

Molecular alterations of *KIT* and *PDGFRA* in GISTs: evaluation of a Portuguese series

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ABSTRACT

Aim: To assess *KIT* and *PDGFRA* mutations frequencies in a Portuguese series of gastrointestinal stromal tumours (GISTs).

Methods: 78 GISTs were evaluated for CD117 expression and screened for mutations in *KIT* (exons 9, 11, 13, 14 and 17) and *PDGFRA* (exons 12, 14 and 18) genes.

Results: *KIT* activating mutations were identified in 44 (56%) of the 78 GISTs. Forty cases (91%) presented a mutation in *KIT* exon 11, and 4 (9%) in exon 9. One case showed a 4 bp deletion in intron 14. *PDGFRA* mutations were observed in 5 cases (6%): 2 (3%) in exon 12 and 3 (4%) in exon 18. Survival analysis was performed in 63 of the 78 GISTs. The presence of mutated *KIT* was significantly correlated with shorter survival of patients ($p = 0.0460$), and inversely associated with epithelioid histological type of GISTs ($p = 0.0064$).

Conclusions: Overall, the incidence of both *KIT* and *PDGFRA* mutations in these Portuguese series was 63%, being in agreement with other studies, mainly of Iberian populations. The great majority of mutations were located in *KIT* exon 11, statistically associated with worse prognosis and indicative of favourable response to imatinib-based therapy in this Portuguese series of GISTs.

Gastrointestinal stromal tumours (GISTs) although rare, are considered to be the most frequent gastrointestinal mesenchymal tumours in humans.¹ A Scandinavian study estimated the incidence of GISTs to be between 20 and 40 per million.² In Portugal, as far as we know, an epidemiological study is yet to be done.

The cellular origin of GISTs is not fully understood, but they are thought to arise from interstitial cells of Cajal or their precursors, due to their similar positive *KIT* (CD117) and CD34 staining and negative staining for both desmin and S-100 protein immunostaining.^{1–3} GISTs are rarely found outside the gastrointestinal tract, being most commonly found in the stomach (40–70%), small intestine (20–50%) and colon or rectum (5–15%).^{1,2,4} Nowadays, the diagnosis of GISTs is partially dependent on tumour cells overexpression of CD117 together with CD34.⁵ The expression of such immunohistochemistry features is useful to differentiate GISTs from other mesenchymal tumours of the gastrointestinal tract, namely leiomyomas and leiomyosarcomas, nerve sheath tumours, and other primary and metastatic tumours possibly occurring in this location.^{1,2,4}

KIT belongs to the class III receptor tyrosine kinases (RTKs), which also include platelet-derived growth factor A and B (*PDGFRA*, *PDGFRB*), colony stimulating factor-1 receptor (*CSF1R*) and

FMS-related tyrosine kinase 3 (*FLT3*).⁶ These RTKs are characterised by the presence of an extracellular domain, a transmembrane domain, a juxtamembrane domain, and an intracellular domain where the two kinase domains are lodged.⁷ RTK activation occurs when by ligand binding, the receptor dimerises and suffers conformational transformations, which induce activation of the kinase domains. These, in turn, lead to activation of important intracellular signalling pathways, such as RAS/mitogen activated protein kinase (*RAS*/*MAPK*), phosphoinositide-3 kinase (*PI3K*), and signal transducers and activators of transcription (*STAT*), which regulate many physiological functions such as cell survival, proliferation, differentiation, adhesion and apoptosis.^{7,8}

