Original Article

High proportion of wild-type gastrointestinal stromal tumor in a cohort of Chilean patients screened by KIT and PDGFRA exome profiling

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Background: About 80–90% of gastrointestinal stromal tumor (GIST) patients harbor KIT proto-oncogene (*KIT*) and/or platelet-derived growth factor receptor alpha (*PDGFRA*) gain-of-function mutations. The *KIT* gene also encodes a tyrosine kinase receptor; therefore, *KIT/PDGFRA* alterations not only serve as hallmarks, but also as potential therapeutic targets. Previous reports have demonstrated that differences in the *KIT/PDGFRA* mutation rates are generally attributed to ethnic and/or technical factors. Herein, we report a molecular profiling of *KIT/PDGFRA* in a Latin American cohort of GIST patients.

Methods: In this observational study, DNA samples were obtained from paraffin blocks in 42 GIST patients. We performed *KIT/PDGFRA* molecular profiling by Sanger sequencing. Patients' clinical characteristics were obtained from their medical records. A single case was further analyzed with next-generation sequencing (NGS).

Results: Patients were predominantly females (n=22; 52.4%). Median age at diagnosis was 53 years old. As expected, the stomach was the most frequent primary location (47.6%), and 38.1% of cases were metastatic. We detected *KIT* and *PDGFRA* alterations in 64.3% and 4.8% of patients, respectively. Within this subset (n=29), 82.8% had exon-11 *KIT* mutations, and 6.9% had exon 18 *PDGFRA* mutations. As predicted, *KIT* and *PDGFRA* mutations were mutually exclusive, and 31% (n=13) were wild-type *KIT/PDGFRA*. These results could be attributed to ethnic and methodological differences. Therefore, we presented a case of a metastatic patient analyzed by NGS to illustrate the clinical utility of an alternative screening strategy to Sanger sequencing.

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Conclusions: There were a high proportion of wild-type GISTs in this cohort. This could be attributed to technical/methodological and/or ethnic/genetic differences. Our findings also encourage the use of alternative techniques, such as NGS for *KIT/PDGFRA* screenings, particularly in the case of advanced-stage patients.

Keywords: KIT; PDGFRA; gastrointestinal stromal tumor (GIST); tyrosine kinase inhibitor (TKI); molecular profiling

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Introduction

Although gastrointestinal stromal tumors (GIST) are rare, they are the most frequent mesenchymal malignancies of the GI tract with an estimated incidence of 1.5 per 100,000/year (1). Studies suggest these tumors may arise or differentiate from interstitial Cajal cells or their precursor stem cells (2). The most frequent primary site is the stomach (45–59%), followed by the small intestine (30–45%) (1,3,4). In terms of survival, GIST patients usually display prolonged overall survival (OS) rates ranging from 8.9 to 13 years. Similar to many cancer types, the incidence and prevalence of GIST is geographically heterogeneous. Recently, we reported that the proportion of metastatic patients displayed significant differences when comparing Chilean and Mexican GIST registries (5).

Several reports have indicated that KIT proto-oncogene (*KIT*) and platelet-derived growth factor alpha (*PDGFRA*) mutations are very common in GISTs, affecting 80–90% of patients (6). Indeed, given its high mutation rate, *KIT* serves both as a hallmark for GISTs and as an actionable target. Imatinib is a tyrosine kinase inhibitor (TKI) originally designed to target the BCR-ABL fusion kinase in the late 1990s; however, subsequent studies revealed that imatinib also can also target *KIT* and *PDGFRA*. Consequently, in 2002, imatinib mesylate became the standard of care for advanced stage GISTs (7,8). Studies have confirmed the efficacy of imatinib, demonstrating up to 13% of disease control in metastatic or unresectable patients even after 10 years (9). Previous reports have also demonstrated that *KIT* and *PDGFRA* mutant patients experience the most benefits from imatinib treatment (10,11). Despite this, a proportion of patients are refractory to imatinib treatment including exon 9 *KIT* mutants and *KIT/PDGFRA* wild-types (10,12). Moreover, a subset of *KIT*-mutants are characterized by good initial responses to imatinib, but later these patients become refractory (12). The therapeutic alternative for these patients is sunitinib malate, a broad-spectrum TKI that also exhibits anti-angiogenic properties (13). Interestingly, a study demonstrates that some of these patients harbor pathogenic *KIT* mutations and are therefore eligible for *KIT*-specific therapies (14).

