Undetected KIT and PDGFRA mutations: an under-recognised cause of gastrointestinal stromal tumours (GISTs) incorrectly classified as wild-type

Undetected KIT and PDGFRA mutations – an under recognized cause of GISTs incorrectly classified as wild type

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Running Title: Undetected KIT and PDGFRA mutations in GIST

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Sir,

Approximately 10%–15% of gastrointestinal stromal tumors (GIST) in adults and the vast majority of pediatric GISTs lack mutations in KIT or platelet-derived growth factor receptor alpha (PDGFRA).[1] This subgroup is often referred to as KIT/PDGFRA wild-type (WT) GIST, but recent advances in molecular biology have provided further insights into this group, demonstrating that it is heterogeneous and composed of several distinct entities with multiple unique molecular alterations.[2] Approximately 15% of these cases harbor activating mutations in BRAF, or, more rarely, RAS.[2] In addition, KIT/PDGFRA WT GIST can arise in the context of syndromic neurofibromatosis type I (NF1) disease, associated with loss of function of the NF1 protein due to genomic inactivation of both NF1 alleles.[2] Collectively, GISTs with oncogenic mutations in BRAF/RAS or NF1 can be referred to as RAS-pathway (RAS-P) mutant GISTs. Between 20% and 40% of KIT/PDGFRA WT GIST are driven by loss of function of the succinate dehydrogenase complex (SDH) and identified in clinical practice by the loss of subunit B (SDHB) protein expression as determined by immunohistochemistry. These tumors are designated as SDH-deficient GIST.[3,4] In recent years, oncogenic fusions involving neurotrophic tyrosine receptor kinase (NTRK) and FGFR1 have been reported in KIT/PDGFRA WT GIST.[2]

The lack of mutations in KIT and PDGFRA confer resistance to imatinib and other second- and third-generation KIT and PDGFRA inhibitors (TKI), which still represent the most successful therapies for GIST. Thus, identifying KIT and PDGFRA mutant GIST is essential for patients, avoiding the exclusion of imatinib treatment in both adjuvant and metastatic settings. The aim of this report is to review the current knowledge on undetected KIT and PDGFRA mutations in GIST patients incorrectly classified as wild-type and to describe in detail two novel wild-type GIST cases that turned out to harbor KIT mutations by next-generation sequencing (NGS) analysis.

Recent evidence of missed KIT mutations in GIST highlights the need to improve protocols for sequencing of KIT and PDGFRA as well as the current algorithm of patient referral.[5-8] In a series of 26 sporadic KIT/PDGFRA/SDH/RAS-pathway WT GISTs profiled by a targeted NGS approach, five patients were found to carry pathogenic alterations in the KIT gene.[5] The molecular findings were validated by alternative amplicon-

based targeted sequencing, immunohistochemistry, gene expression profiling and Sanger sequencing. The mutations included a large deletion of 32 nucleotides (c. 1648-7_1672del) overlapping the intron-exon boundary upstream of exon 11 in one case, while the others were low-allele-fraction KIT mutations, with a detected altered allele frequency of 12–16% (Table 1).

A large series of 162 GIST patients were profiled by three different targeted NGS assays with their associated bioinformatics pipelines (Agilent GIST MASTR, Illumina TruSight 26 and an in-house developed cancer gene panel). Within the 17 KIT/PDGFRA wild-type cases, 5 KIT mutations were found (Table 1), including 3 cases harboring an intron 10-exon 11 deletion and one case carrying a large (>24bp) exon 11 in/del.[6] At least two other studies highlighted the potential of the targeted NGS approach in identifying unrecognized KIT and PDGFRA mutations in GIST (Table 1).[7,8]

The two novel cases reported here are described in Table 2 and show a KIT exon 11 c.1648-3_1670delins GTTTCT mutation in one case and a KIT exon 11 c.1648-3_1673del in the other. The cases harbored a large deletion at the splice acceptor site, overlapping the intron-exon boundary upstream of exon 11. In both cases the deletion removes the 3' end of the intron and is predicted with more than 80% confidence to introduce a new splice site at position c.1671 and c.1674 respectively, thus retaining protein reading frame. Both cases were detected with low mutant allele frequency (6.8% and 9.4%, respectively), likely reflecting the reduced efficiency in amplification or capture of a largely deleted region and in the alignment to the reference genome. This mutation is challenging to detect in routine molecular diagnostics testing because of its large size and because it removes nucleotides from the flanking intronic region where sequencing primers are typically located.

