10 patients or disease stabilization in one patient at three months. This further supported ctDNA role as an early pharmacodynamic biomarker, potentially preceding radiological evaluation (Boonstra et al., 2018).

Namlos *et al.* also confirmed the value of serial plasma samples collected during treatment, demonstrating that ctDNA could capture tumor molecular heterogeneity throughout the disease course and might inform treatment decisions, particularly at the time of progression (Namløs et al., 2018). In one patient, it showed how mutations disappeared or diminished depending on the inhibitor, showing different responses to treatments.

This was corroborated by Jilg *et al.*, who reported that fluctuations in circulating *KIT* and *PDGFRA* ctDNA levels closely mirrored clinical outcomes. ctDNA levels rose during disease progression and decreased during response, with ddPCR showing high specificity (96 %) for distinguishing active disease from complete response, although sensitivity remained modest (44.7 %). For progression detection, ddPCR achieved a specificity of 79.2 % and sensitivity of 55.2 %, highlighting its potential as a complementary tool alongside imaging (Jilg et al., 2019).

In the phase I trial by Serrano *et al.*, clonal dynamics assessed by TEC-seq in three *KIT*-mutant patients with clinical benefit revealed an early and sustained decline in both primary and secondary mutations during the first treatment cycle. These suppressed clones remained undetectable until disease progression, when they reemerged, offering direct evidence that ctDNA monitoring could predict treatment response and anticipate progression (Serrano et al., 2019). Contrariwise, this pattern was not observed in a patient with rapid disease progression, underscoring the predictive potential of ctDNA kinetics.

Serrano *et al.* further confirmed that serial ctDNA analysis could accurately reflect disease course, subclonal dynamics, and resistance evolution. Importantly, monitoring known plasma *KIT* or *PDGFRA* mutations with ddPCR could anticipate radiological progression, providing clinicians with an earlier signal of treatment failure (Serrano et al., 2020).

George *et al.* integrated ctDNA assessment into biomarker analyses, observing distinct patterns of clonal response and emergence of resistance mutations. ctDNA levels of the *KIT* exon 17–18 mutations decreased during ponatinib treatment, reflecting its efficacy. Conversely, ctDNA levels of *KIT* exon 9 primary and exon 13 V654A secondary alterations increased during therapy, suggesting these events as potential mechanisms of acquired resistance, with implications for subsequent therapeutic strategies (George et al., 2022).

More recently, Bleckman *et al.* evaluated ctDNA during the treatment of GIST patients using a *KIT* exon 11 ddPCR drop-off assay. They compared changes in ctDNA levels with radiologic responses defined by RECIST criteria and tumor volumes measured by CT scan. The aim was to assess the clinical utility of ctDNA for monitoring long-term treatment response and detecting disease progression (Bleckman et al., 2024). Using a total of 94 samples from 6 patients, the study demonstrated that an increase in tumor volume was more accurately mirrored by a rise in ctDNA. Specifically, in patients with large tumor volumes during disease progression, higher ctDNA levels were more frequently observed. Furthermore, in cases of disease progression with undetectable ctDNA, the tumor volumes were typically smaller compared to those with detectable ctDNA. Importantly, a rapid ctDNA drop was often observed within 3 weeks of initiation of systemic therapy, preceding the first radiologic responses. Additionally, short-lived “ctDNA spikes” were frequently seen within 2 weeks of starting treatment, followed by ctDNA disappearance in patients who subsequently responded to imatinib or sunitinib. These findings underscored the ctDNA role in closely and non-invasively monitoring GIST kinetics and the importance of identifying the optimal timing for blood sampling to maximize the clinical information.

4.4. Minimal residual disease (MRD) detection and recurrence monitoring

The detection of MRD and early recurrence remains a significant clinical challenge in GIST management, particularly after curative-intent surgery. ctDNA analysis has emerged as a promising tool for this purpose.

Jilg *et al.* investigated the utility of ctDNA in monitoring MRD in five patients who achieved complete remission following radical surgery. ctDNA levels dropped rapidly post-surgery and remained undetectable during follow-up in 4 cases, in concordance with imaging studies. Thus, ctDNA monitoring could offer a non-invasive, highly sensitive method to surveil patients in remission, with the potential to detect recurrence earlier than conventional imaging or clinical assessment (Jilg et al., 2019).

Despite these promising results, data on ctDNA-based MRD detection in GISTs remain limited, and larger, prospective studies are warranted to validate its clinical utility, define optimal sampling intervals, and determine thresholds for intervention upon ctDNA reappearance. Therefore, future studies should investigate whether ctDNA-guided surveillance could lead to earlier therapeutic intervention and improved patient outcomes compared to standard monitoring strategies.