In addition to first-line imatinib and sunitinib, other TKIs, such as larotrectinib and entrectinib, are recommended for GIST patients that display *NTRK* fusions (15). Others, such as avapritinib, are specifically designed for *PDGFRA* mutations, such as D842V on exon 18 or ripretinib for *KIT/PDGFRA* mutations that affect exons 9, 11, 13, 14, 17 or 18, including D842V (3,16,17). Therefore, the identification of primary/secondary *KIT/PDGFRA* mutations provides key therapeutic information for these treatments, and could identify alternative approaches in anticipation of the development of drug resistance in GIST patients (18-20).

Here, we report the mutational profiling of *KIT/PDGFRA* in a group of 42 Chilean GIST patients, describing their main characteristics and specific alterations. Expectedly, our analysis found that most patients benefited from first-line imatinib or sunitinib treatment. We hypothesized that complementary analyses that encompassed all *KIT* and *PDGFRA* genes could expand this benefit to a particular subset of patients categorized as wild-type (WT) due to the absence of alterations in specific *KIT/PDGFRA* exons. As a proof of principle, we present the case of an advanced stage GIST patient initially classified as WT and analyzed with next-generation sequencing (NGS). In view of our results that demonstrate the clinical utility of precision medicine tools (such as NGS), we support the use of NGS for metastatic or recurrent advanced stage GISTs. We present the following article in accordance with the STROBE reporting checklist (available at https://gist.amegroups.com/

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Methods

Patients

In this observational study, we included the *KIT/PDGFRA* mutational status reports of 42 GIST patients of the Centro de Cancer UC-CHRISTUS of the Pontificia Universidad Catolica de Chile (PUC), who had been diagnosed with GIST from February 2002 to January 2019. These patients underwent *KIT/PDGFRA* mutational profiling during their diagnosis or treatment, with molecular testing results from November 2015 to February 2019. All mutational status reports were considered for the study size. For this study, patients were registered between May 2017 and December 2019 in a patient registry led by "Fundacion GIST Chile", a patient advocacy group dedicated to accompanying and educating GIST patients, developing public policies to include GISTs and their treatments to the Chilean public health system, and contributing to research efforts studying this entity. Patients were included if their data was available from clinical records, the had a confirmed histopathologic diagnosis of GIST, and they were ≥18 years old. Patients with missing or incomplete clinical records, including follow-up data, or who were unable to read and write an informed consent form were excluded from this study.

Recorded variables of interest included *KIT/PDGFRA* alteration per exon, patient gender, age at diagnosis, disease stage at diagnosis, primary tumor location, mitotic index, tumor size, and the modified National Institutes of Health (NIH) risk of recurrence. These data were obtained from medical records and available reports. Quantitative data were grouped and handled by intervals.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Internal Review Board and the Ethics and Scientific Committee of the Pontificia Universidad Católica de Chile (approval #16-046) and individual consent for this retrospective analysis was waived.

Molecular analysis

Formalin-fixed paraffin-embedded (FFPE) tissue samples from primary or secondary GISTs were obtained from all 42 participants. Hematoxylin-eosin staining and light microscopy inspection were used to recognize areas abundant with tumor cells. These areas were dissected from the FFPE blocks for the enrichment of cellular subpopulations. Later, DNA was extracted with the QiAMP DNA Micro Kit (Qiagen, Hilden, Germany). The *KIT/PDGFRA* mutational status assessment was performed on the ABI PRISM 3500XL Genetic Analyzer sequencer (Thermo Fisher Scientific, Waltham, MA, USA). The procedure involved DNA amplification by polymerase chain reaction (PCR), followed by bidirectional sequencing of *KIT* gene exons 9, 11, 13, and 17 and *PDGFRA* gene exons 12, 14, and 18. Comprehensive NGS sequencing was performed using a panel of 688 cancer-related genes (Sentis Cancer + Discovery; BGI, Shenzhen, China).