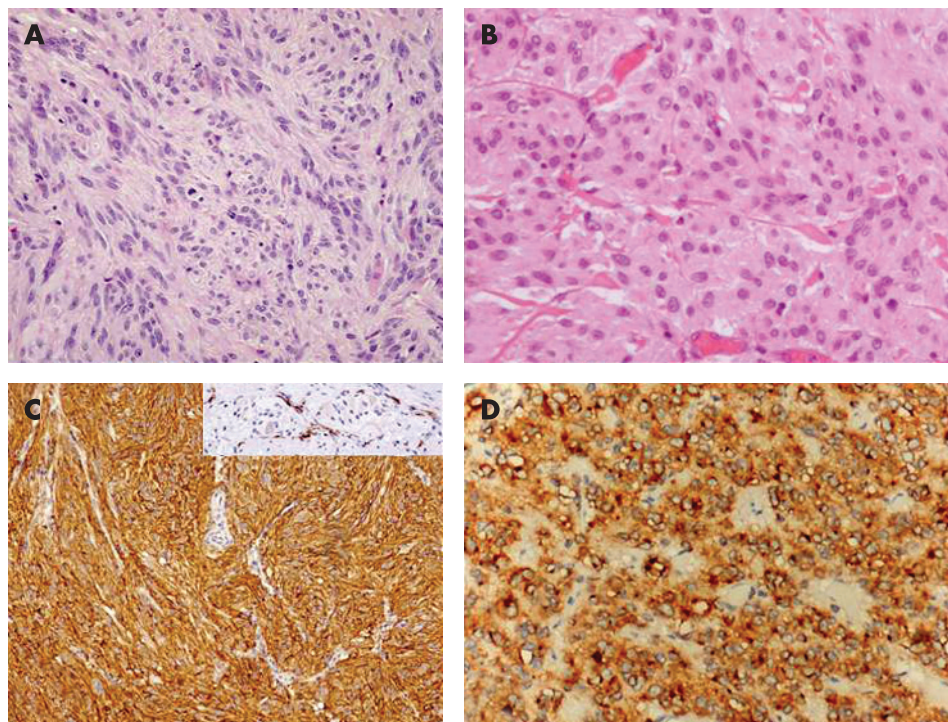
GISTs are molecularly characterised by mutations in *KIT* oncogene, located in the long arm of chromosome 4 (4q11–12).⁹ There is a broad spectrum of *KIT* mutations in GISTs, ranging from 20% to 80%, most of them being located in the juxtamembrane domain (exon 11), followed by mutations in the extracellular domain (exon 9), and seldom in the kinase (exon 13 and 17) and ATP pocket (exon 14) domains.^{10–12} Later studies reported the presence of activating mutations in the *PDGFRA* oncogene in wild-type *KIT* bearing GISTs.^{13,14} *PDGFRA* is also located at 4q11–12 and exhibits similar RTK cellular functions.^{13,14} The hotspot regions in this gene lie in the juxtamembrane (exon 12) and kinase (exons 14 and 18) domains, and have been reported in 5–12% of cases.^{12,14} The frequency of *KIT*/*PDGFRA* mutations in GISTs varies from series to series, probably reflecting epidemiological and methodological differences in the various studies on record.^{10,12}

Until recently, the treatment of GISTs was limited to surgical removal of the tumour. Unfortunately, even in patients where the tumour was completely and successfully removed, there was a high probability of recurrence.¹ The development of imatinib mesylate (*Glivec*/*Gleevec*, Novartis, Basel, Switzerland), a selective inhibitor of RTKs, has brought new hope for GIST patients. Imatinib targets *KIT* by competing with its ATP binding site, preventing further phosphorylations of downstream intracellular signalling molecules responsible for its oncogenic properties.^{15,16} Several studies have showed the importance of *KIT* and *PDGFRA* molecular status in the imatinib response.^{10,12} It has been reported that patients with tumours harbouring exon 11 *KIT* mutations are more likely to respond to imatinib therapy than those with either exon 9 *KIT* mutations or undetectable mutations.^{10,12}



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Figure 1 Morphological and immunohistochemical features of gastrointestinal stromal tumours (GISTs). (A) spindle cell and (B) epithelioid tumour cells with (C) membranar/cytoplasmic and (D) cytoplasmic/paranuclear dot immunoreactivity for CD117. Note immunoreactivity of interstitial cells of Cajal (C, inset). H&E and ABC immunohistochemistry (200×).



In Portugal, the incidence of both *KIT* and *PDGFRA* mutations in GISTs is, to the best of our knowledge, unknown. Since different genotypic features give rise to different drug responses and thus different prognosis, it becomes important to define which patients will positively respond to imatinib treatment. Therefore, we characterised the occurrence of *KIT* and *PDGFRA* mutations in a series of Portuguese GIST patients.

