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REVIEW

Using molecular diagnostic testing to personalize the treatment of patients with gastrointestinal stromal tumors

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ABSTRACT

Introduction: The diagnosis and treatment of gastrointestinal stromal tumor (GIST) has emerged as a paradigm for modern cancer treatment ('precision medicine'), as it highlights the importance of matching molecular defects with specific therapies. Over the past two decades, the molecular classification and diagnostic work up of GIST has been radically transformed, accompanied by the development of molecular therapies for specific subgroups of GIST. This review summarizes the developments in the field of molecular diagnosis of GIST, particularly as they relate to optimizing medical therapy.

Areas covered: Based on an extensive literature search of the molecular and clinical aspects of GIST, the authors review the most important developments in this field with an emphasis on the differential diagnosis of GIST including mutation testing, therapeutic implications of each molecular subtype, and emerging technologies relevant to the field.

Expert commentary: The use of molecular diagnostics to classify GIST has been shown to be successful in optimizing patient treatment, but these methods remain under-utilized. In order to facilitate efficient and comprehensive molecular testing, the authors have developed a decision tree to aid clinicians.

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1. Introduction

Although not widely recognized before 1998, gastrointestinal stromal tumor (GIST) now represents the most common mesenchymal tumor of the gastrointestinal (GI) tract, with more than 5000 new cases diagnosed annually in the United States [1]. GISTs have become a paradigm for the use of molecular diagnostics and targeted therapy. The molecular classification of a patient's GIST informs therapeutic decision-making and predicts treatment responses. Unfortunately, less than 15% of patients have their tumors genotyped, potentially leading to suboptimal care [2].

Most GISTs are driven by activating mutations in either of two receptor tyrosine kinases (RTKs), KIT or platelet-derived growth factor receptor alpha (PDGFRA). Following the discovery of these mutations as tumor drivers, GIST was the first solid tumor to be successfully treated with small molecule tyrosine kinase inhibitors (TKIs). We now know that there are other molecular drivers of GIST pathogenesis including deficiency of succinate dehydrogenase (SDH) or neurofibromatosis type I (NF1), activating mutations in the RAS/RAF/MEK pathway, and translocations involving the kinase domain of RTKs other than KIT/PDGFRA (e.g. NTRK3). Optimal treatment of patients with GIST requires molecular subclassification. This review focuses on advances in the diagnosis and characterization of GIST and how molecular testing should be used to guide patient care.

2. Initial diagnosis using immunohistochemistry (IHC)

GISTs most commonly arise in the stomach (60%), but can also be found in the small intestine (25%), rectum (5%), and elsewhere in the GI tract, including esophagus, colon, appendix, and gallbladder. Occasionally, GISTs arise outside the wall of the gut, designated extraintestinal GIST. Even more rare are reports of primary GISTs that originate outside the abdominal cavity, including reports of a primary GIST of the pleura or pericardium [3–5].

Historically, GISTs were classified as smooth muscle tumors (leiomyoma or leiomyosarcoma) because of their predominantly spindle cell morphology and their association with the muscularis propria of the bowel wall. However, studies using electron microscopy and IHC differentiated these tumors from classic leiomyosarcoma, leading Mazur and Clark to propose the term 'stromal tumor' in 1983 [6]. The subsequent discovery that most stromal tumors arising in the GI tract are CD34-positive provided further evidence for their distinction from CD34-negative leiomyosarcoma.

During the 1990s, several investigators observed similarities between GIST cells and a unique population of cells in the gut wall known as the interstitial cells of Cajal (ICC). ICC are the pacemaker cells of the gut, responsible for coordinated peristalsis. Normal ICC express KIT (CD117) and are developmentally dependent on the expression of both KIT and its cognate ligand, stem cell factor (SCF). Mice deficient in KIT or SCF expression have a marked reduction in certain populations

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Table 1. Immunohistochemistry in differential diagnosis of GIST.

Diagnosis	KIT (CD117)	ANO1	SDHB	SDHA	Desmin	S-100
KIT-, BRAF-, NF1- mutant GIST	Positive	Positive	Positive	Positive	Negative	Negative
PDGFRA-mutant GIST	Sometimes low[a]	Positive	Positive	Positive	Negative	Negative
SDHB/C/D-mutant GIST	Positive	Positive	Negative	Positive	Negative	Negative
SDHA-mutant GIST	Positive	Positive	Negative	Negative or positive[b]	Negative	Negative
RTK-WT/SDHB positive	Positive	Positive	Positive	Positive	Negative	Negative
Quintuple WT	Positive	Positive	Positive	Positive	Negative	Negative
Leiomyoma	Negative	Negative	Positive	Positive	Positive and uniform	Negative
Leiomyosarcoma	Negative	Negative	Positive	Positive	Usually positive	Negative
Schwannoma	Negative	Negative	Positive	Positive	Negative	Positive and uniform
Desmoid fibromatosis	Negative	Negative	Positive	Positive	Negative	Negative

Multiple immunohistochemical markers can be used in the differential diagnosis of GIST. However, the combination of CD117 (KIT) and ANO1 expression by an abdominal sarcoma is essentially diagnostic of GIST. SDH IHC is useful in further distinguishing subtypes of GIST.

[a]Sometimes low KIT indicates that some cases of PDGFRA-mutant GIST are KIT low/negative, whereas many cases are KIT positive.

[b]Negative SDHA indicates the presence of an *SDHA* mutation but *SDHA* mutation can also be present when SDHA IHC is positive. *SDHA* mutation is always accompanied by SDHB deficiency.

ANO1: anoctamin-1; GIST: gastrointestinal stromal tumor; NF1: neurofibromatosis type I; PDGFRA: platelet-derived growth factor receptor alpha; RTK: receptor tyrosine kinase; SDH: succinate dehydrogenase; SDHA: succinate dehydrogenase subunit A; SDHB: succinate dehydrogenase subunit B; WT: wild type.

of ICC [7]. In 1998, two separate groups reported that GISTs commonly express CD117 [8,9]. It is now well established that 95% of GISTs are immunohistochemically positive for CD117.

In 2004, another highly specific marker for GISTs was described. Anoctamin-1 (ANO1 or DOG1) is a calcium-activated chloride channel that is highly expressed in ICC and in 98% of GIST, regardless of CD117 expression levels [10]. Conveniently, only a small number of non-GIST sarcomas express ANO1. The combination of CD117 and ANO1 expression by an abdominal sarcoma is essentially diagnostic of GIST [10,11].