5. Liquid biopsy in large clinical trials: VOYAGER, INVICTUS, INTRIGUE and INSIGHT

While numerous smaller studies have demonstrated the feasibility and potential clinical utility of ctDNA analysis in GISTs, the integration of liquid biopsy into large, randomized clinical trials marked a critical step toward broader clinical adoption. Notably, several phase III trials in advanced GISTs, such as VOYAGER, INVICTUS, INTRIGUE, and INSIGHT, have incorporated liquid biopsy components to explore its role in molecular profiling, treatment monitoring, and detection of secondary resistance mutations. These studies collectively offer real-world insights into how liquid biopsy may support precision oncology in GISTs by enabling noninvasive, serial assessments of tumor genomics. Below, we summarize the contribution of each trial to the growing evidence regarding liquid biopsy in this disease (Table 2).

5.1. VOYAGER trial

The VOYAGER trial investigated the efficacy of avapritinib, a potent and selective *KIT* and *PDGFRA* inhibitor, compared with regorafenib in patients with advanced GISTs previously treated with imatinib and at least one other TKI (Kang et al., 2021). Although the trial did not meet its primary endpoint of improved progression-free survival (PFS), it incorporated exploratory biomarker analyses, including ctDNA profiling, to gain insights into resistance mechanisms and treatment dynamics in this heavily pretreated population (Serrano et al., 2023). Baseline ctDNA analysis using a 74-gene NGS panel was performed in 386 of the 476 enrolled patients, revealing a 69 % overall detection rate of *KIT* and *PDGFRA* mutations in plasma, with a mean VAF of approximately 1 %. Firstly, ctDNA profiling revealed a shift in resistance patterns following previous treatments. While ATP-binding pocket mutations are usually associated with imatinib resistance, their frequency was low in this population. Conversely, activation loop mutations predominated, supporting sunitinib activity against ATP-binding pocket mutations and its reduced efficacy against A-loop variants. This suggested a treatment-driven clonal selection, with A-loop mutant clones emerging under selective pressure. Secondly, although GISTs are known for intratumoral heterogeneity, the degree of detectable clonal complexity in ctDNA was relatively limited. 82 % of patients had only 1–3 *KIT* variants identified, while 17 % exhibited a higher mutational burden, harboring 4–14 different resistance mutations. Thus, polyclonality may be potentially underestimated due to assay sensitivity thresholds. Importantly, the ctDNA assay also identified a broader range of *KIT* pathogenic variants than previously reported. Approximately 28 % of the detected *KIT* mutations were novel, underscoring the utility of liquid biopsy to capture the evolving and diverse genomic GIST landscape, particularly in patients with heavily pretreated, heterogeneous disease. Together, these findings reinforce the role of ctDNA as a non-invasive tool for real-time molecular profiling, enabling more precise characterization of resistance mechanisms and potentially informing TKI selection in advanced GISTs.

5.2. INVICTUS trial

The INVICTUS trial evaluated the efficacy of ripretinib in patients with advanced GIST who had received at least three kinase inhibitors (Blay et al., 2020). It demonstrated a significant improvement in PFS and OS with ripretinib compared to placebo, leading to its approval as fourth-line treatment. ctDNA analyses were mainly adopted to better understand the molecular landscape of late-line GISTs (Bauer et al., 2021). Plasma samples were collected at baseline, every other 28-day cycle, and at the end of the treatment. Analysis was performed using an FDA-approved NGS liquid biopsy assay designed to detect GIST-relevant mutations, including *KIT* and *PDGFRA* hotspots. At baseline, *KIT* mutations were detected in 91 % of evaluable patients, with exon 11 (68 %) and exon 9 (16 %) being the most common primary mutations. Secondary resistance alterations were frequently identified in exons 13, 14, and exons 17, 18, with many patients harboring multiple concurrent mutations, highlighting the polyclonal resistance seen in late-line GISTs. A high concordance rate of 93.75 % was observed between ctDNA and archival tumor tissue for known driver mutations. During treatment, a decline or clearance of ctDNA allele frequencies was observed in patients who experienced clinical benefit, suggesting that ctDNA dynamics may serve as a real-time marker of treatment response. However, baseline ctDNA levels and mutational burden did not consistently correlate with PFS or OS indicating that, while liquid biopsy is promising for molecular profiling and disease monitoring, further validation is required to establish its prognostic or predictive utility in this setting.

Table 2
Clinical trials involving liquid biopsy.