These findings demonstrate that mutations in KIT and PDGFRA can be missed by routine analyses in a small subset of cases. Thus, a significant fraction of patients actually referred to as having a KIT/PDGFRA WT GIST could be a consequence of an undetected KIT-mutation due to limitations associated with standard molecular diagnostic techniques. These findings highlight the need for an extensive diagnostic workup for patients with KIT/PDGFRA WT GIST so that those with undetected KIT/PDGFRA mutations can receive appropriate tyrosine

kinase inhibitor therapy. This is important mainly in the adjuvant setting since clinical guidelines do not recommend adjuvant imatinib in KIT/PDGFRA WT GISTs. Moreover, KIT exon 11 homozygous mutations or intron 10/exon 11 junction deletions in GIST have a high recurrence rate and very poor prognosis after surgery; thus, these data have prognostic and therapeutic implications.[9]

There is a need to refer patients with KIT/PDGFRA WT GIST to centers specialized in GIST and where the implementation of appropriate NGS panels and bioinformatics pipelines can be used to find unusual KIT mutations. This recommendation has been included in many clinical guidelines, but it has become increasingly important that it is implemented in the clinical practice. A comprehensive targeted NGS approach should be considered in the molecular diagnostic workup of GISTs since it is useful for capturing complex KIT and PDGFRA mutations and those at low allele frequency that are routinely overlooked by conventional Sanger sequencing. A recent genomic study of more than 5,000 tumor samples showed that mutations with a low-allele-fraction in cancer samples are surprisingly frequent. Hotspot mutations in actionable genes in cancers, such as EGFR, KRAS, PIK3CA, and BRAF were detected with an allele fraction below 10% in approximately 20% of clinical samples.[10] The cases with low mutant allele fraction were responsive to TKI therapy at the same level as cases with high allele fraction mutations, offering direct proof that low-frequency mutations are biologically meaningful and clinically actionable. Moreover, the NGS approach allows identification of other missed genetic alterations in GISTs. Patients harboring NF1, BRAF, and SDHA mutations and one case with in-frame TRIM4-BRAF gene fusion have been reported in the above-mentioned studies.[5,7]

In the last few decades, targeted NGS assays have become widely available in diagnostic laboratories, and their accuracy and feasibility are improving, which has allowed their incorporation into GIST molecular diagnostics.[11] NGS assays can be difficult to interpret due to the complexity of data generated with many variants of unknown significance. Over the years, tumor-molecular-bioinformatics boards have become an integral component of cancer care in referral centers.[12] In this board, the multidisciplinary team discusses the interpretation of the assay results within the clinical context of each patient so that an optimal treatment plan can be formed.

Obviously, extending NGS testing of KIT/PDGFRA WT GIST in a referral center could be time- and cost-consuming and may therefore delay the beginning of therapy and add expensive costs to the management of patients. Anyway, the alternative to start the treatment with imatinib to all KIT/PDGFRA WT patients is equally expensive, especially if continued for months before evaluating the effectiveness, and in addition potentially toxic for the patients.

In conclusion, there should now be increased awareness of missed KIT and PDGFRA mutations in GISTs in that some events are difficult to be found and they are source of missed opportunity for tyrosine kinase inhibitor therapy. Pathologists, molecular biologists, oncologists, and surgeons treating patients with KIT/PDGFRA WT GIST should refer these patients to centers with extensive experience and access to the latest NGS-based tools in molecular diagnostics in GISTs. So, the diagnosis of KIT/PDGFRA/RAS-P/SDH wild type GIST should only be made after the possibility of missed mutations have been excluded by advanced (and often repeated) molecular testing. Twenty years after the advent of imatinib for targeting KIT and PDGFRA mutant GIST, a correct diagnosis of GIST subtypes is now more critical than ever for appropriate therapy.