The use of CD117 and ANO1 has helped define the full range of cellular morphology associated with GIST (see Table 1). Although most GISTs consist of a uniform population of spindled cells, some cases have an epithelioid appearance and others are a mixture of spindled and epithelioid cells. Tumor cellularity varies widely among GISTs. Low-grade lesions may show areas of central calcification, or demonstrate band-like alignment of nuclei mimicking a schwannoma. High-grade tumors often ulcerate the overlying mucosa and may undergo significant hemorrhagic necrosis. This variety in GIST histology dictates a broad morphologic differential diagnosis; therefore, judicious use of CD117 and ANO1 IHC is key to making an accurate diagnosis.

In 2008, a subset of GISTs were found to be immunohistochemically negative for the expression of SDH subunit B (SDHB) [12]. An additional subset of SDHB-deficient tumors also lacks SDH subunit A (SDHA) expression. The implications and importance of these findings are discussed later. The use of IHC markers used in the diagnosis of GIST is summarized in Table 1.

3. Molecular classification

The vast majority of GISTs (75–80%) harbor gain-of-function *KIT* mutations [8]. The second most common class of mutations, representing 5–10% of GIST, affects PDGFRA, an RTK homologous to KIT. The remaining 10–15% of GISTs do not have mutations in *KIT* nor *PDGFRA*; these tumors historically have been referred to as wild-type (WT) GIST (Figure 1). Increasingly, the term WT GIST is confusing, as modern testing can actually identify a pathogenic mutation in most of these cases. An updated molecular classification of GIST summarized in Table 2 may be helpful for diagnostic, prognostic, and treatment planning purposes.

3.1. Kit-mutant GIST

KIT is a type III RTK, belonging to the family that also includes PDGFRA, PDGFRB, Colony stimulating factor 1 receptor (CSF1R), and FLT3. Upon binding its cognate ligand, SCF, two KIT polypeptides dimerize and transactivate each other by tyrosine phosphorylation. The resultant fully activated kinase complex initiates downstream signaling through multiple pro-proliferative and pro-survival pathways, including PI3K/AKT and RAS/RAF/MEK.

The functional importance of *KIT* mutations in GIST pathogenesis is supported by multiple lines of evidence. First, phosphorylated KIT, indicative of activated KIT, is readily detected in extracts from clinical GIST specimens and GIST cell lines [13]. Second, mutant KIT can transform Ba/F3 cells, supporting their growth in nude mice [8]. Third, mice engineered to express KIT with activating mutations like those found in human GISTs develop diffuse ICC hyperplasia of the stomach and intestines and also develop GIST-like tumors [14,15]. The histologic pattern of diffuse ICC hyperplasia and focal GIST formation in these mice is similar to that seen in individuals who inherit germ line KIT-activating mutations [16,17]. Fourth, when exogenously expressed in cell lines, mutant forms of KIT show constitutive kinase activity in the absence of SCF, as shown by auto-phosphorylation and activation of downstream signaling pathways [8,18,19]. Fifth, treatment of GIST cell lines or primary GIST cell cultures with KIT kinase inhibitors or interfering RNA against *KIT* results in decreased proliferation and increased apoptosis [19,20]. Finally, TKI-resistant, *KIT*-mutant GIST typically harbors secondary *KIT* mutations that confer drug resistance but maintain kinase activity, suggesting that even in the advanced state, GISTs require maintenance of KIT signaling (see Section 4.4 for additional discussion of resistance mutations) [20].

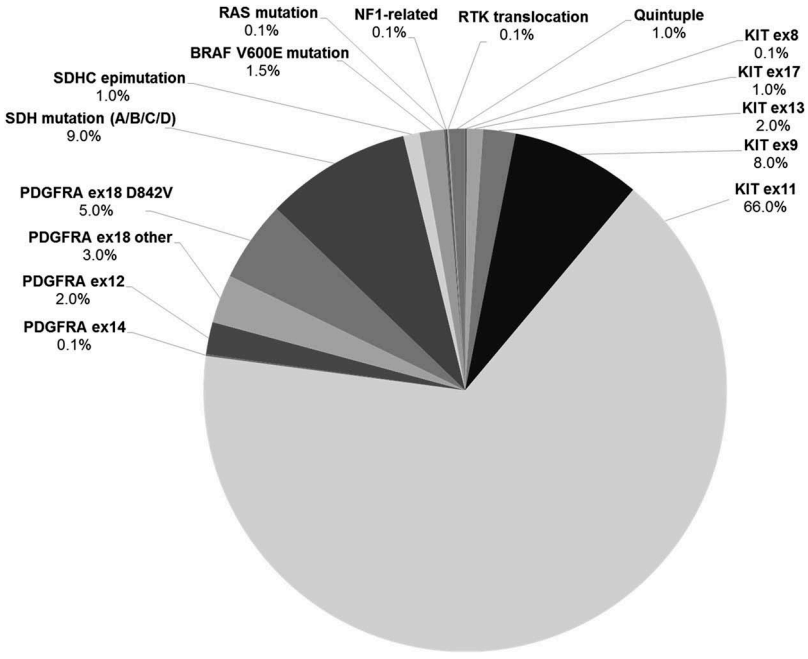

Figure 1. Mutational subclassification of GIST. The percentage of GIST cases within each mutation-based subclass is depicted.

3.1.1. *KIT* exon 11 mutations

Mutations in *KIT* exon 11 are the most common type of oncogenic mutation found in GIST, occurring in approximately 67% of cases. These mutations include point mutations, in-frame deletions and/or insertions. Exon 11 encodes the juxtamembrane portion of KIT that prevents the kinase activation loop from swinging into the active state, thus favoring the auto-inhibited conformation. Mutations of *KIT* exon 11 disrupt this auto-inhibition, allowing spontaneous kinase activation in the absence of SCF ligand [21].

KIT exon 11-mutant GISTs arise throughout the GI tract, but the most common site is the stomach. Tumors with *KIT* exon 11 mutations typically have spindled rather than epithelioid cell morphology. After complete resection, these tumors have a higher rate of recurrence than other genotypically defined GIST subgroups. Correspondingly, GISTs with *KIT* exon 11 deletions, particularly deletions involving codons 557 and/or 558, have a worse prognosis than those with exon 11 and point mutations [22].

3.1.2. *KIT* exon 9 mutations

The second most common class of mutations affects *KIT* exon 9 (8–10% of GISTs), which encodes the KIT proximal extracellular domain. More than 95% of GIST-associated *KIT* exon 9 mutations consist of an insertion of six nucleotides, resulting in duplication of amino acids 502 and 503. Rare cases of amino acid substitutions involving codon 476 have also been reported [23]. Most GISTs harboring *KIT* exon 9 mutations arise from the small or large bowel and *KIT* exon 9-mutant tumors make up 25–30% of intestinal GISTs. In contrast, *KIT* exon 9 mutations make up less than 2% of gastric GISTs [23]. *KIT* exon 9 mutations result in constitutive kinase activation by mimicking the conformational change that the extracellular domain undergoes after ligand binding. The kinase domain conformation in exon 9-mutant KIT is believed to be the same as for WT KIT.