TRIAL	PHASE	DESIGN/ LINE	METHODS	TIMING	AIMS
VOYAGER	III	Avapritinib vs Regorafenib (3rd/4th line)	74 genes NGS panel Guardant360® CDx[1]	Baseline and EOT[2]	• Clinical utility of ctDNA for resistance mutation profiling
INVICTUS	III	Ripretinib vs Placebo (≥4th line)	74 genes NGS panel Guardant360® CDx[1]	Baseline, day 1 of each cycle and EOT[2]	• Clinical utility of ctDNA for resistance mutation profiling • Correlation between clinical benefit of ripretinib and baseline mutations
INTRIGUE	III	Ripretinib vs Sunitinib (2nd line)	74 genes NGS panel Guardant360® CDx[1]	Baseline	• Landscape of KIT mutations at the onset of imatinib failure • Evaluate the efficacy of ripretinib versus sunitinib according to baseline *KIT* mutation status as determined by ctDNA analysis
INSIGHT	III	Ripretinib vs Sunitinib (2nd line)	74 genes NGS panel Guardant360® CDx[1]	Baseline as pre-screening	• Investigate the efficacy of ripretinib versus sunitinib in patients with imatinib-resistant advanced GIST harboring *KIT* exon 11 + 17/18 mutations as identified by ctDNA analysis

[1] **Gene analyzed:** AKT1;ALK; APC; AR; AR AF; ARID1A; ATM; BRAF; BRCA1; BRCA2; CCND1; CCND2; CCNE1; CDH1; CDK12; CDK4; CDK6; CDKN2A; CTNNB1; DDR2; EGFR; ERBB2; ESR1; EZH2; FBXW7; FGFR1; FGFR2; FGFR3; GATA3; GNA11; GNAQ; GNAS; HNF1A; HRAS; IDH1; IDH2; JAK2; JAK3; KIT; KRAS; MAP2K1; MAP2K2; MAPK1; MAPK3; MET; MLH1; MPL; MTOR; MYC; NF1; NFE2L2; NOTCH1; NPM1; NRAS; NTRK1; NTRK3; PDGFRA; PIK3CA; PTEN; PTPN11; RAF1; RB1; RET; RHEB; RHOA; RIT1; ROS1; SMAD4; SMO; STK11; TERT; TP53; TSC1; VHL

[2] **EOT** = end-of-treatment

5.3. INTRIGUE trial

The INTRIGUE trial compared ripretinib with sunitinib in patients with advanced GIST previously treated with imatinib, aiming to evaluate the efficacy of ripretinib as a second-line therapy (Bauer et al., 2022). Although the trial did not meet its primary endpoint of superior PFS with ripretinib compared to sunitinib, a comprehensive ctDNA biomarker analysis was performed to characterize the molecular heterogeneity at the time of imatinib failure and to assess mutation-specific responses to each drug (Heinrich et al., 2024). This exploratory analysis, conducted using a 74-gene ctDNA NGS assay on plasma samples from 362 patients, demonstrated a high rate of ctDNA detection. *KIT* mutations were identified in 59 % of patients, with primary mutations most commonly found in exon 11 (73.7 %) and 9 (16.9 %). Secondary resistance mutations were detected in exons 13/14 (38.0 %) and 17/18 (41.8 %), highlighting the molecular heterogeneity of this imatinib-resistant GIST population.

Moreover, patients with imatinib-resistant secondary mutations in *KIT* exons 13/14 experienced significantly greater clinical benefit from sunitinib compared to ripretinib, with a median PFS of 15.0 versus 4.0 months, respectively ($P = 0.0005$). In contrast, patients with secondary mutations in *KIT* exons 17/18 derived greater benefit from ripretinib, with a median PFS of 14.2 months versus 1.5 months with sunitinib ($P < 0.0001$).

5.4. INSIGHT trial

The INSIGHT trial is a randomized, multicenter, open-label study evaluating ripretinib versus sunitinib in patients with advanced GIST previously treated with imatinib harboring *KIT* exon 11 mutations along with secondary mutations in exon 17 and/or 18, as determined by ctDNA analyses during prescreening (George et al., 2024). This ongoing study involves a molecularly selected population, based on the hypothesis that ripretinib, which targets *KIT* activation loop mutations, may provide superior clinical benefit compared to sunitinib, which is less effective against these resistance-associated alterations. The primary endpoint is PFS, assessed by an independent radiologic review according to the modified RECIST version 1.1. Secondary endpoints include the objective response rate (ORR) and OS, as well as exploratory analyses of ctDNA kinetics and molecular correlates of response. This genotype-driven approach reflects a growing emphasis on personalized therapy in GISTs and may help define the optimal TKI sequencing strategy for patients with activation loop-mediated resistance following imatinib failure.