Results

Patient characteristics

A total of 42 patients enrolled between 2017 and 2019 were included to this study. The median age at diagnosis was 53 years old, and patients were predominantly female (n=22; 52.4%). As expected, the stomach was the most frequent primary tumor location (n=20; 47.6%) followed by the small intestine (n=16; 38.1%) and 16 patients (38.1%) had metastatic disease at diagnosis. Regarding the risk of recurrence, 73% (n=16) of patients with resected localized disease were classified as high risk according to the modified NIH risk stratification criteria. Clinical data, including mitotic index and tumor size are summarized in *Table 1*.

Molecular profile in KIT and PDGFRA genes

As expected, *KIT* and *PDGFRA* mutations were mutually exclusive (*Table 2*); 64.3% and 4.8% harbored *KIT* and *PDGFRA* alterations, respectively. In the case of *KIT*, the mutations were mainly deletions located at exon 11 (n=24; 57.1%) and exon 9 (n=3; 7.1%). No alterations were found at exons 13 or 17 (*Table 2*). For *PDGFRA*, most alterations were synonymous mutations (P567P and V824V) on exons 12 and 18, and only 2 cases harbored missense mutations (D842V) at exon 18 (*Table 2*). When we analyzed the subset of *KIT/PDGFRA* mutants (n=29), 82.8% and 10.3% harbored exon 11 and exon 9 *KIT* mutations, respectively; 6.9% displayed exon 18 *PDGFRA* mutations. Interestingly, a high percentage of patients (n=13; 31.0%) had no alterations on either *KIT* or *PDGFRA* and therefore, they could be categorized as WT GISTs. *Figure 1* summarizes our findings and their clinical implications.

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Table 1 Demographic and histopathologic characteristics of study population (n=42)

Characteristics	Study population, n (%)
Gender	
Male	20 (47.6)
Female	22 (52.4)
Age at diagnosis, years	
18–40	6 (14.3)
41–60	23 (54.8)
>60	13 (31.0)
Median (IQR)	53 (16.8)
Stage at diagnosis	
Localized	26 (61.9)
Metastatic	16 (38.1)
Primary tumor location	
Stomach	20 (47.6)
Small intestine	16 (38.1)
Rectum	5 (11.9)
EGIST	1 (2.4)
Mitotic index	
≤5/50 HPF	16 (38.1)
>5/50 HPF	18 (42.9)
NA	8 (19.0)
Tumor size*, cm	
2.0–4.9	7 (26.9)
5.0–10.0	12 (46.2)
>10.0	4 (15.4)
NA	3 (11.5)
Modified NIH risk of recurrence**	
Low	0 (0.0)
Intermediate	6 (27.3)
High	16 (72.7)

*, tumor size assessed in localized, surgically resected GISTs (n=26); **, modified NIH risk of recurrence assessed in localized, surgically resected GISTs with available data only (n=22). IQR, interquartile range; EGIST, extra gastrointestinal stromal tumor; HPF, high power field; NA, not available.

NGS in a metastatic GIST patient

Exons 9, 11, 13, 14, and 17 from *KIT* and exons 12, 14, and 18 from *PDGFRA* were analyzed by Sanger sequencing to determine the mutational status of GIST patients. Given the high proportion of WT GISTs in our cohort we decided to perform NGS in a metastatic GIST patient to search for alterations that were not detected by our initial *KIT/PDGFRA* exome profiling. This patient was initially classified as *KIT/PDGFRA* WT by the Sanger method. In general, WT patients are usually refractory to imatinib/sunitinib treatment. *Figure 2* summarizes the case. The patient was a 35-year-old pregnant female diagnosed with metastatic small bowel GIST during her cesarean delivery. Due to its aggressiveness and the early onset, a comprehensive molecular test was ordered. Our analyses with NGS found 2 clinically relevant alterations: a germline pathogenic alteration in *ATM* (K3016Sfs*43) and a *KIT* deletion (c.1648-3_1673del). The latter was a 1–14 bp deletion of intron 10 that resulted in loss of the acceptor splice site at exon 11 and produced a mutant isoform of the KIT protein and a deletion of 9 amino acids (550-KPMYEVQWK-558). Based on the reports, the patient received imatinib and has remained under this treatment for >10 months. Follow-up computed tomography (CT) scans demonstrated a sustained complete response assessed by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1.