3.1.3. Other *KIT* mutations

Primary mutation of *KIT* exon 13, which encodes part of the kinase adenosine triphosphate (ATP)-binding pocket, occurs in approximately 1% of GISTs. The substitution K642E accounts for the vast majority of primary *KIT* exon 13 mutations. *KIT* exon 13-mutant GISTs are most commonly found in the stomach but can arise throughout the GI tract. *KIT* exon 13-mutant tumors typically have a spindle cell appearance, but occasionally have epithelioid or mixed histology.

Primary mutations affecting *KIT* exon 17, which encodes the kinase activation loop, are found in approximately 1% of GISTs. Substitutions at codons 820, 822, or 823 are the most common mutation sites in this exon. Almost all of these tumors have a spindle cell appearance and most are located in the small bowel, but can arise in the stomach as well [24].

Rarely, mutations occur in *KIT* exon 8, which encodes part of the KIT extracellular domain. The majority of these GISTs occur outside of the stomach, in the duodenum or small intestine [25].

3.2. *PDGFRA*-mutant GIST

Mutations in *PDGFRA* are the most common non-*KIT* oncogenic mutations associated with GIST. *PDGFRA* is a close homolog of KIT and uses similar downstream signaling pathways to drive proliferation. *PDGFRA* mutations found in GIST result in constitutive kinase activation and are mutually exclusive with *KIT* mutations [26,27]. The most common location for *PDGFRA*-mutant GISTs is the stomach, but they can arise in the small or large intestine. Histologically, *PDGFRA*-mutant GISTs usually have an epithelioid or mixed epithelioid/spindle appearance, commonly accompanied by a myxoid stroma [28]. Some, but not all, *PDGFRA*-mutant GISTs express low or undetectable levels of KIT as assessed by IHC (so-called KIT-negative GIST); however, these tumors retain expression of

Table 2. Molecular classification of GIST.

Molecular classification	Relative frequency (%)	Clinical presentation	Treatment or notable features
***KIT* mutation**	77		
Exon 8	Rare	Small bowel	Rare, reports of favorable response to imatinib
Exon 9	8	Small bowel, colon	Better responses with high-dose imatinib (800 mg/day) or sunitinib than with standard dose imatinib
Exon 11	67	All sites	Sensitive to imatinib (400 mg per day), no advantage to higher dose therapy
Exon 13	1	All sites	Sensitive to imatinib but limited treatment data; need for doses higher than 400 mg
Exon 17	1	All sites	Some are sensitive to imatinib; consider dose escalation if no response to imatinib 400 mg
***PDGFRA* mutation**	10		
Exon 12	1	All sites	Sensitive to imatinib
Exon 14	<1	Stomach	Sensitive to imatinib
Exon 18 D842V	7	Stomach, mesentery, omentum	Resistant to all agents approved for GIST, consider clinical study
Exon 18 other	1	All sites	Most are sensitive to imatinib (D842V is resistant)
RTK-WT	13	All sites	
SDHB-negative			Treat with standard paradigm of imatinib → sunitinib → regorafenib. Needs closer follow-up than kinase mutation GIST as this is much less likely to respond to conventional GIST TKIs
SDH mutation (A/B/C/D)	9	Stomach only; Some cases with germ line mutations (Carney–Stratakis), rarely Carney triad	Carney–Stratakis syndrome; rarely Carney triad
SDHC epimutation	<1	Stomach only, Carney triad (not heritable) or sporadic	Carney triad, not heritable
SDHB-positive			
BRAF V600E mutation	<1	All sites	May respond to BRAF inhibitor ± MEK inhibitor
RAS mutation	<1	Stomach	No established therapy for advanced medical disease. Consider clinical study of MEK inhibitor or other investigational approaches
NF1-related	~1	Small bowel	Multiple lesions, rarely malignant. No established medical therapy for advanced disease
RTK translocation	<1	Non-gastric	Reports of response of an *NTRK3*-translocated GIST to an investigational NTRK3 TKI. Other forms may respond to appropriate inhibitors for the translocated kinase. Enrollment in a clinical study of an appropriate kinase inhibitor is recommended.
Quintuple WT	<1	All sites	Response to standard GIST therapy is poorly described. Reasonable to try one or more approved GIST agents in cases of advanced disease. As this entity becomes better understood, additional options may emerge.

An updated molecular classification of GIST subtypes, including relative frequency, clinical presentation, treatment, and notable features, is provided.

GIST: gastrointestinal stromal tumor; PDGFRA: platelet-derived growth factor receptor alpha; RTK: receptor tyrosine kinase; WT: wild type; SDHB: succinate dehydrogenase subunit B; SDH: succinate dehydrogenase; SDHC: succinate dehydrogenase subunit C, TKI: tyrosine kinase inhibitor.

ANO1. Other similarities between *PDGFRA*-mutant and *KIT*-mutant GISTs include expression of Protein kinase C (PKC)-theta and activation of the RAS/MAPK and PI3K pathways [23]. In addition, these tumors tend to have similar cytogenetic abnormalities, including monosomy of chromosome 14 [26]. However, gene expression profiling of *KIT*-mutant and *PDGFRA*-mutant GISTs has shown subtle differences that may relate to some of the differences in clinical behavior [29]. In a population-based series of 492 primary GISTs in France, the frequency of *PDGFRA* mutations was 15%, whereas only 2% of cases in two large clinical series of metastatic GIST were driven by *PDGFRA* mutations [30]. These observations, which have been confirmed in other series, suggest that *PDGFRA*-mutant GISTs generally have a lower risk of recurrence than *KIT*-mutant GIST. As with *KIT* mutations, rare families with germ line *PDGFRA* mutations and susceptibility to developing GIST have been reported [31].

3.2.1. PDGFRA exon 18 mutations

The most common *PDGFRA* mutations in GIST involve exon 18, and are thought to stabilize the kinase activation loop in a conformation that favors kinase activation [32]. A single mutation, D842V, accounts for at least 70% of all *PDGFRA* mutations seen in GIST [33]. Curiously, D842V mutations are found only in tumors arising in the stomach, omentum, and mesentery.