6. Conclusions: critical view

In this review we have portrayed the multifaceted role of liquid biopsy in GIST research, retracing its applications from the earliest publications to the most recent studies, including large-scale clinical trials, to highlight both the progress achieved and the current limitations that still need to be addressed. Overall, liquid biopsy is emerging as an innovative tool in the GIST management, offering a non-invasive approach for real-time genomic profiling of the disease. Its potential clinical applications include the identification of resistance mechanisms, monitoring of treatment response, and detection of MRD. Indeed, across multiple studies, ctDNA has shown strong concordance with tissue-based genotyping, particularly in metastatic or high tumor burden settings, and its dynamic nature allows detection of emerging mutations and clonal evolution under therapeutic pressure, often preceding radiological progression. Notably, the integration of liquid biopsy into large trials, such as VOYAGER, INVICTUS, INTRIGUE, and INSIGHT, has further validated its feasibility at scale and underscored its potential to support genotype-driven treatment strategies.

However, several technical and biological limitations currently hamper the widespread clinical implementation of liquid biopsy. ctDNA sensitivity is strongly influenced by tumor burden and metastatic distribution, with low-volume or anatomically confined disease often associated with limited tumor DNA shedding and an increased risk of false-negative results. Pre-analytical variables, including blood collection, processing, and storage further contribute to variability in ctDNA yield and quality. Additionally, while NGS-based approaches enable broader mutational profiling, they may lack sensitivity for low-frequency subclones, whereas highly sensitive techniques such as BEAMing, allele-specific PCR, and ddPCR require prior knowledge of target mutations and may struggle to detect complex alterations, including large insertions or deletions. Costs, technical complexity, and the need for dedicated bioinformatic expertise remain additional barriers to routine clinical adoption.

Furthermore, the concept of ctDNA-based MRD assessment, although appealing, requires particular caution in GISTs. Unlike hematologic malignancies, where MRD negativity is closely associated with complete remission and directly informs therapeutic decisions, the biological and clinical significance of MRD negativity in oncogene-addicted solid tumors such as GISTs remains uncertain and cannot yet be considered a reliable guide for clinical decision-making, particularly with regard to treatment interruption. Similarly, while liquid biopsy enables early detection of secondary resistance mutations and provides insights into tumor heterogeneity, their immediate impact on clinical decision-making may be limited, as treatment modifications in advanced GISTs are driven mainly by clinical and radiological progression rather than molecular findings alone.

Another aspect that should be addressed is the role of alternative circulating biomarkers and epigenetic alterations, which are still largely unexplored, in GIST diagnosis and prognosis. Finally, key questions remain unresolved, including the definition of optimal detection thresholds, the most appropriate timing and frequency of sampling, and the actual clinical benefit of ctDNA-guided interventions on long-term patient outcomes.

Addressing these issues through well-designed prospective studies will be essential to standardize methodologies and to fully establish liquid biopsy as a reliable and clinically actionable tool in routine GIST management.

Author contributions

Margherita Nannini: Conceptualization, Investigation, Project Administration, Resources, Visualization, Writing – original draft, Writing – review and editing. **Livia Gozzellino:** Conceptualization, Investigation, Project Administration, Resources, Visualization, Writing – original draft, Writing – review and editing. **Alice Costa:** Visualization, Writing – review and editing. **Maria Concetta Nigro:** Writing – review and editing. **Dario de Biase:** Supervision, Writing – review and editing. **Thais Maloberti**: Writing – review and editing. **Annalisa Astolfi:** Conceptualization, Project Administration, Supervision, Writing – review and editing. **Maria Abbondanza Pantaleo:** Conceptualization, Project Administration, Supervision, Writing – review and editing.

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Data availability

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Glossary

BEAMing: Beads, emulsion, amplification, magnetics
cfDNA: Circulating Free DNA
cfRNA: Circulating Free RNA
CTC: Circulating Tumor Cell
ctDNA: Circulating Tumor DNA
dPCR: Digital Polymerase Chain Reaction
ddPCR: Digital Droplet Polymerase Chain Reaction
EV: Extracellular Vesicle
FFPE: Formalin-Fixed Paraffin-Embedded
GIST: Gastrointestinal Stromal Tumor
L-PCR: Long-range Polymerase Chain Reaction
MAF: Mutant Allele Fraction
MRD: Minimal Residual Disease
NGS: Next Generation Sequencing
ORR: Objective Response Rate
OS: Overall Survival
PCR: Polymerase Chain Reaction
PFS: Progression Free Survival
PPV: Positive Predictive Value
SNV: Single Nucleotide Variant
TEC-Seq: Targeted Error Correction Sequencing
TEP: Tumor-Educated Platelets
TKI: Tyrosine Kinase Inhibitor
VAF: Variant Allele Frequency

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