MATERIALS AND METHODS

Tissue samples

Seventy-eight formalin-fixed and paraffin-embedded consecutively diagnosed primary, previously untreated, sporadic GISTs, classified according to World Health Organization criteria⁵ and risk group,¹⁷ were retrieved from files (1989–2005) from the Pathology Department of S. João Hospital, Porto, Portugal. All patients were Caucasian and of Portuguese origin, with a mean age of 61.7 years (range 20–88). Thirty-eight (48.7%) patients were female and 40 (51.3%) were male. Follow-up data, managed according to the guidelines of the European Society of Medical Oncology,¹⁸ were available in 63 patients (range 0.2–206.0 months, mean 122.8 (12.1) months, median 132.6 (26.8) months) in September 2006.

Immunohistochemistry

The immunohistochemistry procedure was performed according to the streptavidin–biotin–peroxidase complex principle, using rabbit polyclonal anti-human antibodies raised against CD117 (dilution 1:500; clone A 4502, DAKO, Carpinteria, Denmark), actin (dilution 1:100; clone HHF35, DAKO), desmin (dilution 1:50; Zymed Laboratories, San Francisco, California, USA), S100 protein (dilution 1:1000; DAKO) and endothelial cell marker CD34 (dilution 1:40; clone QBEnd/10, NovoCastra Laboratories, Newcastle-upon-Tyne, UK). Briefly, deparaffinised and rehydrated slides were subjected to 10 min incubation in 3% hydrogen peroxide in methanol, in order to inhibit endogenous peroxidase. No antigen retrieval was used. After incubation with primary

antibody at room temperature for 30 min, the secondary biotinylated goat anti-polyvalent antibody was applied for 10 min, followed by incubation with streptavidin–peroxidase complex. The immune reaction was visualised by DAB as a chromogen (Ultravision Detection System Anti-polyvalent, HRP/DAB; Lab Vision, Fremont, California, USA). Any (strong/weak, focal, moderate or diffuse) membrane (CD117) and/or cytoplasm (CD117, actin, desmin, and CD34), and nuclear (S100 protein) immunoreactivity of the cells was considered as positive staining. Appropriated positive and negative controls were included in each run: interstitial cells of Cajal in a section of normal intestine were used as positive control for CD117, smooth layers for actin and desmin, small nerves for S100 protein, and vessels for CD34. For negative controls, primary antibodies were omitted. Mast cells, smooth layers, small nerves, and vessels were used as internal positive controls in the cases tested. All sections were counterstained with haematoxylin.

DNA isolation

Selected areas containing at least 85% of tumour tissue were macrodissected into a microfuge tube using a sterile needle (Neolus, 25 G, 0.5 mm). DNA isolation was performed as described previously.¹⁹ Briefly, the dissected tissue was deparaffinised by a serial extraction with xylol and ethanol (100%–70%–50%) and allowed to air-dry. DNA was extracted using Qiagen's QIAamp DNA Micro Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. DNA samples were stored at -20°C for further analysis.

KIT mutation analysis

KIT mutation analysis was performed as described previously.^{19, 20} DNA was subjected to PCR amplifications followed by direct sequencing for exon 11, and pre-screening by single strand conformational polymorphism (SSCP) analysis for exons 9, 13, 14 and 17, followed by direct sequencing of SSCP positive cases. Briefly, the PCR reaction was carried in a final volume of

Table 1 Correlation analysis of CD117 with clinical and pathological features of gastrointestinal stromal tumours (n = 63)

Parameter (n = 63)	CD117 negative (%)	CD117 positive (%)	p value
Age, y (SD)	54.6 (12.1)	61.2 (15.5)	0.37
Gender			0.13
Male	13.7	86.3	
Female	3.3	96.7	
Location			0.18
Gastric	5.9	94.1	
Small intestine	5.0	95.0	
Other	25.0	75.0	
Dimension (cm)			0.76
<5	4.0	96.0	
≥5	5.7	94.3	
Mitotic index (HPF)			0.34
<5	11.4	88.6	
5–10	0.0	100.0	
>10	0.0	100.0	
Risk grade			0.93
VLR–LR	5.0	95.0	
IR	6.7	93.3	
HR	4.0	96.0	
Histological type			0.02
Spindle cell	2.5	97.5	
Epithelioid	0.0	100.0	
Mixed	25.0	75.0	
Follow-up, months (SD)	92.3 (32.8)	120.8 (12.6)	0.96

HPF, high power field ($\times 400$); VLR, very low risk; LR, low risk; IR, intermediate risk; HR, high risk.