3.2.2. PDGFRA exon 12 mutations

Mutations affecting exon 12 of *PDGFRA* are found in approximately 1% of GISTs [30,33]. *PDGFRA* exon 12 is homologous to *KIT* exon 11 and point mutations or in-frame insertion/deletion mutations of this region lead to loss of the auto-inhibitory function of the juxtamembrane domain [23,32].

3.2.3. PDGFRA exon 14 mutations

Less than 1% of GISTs have activating mutations in *PDGFRA* exon 14, making these tumors some of the rarest types of RTK-mutant GIST. By homology with *KIT* exon 13, mutations in *PDGFRA* exon 14 may interfere with the auto-inhibitory function of the juxtamembrane domain.

3.3. RTK-WT GIST

3.3.1. Historical perspective

Beginning in 1998, GISTs were classified as *KIT*-mutant versus WT, based on the original description of *KIT* exon 11 mutations. When *PDGFRA*-mutant GISTs were identified in 2003, the definition of WT GIST was revised to mean those tumors lacking *KIT* or *PDGFRA* mutations. As detailed later, other gain- or loss-of-function pathogenic mutations have since been discovered in GIST lacking *KIT* or *PDGFRA* mutations. In light of these newer mutations, categorizing GISTs as WT has become confusing and misleading. They are perhaps better referred to as RTK-WT with further subclassification dependent on the results of additional molecular testing, as discussed later (see Table 2).

3.3.2. SDHB-deficient, RTK-WT GIST

A major breakthrough in the understanding of non-RTK oncogenic mechanisms in GIST arose from studies of patients with Carney–Stratakis syndrome. This autosomal-dominant syndrome manifests as a susceptibility to develop both paraganglioma and GIST. Previous studies of familial paraganglioma syndromes revealed germ line-inactivating mutations in the genes encoding the SDH complex, which is composed of four subunits: SDHA, SDHB, SDHC, and SDHD (collectively termed SDHx) [34,35]. The *SDHx* genes are classic tumor suppressor genes, requiring inactivation of both alleles of a specific SDH subunit for loss of SDH activity. Typically, this is the result of a combination of an inactivating germ line mutation (first hit) with a somatic loss of heterozygosity or other inactivating mutation affecting the other allele (second hit). Inactivation of any of the SDHx subunits causes destabilization and loss of enzymatic function of the entire complex, resulting in SDH deficiency [12].

The mechanisms by which SDH deficiency initiates the formation of GIST are incompletely understood. Loss of SDH activity prevents the conversion of succinate to fumarate, which leads to accumulation of succinate, an oncometabolite that has been connected to two mechanisms of cancer pathogenesis: (1) inhibition of prolyl hydroxylase, which leads to the accumulation of the transcription factor Hypoxia-inducible factor 1-alpha (HIF1α); and (2) inhibition of DNA demethylases, resulting in DNA hypermethylation and gene deregulation. Oncometabolite initiation of GIST is believed to be independent of KIT signaling and this has implications for treatment of SDH-deficient GIST as noted later.

Several investigators have shown that the absence of immunohistochemical staining for SDHB is a reliable method to identify SDH-deficient GIST [36–39]. Overall, SDHB-deficient GISTs have distinct clinical and pathologic characteristics, including gastric origin, epithelioid morphology, a multifocal nodular growth pattern, and frequent involvement of local lymph nodes [40,41]. Miettinen et al. reported that 7.5% of 756 gastric GISTs were SDHB immunonegative, while no cases of SDHB deficiency were found among 378 non-gastric GISTs [39]. Many SDHB-deficient GISTs arise in patients younger than 20 years. In contrast, gastric GIST diagnosed in patients older than 40 years is rarely SDHB-deficient [38,39]. SDHB staining is retained in GIST with *KIT* or *PDGFRA* mutations and in GISTs with other oncogenic mutations, as discussed later and in Table 1.

Absence of SDHA immunostaining generally correlates with loss-of-function SDHA mutations, most of which seem to be inherited [41–45]. In rare cases, SDHA staining is retained in an *SDHA*-mutant tumor that lacks SDHB staining [41]; presumably, this is because the mutant-SDHA protein, although dysfunctional, is not degraded. Of 127 SDHB-deficient GISTs, 28% also lacked SDHA expression, suggesting that *SDHA* mutations account for more than a quarter of SDH-deficient GISTs [41]. In contrast, 0 of 556 cases of SDHB-positive GISTs lacked SDHA protein expression. Compared to patients with SDHA-positive/SDHB-negative GISTs, those with SDHA-/SDHB-negative GISTs have an older median age (34 vs. 21 years), lower female/male ratio, and a slower course of disease, despite a slightly higher rate of liver metastases.

Unlike mutations in *KIT* and *PDGFRA*, the mutations seen in *SDHx* are varied. Loss-of-function mutations are found throughout the coding regions and do not cluster around

specific amino acids. Indeed, one of the challenges in evaluating *SDHx* variants in SDH-deficient tumors is determining which ones are responsible for the disease. Functional assays to determine their effects on SDH complex activity would be useful to guide genetic counseling for these patients and their families [46,47].

A subset of SDHB-deficient GISTs have no detectable *SDHx* mutations. The majority of these are thought to have *SDHC* promoter-specific CpG island hypermethylation, referred to as *SDHC* epimutation. Tumors with *SDHC* epimutation have decreased *SDHC* mRNA expression compared with normal controls [48]. Decreased SDHC protein expression leads to SDH complex instability and the secondary loss of SDHB protein expression as assessed by IHC. All known cases of GIST with an *SDHC* epimutation are SDHB-deficient. The mechanisms leading to increased *SDHC* promoter hypermethylation remain unknown at this time, but are likely post-zygotic because the risk for these tumors is not inherited [48]. Patients with *SDHC* epimutation often manifest Carney triad, which consists of gastric GIST, paraganglioma, and pulmonary chondroma [49]. Given that both Carney–Stratakis syndrome and Carney triad are characterized by GIST and paraganglioma, it is challenging to determine which condition is responsible for development of these tumors in a given patient without appropriate molecular testing.

Identification of SDHB immunonegative GISTs is important for several reasons. First, given the increased frequency of *SDHx* mutations in these tumors, genotyping can be used to guide subsequent testing for the presence of germ line mutations (Carney–Stratakis syndrome). Clinical screening guidelines have been described for patients with familial paraganglioma/GIST [50]. Second, conventional risk stratification of SDHB-deficient tumors using tumor size and mitotic index is poorly predictive of tumor behavior. These tumors frequently metastasize but often have an indolent clinical course. In addition, lymph node metastases are common in SDHB-deficient tumors, but extremely rare in SDHB-positive tumors [40,51].