Discussion

Recently, our research group reported clinical and pathological characteristics on >600 GIST patients from Chile and Mexico, revealing geographical differences in terms of stage at diagnosis, primary tumor location and tumor size (5). Herein, we expanded on these findings and delivered a *KIT/PDGFRA* mutational profile of Chilean GIST patients. Overall, 69% of patients in our series displayed *KIT* and/or *PDGFRA* alterations. In particular, 64.3% and 4.8% carried *KIT* and *PDGFRA* mutations, respectively (*Table 2*). In contrast, clinical trials have shown that *KIT* and *PDGFRA* mutation rates range between 80% and 85% and 5% and 10%, respectively (21,22). These differences could be attributed to the characteristics of the patients in each case; while clinical trials included

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Table 2 Genomic alterations in Chilean GIST patients with available mutational status (n=42)

No.	KIT				PDGFRA			KIT status	PDGFRA status
	Exon 9	Exon 11	Exon 13	Exon 17	Exon 12	Exon 14	Exon 18		
1	WT	DEL	WT	WT	WT	WT	WT	ALT	WT
2	WT	NSA	WT	WT	WT	WT	WT	ALT	WT
3	WT	E562_P573 DEL	WT	WT	WT	WT	V824V	ALT	WT
4	NSA	WT	WT	WT	WT	WT	WT	ALT	WT
5	WT	WT	WT	WT	WT	WT	V824V	WT	WT
6	WT	V559D	WT	WT	WT	WT	WT	ALT	WT
7	WT	K558_V560 DEL	WT	WT	WT	WT	WT	ALT	WT
8	WT	K556-558 DEL	WT	WT	WT	WT	WT	ALT	WT
9	WT	WT	WT	WT	WT	WT	WT	WT	WT
10	WT	NSA	WT	WT	WT	WT	WT	ALT	WT
11	WT	WT	WT	WT	WT	WT	V824V	WT	WT
12	WT	V559_E561 DEL	WT	WT	WT	WT	WT	ALT	WT
13	WT	WT	WT	WT	P567P	WT	WT	WT	WT
14	Y503_F503 INS	WT	WT	WT	WT	WT	V824V	ALT	WT
15	WT	WT	WT	WT	P567P	WT	WT	WT	WT
16	WT	M552_W557 DEL	WT	WT	WT	WT	WT	ALT	WT
17	WT	V560D	WT	WT	WT	WT	WT	ALT	WT
18	WT	W557 DEL	WT	WT	WT	WT	WT	ALT	WT
19	WT	W557_K558 DEL	WT	WT	P567P	WT	WT	ALT	WT
20	WT	WT	WT	WT	WT	WT	D842V	WT	ALT
21	WT	WT	WT	WT	WT	WT	WT	WT	WT
22	WT	D579 DEL	WT	WT	WT	WT	WT	ALT	WT
23	WT	W557_K558 DEL	WT	WT	WT	WT	WT	ALT	WT
24	WT	P551_Q556 DEL	WT	WT	WT	WT	WT	ALT	WT
25	WT	NSA	WT	WT	WT	WT	WT	ALT	WT
26	WT	WT	WT	WT	WT	WT	D842V	WT	ALT
27	WT	V559D	WT	WT	WT	WT	V824V	ALT	WT
28	WT	WT	WT	WT	P567P	WT	V824V	WT	WT
29	WT	WT	WT	WT	P567P	WT	WT	WT	WT
30	WT	WT	WT	WT	WT	WT	WT	WT	WT
31	WT	V559G	WT	WT	WT	WT	WT	ALT	WT
32	WT	WT	WT	WT	WT	WT	WT	WT	WT
33	WT	W557_K558 DEL	WT	WT	WT	WT	WT	ALT	WT
34	WT	K550 _K558 DEL	WT	WT	WT	WT	WT	ALT	WT