25 μ l, under the following conditions: 1 \times buffer (Bioron, Ludwigshafen, Germany); 1.5 mM MgCl₂ (Bioron); 200 μ M dNTPs (Fermentas, Hanover, Maryland, USA); 0.5 μ M primers (previously described by Corless *et al.*,²¹ except for exon 14: 5'-TCT CAC CTT CTT TCT AAC CTT TTC TT-3' (forward); 5'-CCC ATG AAC TGC CTG TCA AC-3' (reverse); MWG-Biotech, Ebersberg, Germany); and 1 unit of Super Hot Taq Polymerase (Bioron, Germany). SSCP analysis of exons 9, 13, 14 and 17 was performed in a 1 \times MDE gel (MDE: mutation detection enhancement, Cambrex, Charles City, Iowa, USA), with 6% glycerol addition in the exon 13 analysis, and 3% glycerol addition in exon 14 analysis. PCR product (20 μ l) was incubated at 95°C for 10 min with an equal volume of formamide loading buffer (98% formamide, 10 mM EDTA, and 1 mg/ml bromophenol blue and xylene cyanol). SSCP gels were run at 20°C. Samples with a SSCP pattern different from the normal pattern were directly sequenced. All cases were confirmed twice with a new PCR amplification, SSCP and direct sequencing analysis.

PDGFRA mutation analysis

Tumours bearing a wild-type *KIT* gene were further screened for hotspot *PDGFRA* mutations (exons 12, 14 and 18) as previously described.^{19, 20} Briefly, the PCR reaction was carried out in a final volume of 25 μ l, under the following conditions: 1 \times buffer (Bioron); 1.5 mM MgCl₂ (Bioron); 200 μ M dNTPs (Fermentas); 0.5 μ M primers (previously described by Heinrich *et al.*¹³; MWG-Biotech) and 1 unit of Super Hot Taq Polymerase (Bioron). PCR was followed by direct sequencing. All cases were confirmed twice with a new PCR amplification and direct sequencing analysis.

Statistical analysis

The available clinical and molecular data were analysed with StatView for Windows, V.5.0. Overall survival time analysis using

Kaplan–Meyer and log rank tests was performed with SPSS for Windows, V.14.0. Probability values <0.05 were considered significant.

RESULTS

Immunohistochemistry

Strong membrane and/or cytoplasm tumour cells immunoreactivity for CD117 was found in variably focal, moderate or diffuse areas in 72 (92%) GIST cases (fig 1). In three cases, CD117 immunoreactivity was weak. Six GIST cases (8%) did not show CD117 immunoreactive tumour cells. Interstitial cells of Cajal and mast cells, used as internal positive controls, were always variably observed in each case. Table 1 summarises statistical analysis of CD117 immunostaining and clinical–pathological features. CD117 immunoreactivity was significantly associated ($p = 0.015$) with spindle cell and epithelioid GIST subtypes. The frequency and expression features of the other antibodies was variable from case to case, and within the same tumour, as follows: actin (51%), desmin (6%), S100 protein (18%), and CD34 (73%); immunoreactivity was observed in focal areas/rare tumour cells for actin, desmin, and S100 protein, whereas CD34 immunoreactivity was found in moderate or diffuse areas (data not shown). Four of the six CD117 negative GISTs expressed CD34 without any tumour cell expression for the other markers tested.

KIT mutation analysis

Mutation screening analysis revealed that 44 of 78 GISTs (56%) presented *KIT* activating mutations (table 2). Forty cases showed mutation in exon 11 (91%, 40/44) and four cases in exon 9 (9%, 4/44). Among the exon 11 mutations, we observed 3–54 bp in-frame deletions in 24 tumours (60%, 24/40), either alone (62%, 15/24) or associated with missense mutations or insertions (38%, 9/24), single base substitutions in 15 tumours (38%, 15/40) and an in-frame insertion associated with a point mutation in 1 tumour (2%, 1/40). Additionally, a silent mutation (Y570Y) was detected in two GISTs. The exon 9 sequence alterations consisted of Ala–Tyr duplication between codons 502 and 503 in three GIST cases, and a point mutation (G470R) in one case. One silent mutation was detected in both exons 13 and 17 (P627P and S865S, respectively). Also, a 4 bp deletion was detected affecting the intronic sequence following exon 14 (IVS14+24:del4). In addition, to exclude the possibility of false-negatives in the SSCP screening at exons 9, 13, 14 and 17, 10 *KIT* wild-type GISTs were direct sequenced for all exons. No additional mutations were identified.