3.3.3. RTK-WT/SDHB-positive GIST

3.3.3.1. RAS/RAF/MAPK. GISTs that are RTK-WT and SDHB-positive are uncommon, but nevertheless comprise a genetically diverse group. Some harbor alterations that hyperactivate the RAS/RAF/MAPK pathway. Among these are mutations in the gene *NF1*, which encodes the tumor suppressor neurofibromin that serves as a negative regulator of the activity of the RAS pathway. Approximately 7% of patients with germ line *NF1* mutations develop RTK-WT GIST (frequently multiple) of the small bowel [52,53]. As expected, *NF1*-associated GISTs are uniformly SDHB-positive [54]. Interestingly, there have been reports of sporadic *KIT*-mutant GISTs in patients with NF1, and this has treatment implications, as discussed later [55]. Therefore, GIST developing in patients with clinical NF should be subjected to molecular analysis for *KIT/PDGFRA* mutations, to distinguish between sporadic GIST and NF-associated GIST due to loss of the WT *NF1* allele. *NF1* mutations have also been reported in GIST in patients without clinical NF; this may occur either due to unrecognized NF or as a sporadic cause of GIST without germ line *NF1* mutation [56,57]. NF1 loss may be under-recognized as a potential molecular cause of GIST, due to the large size of the *NF1* gene and the frequent occurrence of intragenic (and sometimes flanking region) microdeletions that are not detectable by sequencing, especially using exome-focused sequencing panels [58].

BRAF V600E mutations have been reported in 7–15% of RTK-WT GISTs, but comprise less than 2% of overall GIST diagnoses [59–61]. There do not seem to be any common anatomic or pathologic associations for BRAF-mutant GIST. Rare cases of RAS-mutant GIST have also been described [23,62].

3.3.3.2. RTK translocations. Approximately 5% of GISTs lack mutations in all genes currently linked to GIST development (*KIT, PDGFRA, SDHx*, and RAS pathway). These GISTs have been termed 'quadruple WT' [63]. In 2016, two groups identified oncogenic RTK translocations in a subset of quadruple-WT GISTs. Brenca et al. used transcriptome sequencing to identify the fusion of exon 4 of *ETV6* to exon 14 of *NTRK3* in one quadruple-WT GIST that arose in the rectum [64]. Shi et al. identified four cases of quadruple-WT GIST with oncogenic RTK translocations: two with *ETV6-NTRK3* fusions, one with a *fibroblast growth factor receptors 1 (FGFR1)-TACC1* fusion, and the final case had a *FGFR1-HOOK3* fusion [65]. All of these translocations are known or predicted to result in constitutive kinase activation. Despite the novel molecular findings, these tumors were otherwise typical for GIST in terms of morphology and IHC testing results, indicating that these tumors comprise a new molecular subclass of GIST rather than a different form of sarcoma. Given the small size of these two series, it is possible that additional gene fusions of these or other kinases may be involved in GIST pathogenesis.

3.3.3.3. Quintuple WT. As outlined earlier, it is imperative that the molecular classification of GIST be defined during the diagnostic process to better define therapeutic options for individual patients. However, there is still a small subset of GIST that is wild type for all known genes causing GIST including *KIT, PDGFRA, SDH*, RAS-pathway, and RTK translocations. We propose that these GISTs be termed quintuple-WT GIST. This group likely represents only 1% of GISTs [65].

4. Using molecular classification to optimize clinical treatment

There are three treatment scenarios in which molecular classification of GIST is important: therapy for advanced disease, adjuvant therapy following primary GIST resection, and primary/secondary resistance. Although molecular classification is beneficial in all three circumstances, it is the most powerful for optimizing clinical treatment in advanced disease (Table 2). The relevance of molecular classification in each scenario is discussed later.

4.1. Therapy for advanced disease

While many GISTs are controlled by surgery with or without adjuvant imatinib, treatment of GIST in the advanced setting has improved greatly when patients are stratified by molecular

subtype. Preclinical and clinical data demonstrate therapeutic responses differ significantly between GISTs with different molecular defects or advanced disease; there are currently three approved small-molecule therapies to treat GIST of any classification: imatinib (first line), sunitinib (second line), and regorafenib (third line); all are TKI small molecules that have variable potency against mutations in KIT or PDGFRA. In general, these treatments have been shown to be most effective in RTK-mutant GIST. As we further understand what drives these tumors, we are able to effectively inhibit their growth. The preclinical and clinical studies that inform the treatment of each GIST subtype are summarized later (see also Table 2).

4.1.1. KIT-mutant GIST

KIT exon 11-mutant GISTs have the most robust and durable response to front-line treatment with imatinib compared with other types of GIST. *In vitro* assays of *KIT* exon 11-mutant kinases have confirmed that mutations found in GIST tumors are tenfold more sensitive to KIT inhibitors, such as imatinib, than the WT isoform [66]. These *in vitro* findings are reflected in the clinic, where primary resistance to imatinib treatment (defined by progression within the first 6 months of therapy) is seen in only 5% of cases of advanced *KIT* exon 11-mutant GIST, compared with 16% of *KIT* exon-9 mutant and 43% of *KIT/PDGFRA* WT cases [67,68]. Correspondingly, the objective response rate to imatinib is 67–83% for *KIT* exon 11-mutant GIST versus 35–48% for *KIT* exon 9-mutant GIST [68]. The median time to progression on first-line imatinib therapy for *KIT* exon 11-mutant GIST is approximately 25 months, and the current median overall survival for patients with *KIT* exon 11-mutant GIST is at least 60 months. The molecular mechanisms leading to secondary drug resistance in *KIT* exon 11-mutant GISTs are discussed later.

Exon 9-mutant *KIT* shows decreased *in vitro* sensitivity to imatinib compared with exon 11-mutant *KIT* [69,70]. In agreement with these data, results from randomized Phase 3 studies showed that patients with *KIT* exon 9-mutant GIST had a significantly improved progression-free survival, approximately 1 year longer, when treated with a higher total daily dose of 800 mg of imatinib compared with patients treated with 400 mg [71]. Sunitinib, the second-line KIT inhibitor approved for GIST, has a greater potency than imatinib against *KIT* exon 9-mutant kinases. Consistent with this, patients with *KIT* exon 9-mutant GIST represent the most likely subset of patients with imatinib-resistant tumors to benefit from second-line sunitinib therapy [66].

Treatment of GIST with mutations in *KIT* exons 13 and 17 can be informed by preclinical and clinical observations as well. *In vitro* data indicate that *KIT* exons 13 and 17 are sensitive to imatinib, but perhaps less so than *KIT* exon 11-mutant kinases [23,24,67,72]. If there is no response to imatinib at 400 mg/day, it is reasonable to consider dose escalation to 800 mg/day, if tolerated.