Table 2 *(continued)*

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Table 2 *(continued)*

No.	KIT				PDGFRA			KIT status	PDGFRA status
	Exon 9	Exon 11	Exon 13	Exon 17	Exon 12	Exon 14	Exon 18		
35	WT	W557R	WT	WT	WT	WT	WT	ALT	WT
36	WT	V560G	WT	WT	P567P	WT	WT	ALT	WT
37	WT	W557_K558 DEL	WT	WT	WT	WT	WT	ALT	WT
38	Y503_F504 INS	WT	WT	WT	WT	WT	WT	ALT	WT
39	WT	N564H/N566_N574 DEL	WT	WT	WT	WT	WT	ALT	WT
40	WT	WT	WT	WT	WT	WT	WT	WT	WT
41	WT	WT	WT	WT	WT	WT	WT	WT	WT
42	WT	WT	WT	WT	P567P	WT	V824V	WT	WT
Total n (%)	3 (7.1)	24 (57.1)	0	0	0	0	2 (4.8)	27 (64.3)	2 (4.8)

P567P and V824V in PDGFRA were not considered in the mutation frequency calculation as they were not classified as pathogenic according to current evidence. GIST, gastrointestinal stromal tumor; WT, wild-type; NSA, non-specified alteration; INS, insertion; DEL, deletion; ALT, alteration present.

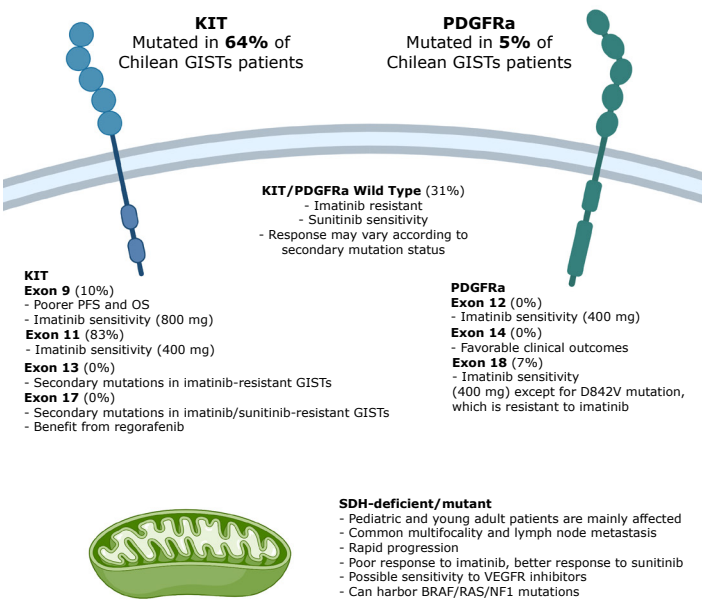

Figure 1 Clinical implications based on mutational profile in KIT and PDGFRA genes in a cohort of GIST patients. KIT, KIT proto-oncogene; PDGFRA, platelet-derived growth factor alpha; GIST, gastrointestinal stromal tumor.

mostly advanced stage patients, a high proportion of patients in our study had localized GISTs (61.9%, *Table 1*). Previous population-based studies have reported similar discrepancies. A study by Braggio *et al.* found 74.5% and 7.3% of *KIT* and *PDGFRA* mutants in a Brazilian cohort of GIST patients (23). Similarly, studies based on Greek (22), Italian (24), and Chinese (25) cohorts have reported important differences in the percentages of *KIT* and *PDGFRA* mutants. Notably, we observed lower mutation rates for both *PDGFRA* and *KIT* in our cohort, and