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Table 1: List of previously published GIST cases reported as KIT/PDGFRA WT by routine molecular diagnostics and then identified as carrying pathogenic mutation by advanced NGS sequencing. Mutation type and mutant allele frequency are reported, when available.

	Mutation (exon, cDNA, protein)	**Type of mutation**	**Mutant allele frequency**
Astolfi et al.[5]	KIT Exon 11, c.T1657C, p.W557R	Missense	14%
	KIT Exon 9, c.1502_1503insTGCCTA, p.S501delinsSAY	In-frame Insertion	12%
	KIT Exon 11, c.1723_1724insAACTTCCTTATG, p.Q575delinsQLPYE	In-frame Insertion	16%
	KIT Exon 11, c.1726insC;1726_1764dup, p.L576_R588dup	In-frame Insertion	12%
	KIT Exon 11, c. 1648-7_1672del, p.550_558del	Intron10 - exon11	49%
Vanden Bempt et al.[6]	KIT Exon 11, c.1648-3_1673del (two cases)	Intron10 - exon11	-
	KIT Exon 11, c.1648-1_1672delinsC	Intron10 - exon11	-
	KIT Exon 11, c.1648_1674del, p.L550_L558del	In-frame deletion	-
	KIT Exon 11, c.1724_1774dup, p.F591_G592ins17	In-frame Insertion	-
Gao et al.[7]	KIT Exon 11, p.L576P (six cases)	Missense	11% - 20%
	KIT Exon 11, p.557_558del (three cases)	In-frame deletion	11% - 17%
	KIT Exon 11, p.W557R (two cases)	Missense	23% - 24%
	KIT Exon 11, p.W557G	Missense	18.4%
	KIT Exon 11, p.579del	In-frame deletion	14.4%
	KIT Exon 11, p.V559D	Missense	23.5%
	KIT Exon 17, p.N822K (four cases)	Missense	10% - 23%
	KIT Exon 17, p.A814S	Missense	10%
	PDGFRA Exon 12, R585K	Missense	23%
	PDGFRA Exon 18, D842V	Missense	18 – 19%
Wu et al.[8]	KIT Exon 17, c.2466T>A (two cases)	Missense	31% - 40%
	KIT Exon 21, c.2828G>T	Missense	4.3%

Table 2: Two novel cases describing a clinical history of KIT/PDGFRA WT GIST patients with KIT mutations undetected by routine molecular diagnostics techniques.

	Case 1	Case 2
Gender	Female	Female
Age	57 years	32 years
Diagnosis	- Diagnosis of a high risk 10 cm gastric GIST, mitotic rate: 15/50 HPF - Mutation analysis: KIT/PDGFRA wild-type	- Diagnosis of pelvic "fibroid" lesion considered "not suspicious" during the first trimester of pregnancy - During c-section, surgeons found a 15x11x7.5 cm tumor attached to the small intestine, with multiple metastases on peritoneum (5.7 x 5.5 cm and 3.5 cm) - Diagnosis of small intestine GIST, mitotic rate: 10/ 50 HPF
Therapy	- Neoadjuvant imatinib 400 mg/die, with apparent response - Surgery	- Imatinib 400mg - Mutation analysis: KIT/PDGFRA wild-type (07/2018) - The treatment was stopped
KIT analysis by NGS	NGS testing on the surgical tumor tissue found KIT exon 11 c.1648-3_1670delins GTTTCT mutation	NGS testing found KIT exon 11 C.1648-3_1673del mutation
Follow up	Patient continues imatinib 400mg	- Re-started imatinib 400mg (09/2018) - Significant shrinkage was observed (01/2019) - No tumors were observed (06/2019)
Notes	*The patient is the treating oncologists first/only GIST patient. Insurance hurdles and COVID have so far prevented consult with a GIST expert.*	