4.1.2. PDGFRA-mutant GIST

The mutations seen in *PDGFRA* exon 18 differ markedly in their imatinib sensitivity [33,73]. D842V confers resistance to imatinib and all other approved KIT TKIs *in vitro* [67]. After D842V, the next most common mutation of exon 18 is deletion of codons 842–845, which is imatinib-sensitive [33,73]. Other more rare mutations in exon 18 are imatinib-resistant, including D846Y, N848K, and Y849K.

While the majority of *PDGFRA* exon 18 mutations are resistant to imatinib and other approved KIT TKIs, a novel PDGFRA-selective kinase inhibitor, crenolanib, was found to have *in vitro* potency against D842V and other imatinib-resistant *PDGFRA* mutations. Based in part on these results, a Phase 3 clinical study of this agent to treat advanced GIST with the *PDGFRA* D842V mutation has been initiated [73] (ClincalTrials.gov Identifier NCT02847429). Even more promising, BLU-285 has demonstrated higher potency and specificity against *PDGFRA* exon 18 mutants than any existing small molecule inhibitors, including crenolanib. Early results from a Phase 1 clinical of this agent reported an impressive objective response rate of this agent for PDGFRA D842V-mutant metastatic GIST [74]. Further study is needed to confirm these results and determine the durability of the reported responses. Based on the lack of response of PDGFRA D842V-mutant GIST to conventional agents, consideration of referral to an appropriate clinical study should be strongly considered, even in untreated patients.

In vitro, PDGFRA exon 12-mutant kinases are as sensitive to imatinib as *KIT* exon 11-mutant kinases. While there are only rare reports of clinical outcomes for patients with metastatic *PDGFRA* exon 12-mutant GISTs treated with imatinib, the available clinical data suggest that patients have high response rates and durable disease control [27,33,67,68]. *In vitro* and clinical study data suggest that exon 14-mutant kinase activity is inhibited by imatinib [33].

4.1.3. RTK-WT GIST

For patients with metastatic GIST that lacks a *KIT/PDGFRA* mutation, we recommend referral to a high-volume-GIST treatment center. Patients with *BRAF* mutant, *NTRK3*-translocated, or *FGFR1*-translocated mutant GIST should be considered for enrollment in a study of an appropriate agent. Based on a single case report as well as our personal results from treating *BRAF*-mutant GIST, consideration of off-label use of a BRAF inhibitor or combined BRAF and MEK inhibitor treatment could be considered.

Currently, there is no validated effective treatment for patients with *RAS*-mutant or *NF1*-mutant GIST. Theoretically, these tumors might respond to a MEK inhibitor, but there are no published data on this approach. In our experience, these patients do not respond to imatinib therapy. Potentially, KIT/vascular endothelial growth factor receptors (VEGFR) inhibitors might have better activity than imatinib in this setting but this has not been proven. In these cases, use of serial surgical debulking to control disease could be considered.

Treatment of SDH-deficient GIST with imatinib results in a very low response rate (~2%) [75]. There are some data to suggest that these patients actually have a better response to second-line sunitinib than front-line imatinib [76]. This could reflect VEGFR inhibition by sunitinib, as increased HIF1α in these tumors leads to VEGF upregulation. In the future, treatments directed at the oncometabolites and/or cellular hypermethylation may yield superior results to current therapy. In

some cases, metastatic disease can behave quite indolently; therefore, selected patients may benefit from observation and/or serial surgical debulking. Given the rarity of this type of GIST and the complexity of molecular classification, genetic counseling, and therapeutic decision-making, referral of such patients to a high-volume GIST treatment center is recommended.

In addition, patients with SDHB-deficient tumors should undergo additional testing to determine if their tumor has loss-of-function mutations involving an SDH subunit. For patients with tumor-associated *SDH* mutations that are felt to result in loss of SDH-complex function, we recommend genetic counseling and consideration of testing for an underlying germ line *SDH* subunit mutation (i.e. Carney–Stratakis). Patients with germ line loss-of-function mutations of an SDH subunit should undergo additional genetic counseling to discuss screening with other family members and to review recommendations for surveillance for the potential development of paraganglioma, pheochromocytoma, or additional GIST [77,78].

RTK translocations in GIST have only recently been identified and the only clinical treatment data using inhibitors targeting these translocations exist in case report format. In that report, a patient with *ETV6-NTRK3*-translocated GIST had a robust clinical response to treatment with LOXO-101 (Loxo Oncology, Stamford, CT, USA), the only selective TRK inhibitor in clinical development, in a Phase I trial (NCT 02122913) [65]. There are no reported data for treatment of *FGFR1*-translocated GIST, but we would predict that such GIST would be inhibited by an FGFR1 inhibitor. When feasible, patients with RTK-translocated GIST should be referred to clinical trials testing appropriate inhibitors of these activated kinases.

Finally, we would note that currently 1% of patients will be classified as having a 'quintuple-WT' GIST. As new molecular classes of GIST are described in the future, tumors from patients with metastatic disease should be retested to see if this would change therapeutic decision-making. This recommendation also applies to patients previously classified as *KIT/PDGFRA* WT GIST based on limited genotyping for only *KIT* or *PDGFRA* mutations.

4.2. Adjuvant therapy following primary GIST resection

Approximately 99% of GISTs can now be categorized based on molecular diagnostics, informing therapeutic decisions in both the adjuvant and advanced disease settings. For example, biomarker analyses of patients treated with adjuvant imatinib after complete surgical resection of primary disease have indicated that patients with primary *KIT* exon 11 mutations, especially deletion mutations are the only proven subgroup to benefit from adjuvant imatinib. Notably, patients with GISTs harboring *KIT* exon 9 mutations, PDGFRA D842V mutations, or those lacking mutations in either *KIT* or *PDGFRA* have no discernible benefit with adjuvant imatinib therapy [79,80]. Based on these results, and extrapolating from the clinical outcomes of patients with advanced GIST treated with imatinib, we recommend that physicians who are considering a recommendation of adjuvant therapy for resected primary GIST should first determine the genotype of the patient's tumor. Patients with moderate to high-risk *KIT* exon 11-mutant GIST should be considered for treatment with 3 years of standard dose imatinib. In contrast, patients with PDGFRA D842V-mutant GIST or whose tumor lacks any *KIT/PDGFRA* mutations should not be treated with adjuvant imatinib. In addition, *KIT* exon 9-mutant GIST patients have not been proven to benefit from the lower standard dose (400 mg daily) of imatinib therapy in the adjuvant setting. It is unknown patients with *KIT* exon 9-mutant GIST would benefit from high-dose imatinib in the adjuvant setting. Finally, we also recommend that patients with high-risk GIST with imatinib-sensitive *PDGFRA* mutations (e.g. those other than D842V) should also be offered at least 3 years of adjuvant imatinib [33].