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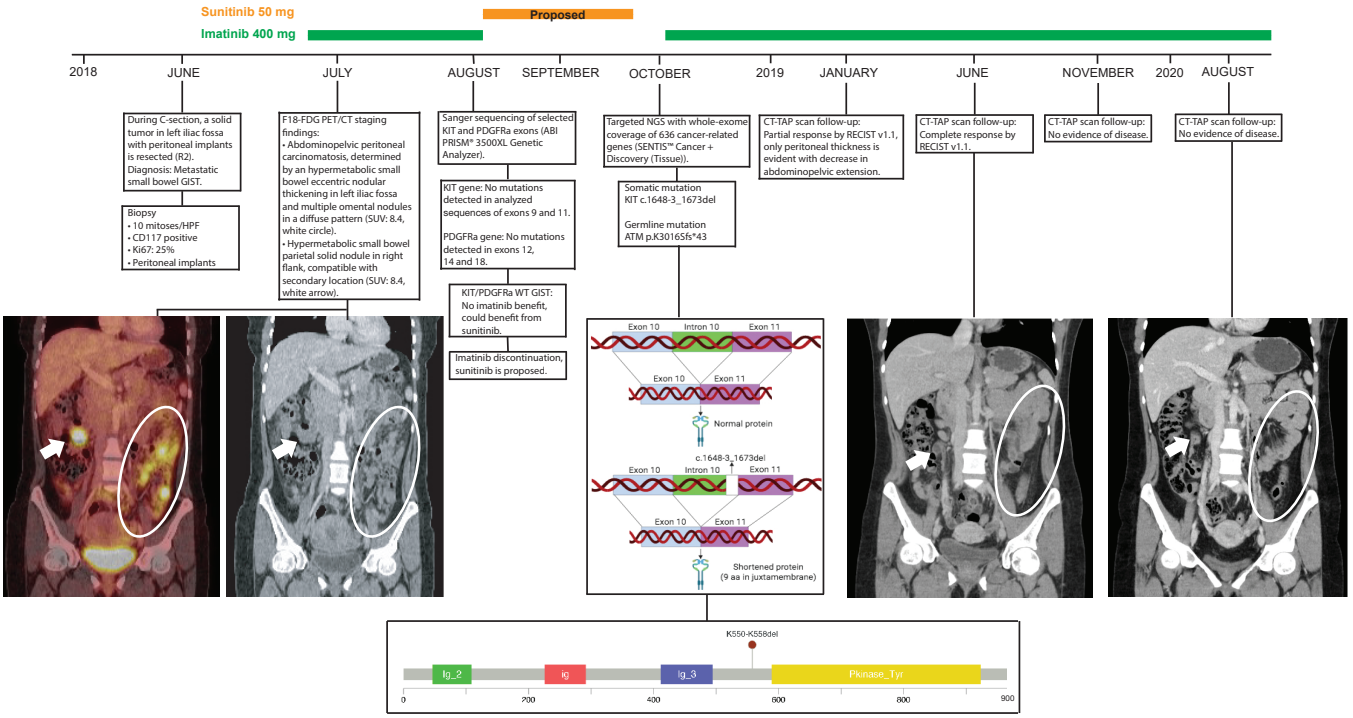

Figure 2 Timeline of diagnosis, treatment, and follow-up of a metastatic GIST patient. The respective changes in clinical practice after the results of the molecular tests are detailed in a timeline. ROIs at diagnosis and follow-up of primary and secondary GIST locations are shown with a white circle and bold white arrow, respectively. GIST, gastrointestinal stromal tumor; ROIs, regions of interest.

consequently a high proportion of wild types (31%, *Table 2*). In this regard, epidemiological studies from Northern Norway (26) and the North American Intergroup (27) found that approximately 15% of GISTs are classified as WT. This discrepancy could be due to ethnic and/or genetic variations in our study population. Genetic studies (28) have already reported the underrepresentation of certain populations which makes a fair comparison difficult. The discrepancy could also be due to differences in technical or methodological approaches. The number of alterations that could be effectively detected by the Sanger method used in our study was much lower compared to massively parallel sequencing techniques such as NGS; therefore, this methodologic bias could underestimate the true mutation rate of these genes (14). Alternately, according to the 1000-G Project, silent *PDGFRA* alterations such as P567P and V824V (rs1873778 and rs2228230) are considered single nucleotide polymorphisms (germline) with an allelic frequency of 1% and 28% in the American population, respectively (29). Within our cohort, 7 out of 42 patients harbored these variants. Unfortunately, since our analyses were performed on tumor tissues, we cannot ensure the germline status of these alterations. Despite this, the high frequency of the *PDGFRA* rs1873778 variant in our cohort warrants further investigation, particularly regarding its association with GIST risk in the Chilean population. Furthermore, silent mutations can also affect protein folding, potentially affecting their function (30,31). However, the functional impact of these highly prevalent variants is yet to be determined.