4.3. Primary resistance to front-line therapy

The treatment of metastatic GIST is limited by the eventual emergence of resistance to one or more TKIs. Resistance to front-line treatment with imatinib can be divided into two categories: primary and secondary. Approximately 10% of patients with GIST have primary resistance, defined as progression within the first 6 months of treatment. With proper molecular subtyping, this resistance is typically foreseeable and therapy can be adjusted in some cases. As discussed earlier, clinical responses to imatinib correlates with the primary tumor genotype, with the probability of primary resistance to imatinib for *KIT* exon 11, *KIT* exon 9, and RTK-WT GISTs being 5%, 16%, and 23%, respectively [67,68,71,81].

Primary resistance is seen at high frequency in *PDGFRA*-mutant GISTs. *In vitro*, the most common *PDGFRA* mutation in GIST, D842V, is strongly resistant to imatinib [73]. This finding is mirrored by clinical results with patients with PDGFRA D842V-mutant GIST having low-response rates and very short progression-free and overall survival during imatinib treatment.

As discussed earlier, RTK-WT GISTs have mutations downstream of KIT or affecting entirely different pathways (e.g. SDH) [38,60,61]. Hence, these GISTs have much lower response rates to imatinib, but may respond to alternative agents, such as KIT/VEGFR inhibitors for treatment of pediatric/*SDH*-mutant GIST, and BRAF/MEK inhibitors for *BRAF/RAS*-mutant GIST [82]. Some patients with RTK-WT GIST have prolonged disease-free and overall survival during front-line imatinib treatment. Whether this situation is due to their underlying indolent biology or by a subgroup of tumors with partial KIT dependency remains unclear [68].

4.4. Secondary resistance to TKI therapy

After an initial benefit from imatinib, most patients eventually experience disease progression caused by secondary resistance. It is now established that acquired mutations in *KIT* or *PDGFRA* account for the vast majority of cases of secondary resistance in RTK-mutant GIST, and that these mutations occur almost exclusively in the same allele as the primary oncogenic driver mutation [23].

In a Phase II imatinib study for advanced GIST, 67% of the patients whose tumor showed imatinib resistance had a secondary or acquired mutation in *KIT*. These mutations were

common among tumors with a primary exon 11 mutation, but were not observed in RTK-WT GISTs [20]. Indeed, secondary mutations of *KIT* have never been reported in RTK-WT GIST. Unlike primary mutations that activate KIT, which are predominantly found in the exon 9 or 11, the secondary mutations associated with TKI resistance are typically concentrated in either the ATP-binding pocket (encoded by exons 13 and 14) or the kinase activation loop (encoded by exons 17 and 18) [20]. Drug resistance has also been observed in *PDGFRA*-mutant GISTs, most commonly by acquiring a D842V mutation (activation loop) [20,83]. However, there have been no reliable reports of a secondary *KIT* mutation arising in a GIST with a primary *PDGFRA* mutation, or vice versa, during treatment with imatinib.

Additional studies using more sensitive assays have identified secondary mutations in more than 80% of drug-resistant GIST lesions [23]. There can be significant heterogeneity of resistance across different metastatic lesions in a patient, and even within different areas of the same lesion [23]. For example, there are reports of up to five different drug resistance mutations in different portions of an individual lesion and up to seven different secondary resistance mutations across multiple tumors in the same patient [84]. This heterogeneity of resistance significantly affects the efficacy of salvage TKI therapy after front-line imatinib, because the diversity of resistant, minority clones precludes the systemic eradication of GIST cells by any particular TKI. Given the problems of tumor heterogeneity and the limited predictive value of lesion genotyping to predict response to changing medical therapy, biopsy of progressive lesions solely to assay for secondary resistance mutations and thereby select subsequent TKI therapy is not recommended. In the future, the use of liquid biopsy techniques to characterize secondary resistance mutations in circulating tumor DNA (ctDNA) may be clinically useful (see Section 6).

5. Expert commentary

As discussed earlier, all GISTs with a significant risk of recurrence should be molecularly tested. It is well established that tumor genotyping plays an important role in defining the prognosis

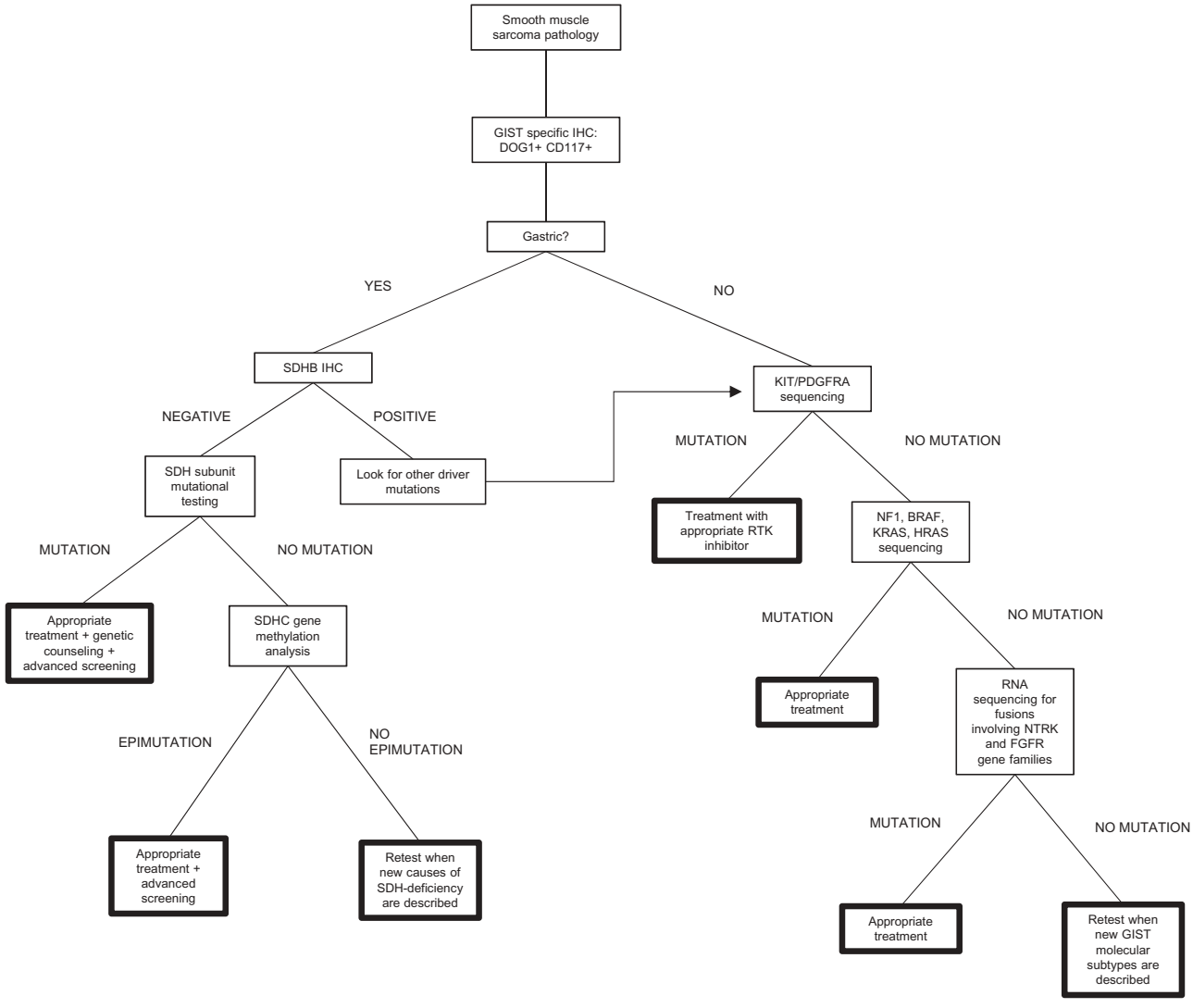

Figure 2. Decision tree for diagnosis and treatment of GIST.
The boxes show the decision nodes and recommended course of action for optimized diagnosis and treatment of GIST based on molecular classification. The end point boxes are indicated by the presence of a thick border.

and treatment of patients with GIST. Nevertheless, molecular diagnostic practices are currently underutilized in the management of GIST patients [2,85]. Because of this, GIST patients may receive less than optimal treatment and inadequate genetic counseling. Figure 2 presents a molecular diagnostic decision tree to help in guide the molecular workup of newly diagnosed GIST. Since SDHB-deficient GISTs are limited to the stomach, we recommend that SDHB IHC should be performed on all gastric GISTs, as it prevents unnecessary sequencing for *KIT* and *PDGFRA* mutations. SDHB-deficient GISTs should be submitted for *SDHx* sequencing so that genetic counseling and follow-up screening can be offered to these patients. If treatment with imatinib is under consideration, then SDHB IHC-positive gastric and all non-gastric tumors should be sequenced for *KIT* and *PDGFRA* mutations. The remaining cases (less than 15%) are candidates for additional testing for mutations in *NF1*, *BRAF*, and the *RAS* genes. Many labs now offer next-generation sequencing panels that cover all the genes relevant to GIST [65,86]. However, screening for fusions involving the *NTRK* and *FGFR* gene families is only currently available from a few specialty labs. Following molecular classification, patients should be treated in the adjuvant or metastatic setting as discussed above for specific molecular subtypes of GIST.

6. Five-year view

As technology advances over the next 5 years, our ability to diagnosis and treat molecular subtypes of resistant GIST will also improve. Mutations that confer resistance to clinically approved TKIs used to treat GIST have emerged as the major factor limiting the survival of patients with metastatic GIST. Currently, an invasive biopsy is needed in order to identify the primary oncogenic mutation and usually is not repeated after a patient relapses solely to identify an acquired resistance mutation. Instead, each patient is treated with TKIs in the same sequence (imatinib followed by sunitinib followed by regorafenib), in accordance with standard professional and health authority guidelines. However, these currently approved inhibitors have serious potency issues against some or all activation loop mutations that are known to be associated with imatinib-resistant GIST. Thus, tumors with secondary activation loop mutations tend to become the dominant clinical problem in patients with resistance to one or more TKIs. Currently, a number of novel inhibitors with activity against KIT activation loop mutations are in Phase 1 clinical studies (NCT02508532, NCT02571036). Assuming that these or other inhibitors prove to be safe and effective for treatment of TKI-resistant GIST, one could envision a clinical scenario where the choice of therapy for a given patient might be informed by having information on which particular resistance mutations exist among different tumors in a single patient.

Liquid biopsy, a technique to identify tumor mutations in ctDNA, could allow a global assessment of the various types of secondary mutations in a given patient with multifocal TKI-resistant GIST. This diagnostic approach has been validated in several types of solid tumors [87–91]. In the case of GIST, *KIT* and *PDGFRA* mutations can be detected from plasma/blood samples of GIST patients [92] including secondary *KIT* mutations in patients undergoing imatinib therapy [93]. There is an ongoing clinical trial to determine if there is an association between changes in ctDNA with GIST disease progression, as measured by conventional methods (NCT02443948). Clinical decision-making based on this technology has not yet been validated. Ultimately, some form of clinical study comparing standard treatment versus ctDNA-guided treatment will be needed to prove that genotype-guided therapy is superior to current treatment guidelines. In addition to guiding management of advanced disease, this technology could also be used to monitor for recurrence after curative intent surgical resection. Monitoring for the primary *KIT* mutation associated with a resected tumor could be used to supplement or replace conventional imaging, assuming that ctDNA detection of recurrence has a sensitivity that is similar or superior to imaging studies.

Key issues

- IHC can distinguish GIST from other tumor types with similar histology but different clinical behavior. Molecular classification based on mutation testing is crucial for the optimal treatment of GIST.
- Some GIST, particularly SDH-deficient tumors, can be caused by a germ line mutation. Identification of individuals with an inherited susceptibility to GIST allows for appropriate genetic counseling, screening of other family member, and surveillance strategies for early detection of other tumors that can independently arise later in life.
- Known oncogenic drivers in GIST include mutations in *KIT, PDGFRA, SDHA/B/C/D, BRAF, RAS, NF1*, and translocations involving RTKs other than KIT/PDGFRA (e.g. NTRK3)
- Oncogenic driver mutations confer unique clinical features requiring different treatment strategies
- The majority of GISTs have mutations in *KIT* or *PDGFRA* and can be successfully treated with tyrosine kinase inhibitors
- In the future, precision medicine treatment of GISTs with molecular abnormalities other than *KIT/PDGFRA* mutations may become clinically available.
- ctDNA offers a potential strategy for detecting and characterizing secondary mutations in patients treated with TKIs. Future clinical studies are required to define the sensitivity and clinical utility of this testing in the management of patients with GIST tested

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Declaration of interest

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