The high proportion of WT GISTs in our cohort also warrants further investigation. From a biological perspective, these cases represent a distinctive phenotype, of which evidence has demonstrated an association with TKI resistance (32). Counterintuitively, WT GISTs are not free of molecular alteration, studies have shown that a proportion of these cases are characterized by succinate dehydrogenase (SDH) deficiencies (33) or by increases in growth factor expression such as VEGF or IGFR1 (34,35). Another subset included SDH-competent cases that displayed *NF1*, *BRAF*, *NRAS*, and *PIK3CA* mutations and, less frequently, *NTRK3* or *FGFR1* fusions, all with important pharmacological implications (32,35).

In addition to their role as first-line systemic treatment

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in advanced stage or recurrent GISTs, imatinib and sunitinib can also be used as adjuvant therapy in patients with a medium/high risk of postoperative recurrence. The clinical benefit of these drugs is determined by the type and location of *KIT* and *PDGFRA* mutations, as shown in *Figure 1*: exon 11 *KIT* mutations are the most prevalent with a 70% frequency (36), and W557_K558del deletions are associated with more aggressive tumors compared to single nucleotide variation (SNV) type mutations (10,37). Nevertheless, several studies have confirmed the sensitivity to imatinib on exon 11 mutants (19,35,38). Regarding exon 9 *KIT* mutations, meta-analyses have shown poorer OS and response rates to imatinib compared to exon 11 mutants (39). However, studies demonstrate better OS, progression-free survival (PFS), and clinical response in these patients using higher imatinib doses (800 mg) (40). Functionally, the kinase domain of *KIT* is divided into 2 cluster regions: the adenosine thiphosphate (ATP)-binding pocket (spanning exons 13 and 14) and the activation loop (exons 17 and 18), and both these regions are associated with secondary mutations that confer imatinib resistance (41). It is common for *PDGFRA* mutations to be found in the exon 18 region. The most common mutation in this exon is p.D842V, a variant associated with imatinib resistance (38). In contrast, exon 12 and exon 14 *PDGFRA* mutations are typically rare, and in most cases maintain imatinib responsiveness (42).

As explained, our technical and methodological approach could have underestimated the proportion of mutant GISTs or led to misinterpretations regarding their true prevalence in our cohort. The patient presented in *Figure 2* is a case in point. This patient was originally diagnosed with metastatic GIST. Initially, the main exons of *KIT* and *PDGFRA* were genotyped using the Sanger method as a part of the routine procedure for GIST patients in our health center. Hence, these studies concluded the patient was a WT (no mutations). However, subsequent NGS analyses found an intronic *KIT* mutation close to the intron 10-exon 11 junction. This alteration has been previously reported and categorized as being sensitive to imatinib. Under treatment with imatinib the patient showed no evidence of disease for 10 months (*Figure 2*). Some important lessons can be learned from this case. First, as recommended by Corless *et al.*, laboratories that usually screen for *KIT* mutations should anticipate intron-10-exon 11-boundary mutations, these are not uncommon and may be overlooked when PCR primers are too proximal to the exon 11 using the Sanger method (43). Second, deep genomic profiling should be considered as an option especially for advanced stage patients given its potential clinical utility (44). Within this context, precision oncology might offer substantial benefits in terms of treatment, particularly on advanced stage or refractory GIST patients. Finally, our work had several limitations. First, this was a retrospective study that reported on the data of a relatively small sample of patients. Second, the access to clinical data was limited and therefore we did not include survival outcomes, such as OS or PFS, or information on the response to treatment or its association with molecular alterations.

Conclusions

We have reported an unexpectedly high proportion of WT GIST in our Chilean cohort. These results could be attributed to technical/methodological and/or ethnic/genetic differences. In view of these findings, we support the use of alternative/complementary methods, such as NGS, for the screening of *KIT* and *PDGFRA* mutations, especially on advanced stage patients. These approaches could also reveal therapeutic alternatives for hard-to-treat patients. Future studies should determine if this is a feasible, cost-effective strategy.

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Footnote

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Data Sharing Statement: Available at https://gist.amegroups.com/article/view/10.21037/gist-21-19/dss

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invited speaker in activities by Novartis, Pfizer, Bayer, BMS, MSD, GBT Biotoscana, and Lilly and has received traveling accommodation and attending support for international meetings by Novartis, MSD, and Bayer. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Internal Review Board and the Ethics and Scientific Committee of the Pontificia Universidad Católica de Chile (approval #16-046) and individual consent for this retrospective analysis was waived.

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