Table 3 shows statistical analysis of *KIT* mutations and clinical–pathological features. No correlation was detected between *KIT* mutation status and CD117 expression ($p = 0.39$). However, all but two GISTs harbouring *KIT* mutation were positive for CD117 expression. Additionally, the three GISTs with weak CD117 immunoreactivity depicted wild-type *KIT*. A statistically significant correlation was obtained between the epithelioid morphology and lack of *KIT* mutation ($p = 0.0064$). The presence of mutated *KIT* was significantly associated with shorter survival of patients ($p = 0.0460$) (fig 2). No correlation was obtained between any specific type of *KIT* mutation (point mutation, deletion, or mixed mutation), or its location (exon 9 or exon 11), and patient survival (data not shown).

PDGFRA mutation analysis

In *KIT* wild-type GISTs, *PDGFRA* activating mutations were identified in five cases; two in exon 12, and three in exon 18

Table 2 Amino acid sequence of exons 9 and 11 of wild-type and mutated KIT protein

	Exon 11										Exon 9																														
	550	560	570	580	590	600	610	620	630	640	470	480	490	500	510	520	530	540	550	560																					
Wild type	K	P	M	Y	E	V	Q	W	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Cases 5, 67, 76, 78	K	P	M	Y	E	V	Q	W	K	D	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 7	K	P	M	Y	E	V	Q	W	-	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	N	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 10	K	P	M	Y	E	V	Q	W	K	V	-	E	E	I	-	-	-	-	-	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Cases 11, 59	K	P	M	Y	E	V	Q	W	K	V	-	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 13	K	-	-	-	E	V	Q	W	K	V	-	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Cases 14, 40	K	P	M	Y	E	V	Q	W	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 15	K	P	M	Y	E	V	Q	F	-	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 16	K	P	M	Y	E	V	Q	W	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 17	K	P	M	Y	E	V	Q	G	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 19	K	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Cases 22, 39, 57, 70	K	P	M	Y	E	V	Q	W	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 24	K	P	M	Y	E	V	Q	W	K	G	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 28	K	P	M	Y	E	V	Q	W	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	H	-	T	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 33	K	P	M	Y	E	V	Q	W	K	V	D	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 37	K	P	M	Y	E	V	Q	W	K	-	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Cases 38, 42, 47	K	P	M	Y	E	V	Q	-	-	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 45	Q	R	-	-	-	-	-	-	-	K	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 48*	-	-	-	-	-	-	-	-	-	K	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 50	K	P	M	Y	E	V	Q	W	K	V	V	E	E	I	-	-	-	-	-	-	Y	I	D	P	T	-	-	-	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 51	K	P	M	Y	E	V	-	-	-	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L
Case 55	K	P	M	Y	E	V	Q	R	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 64	K	P	M	Y	E	V	Q	W	N	-	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 65	I	-	-	-	-	V	Q	W	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 66	K	P	M	Y	E	V	H	-	-	-	-	-	-	-	-	-	-	-	-	-	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 68	K	P	M	Y	E	V	-	-	-	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L
Case 69	K	P	M	Y	E	V	Q	W	K	V	V	E	E	I	N	G	N	N	K	-	-	Y	I	D	P	T	-	-	-	Y	D	H	K	W	E	F	P	R	N	R	L
Case 73	K	P	M	Y	E	V	P	-	-	-	-	-	-	-	-	-	-	-	-	-	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 74	K	P	M	Y	E	V	Q	W	K	V	V	E	E	I	N	G	N	N	Y	V	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 75	K	P	M	Y	E	V	Q	W	-	-	-	-	-	-	-	-	-	-	-	-	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Case 77	K	P	M	Y	E	V	Q	W	-	-	-	-	-	-	-	-	-	-	-	-	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L	
Exon 9	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650																						
Wild type	F	G	K	-	-	T	S	A	Y	F	N	F	A	F	K	G	N	-	-	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L		
Case 2	F	R	K	-	-	T	S	A	Y	F	N	F	A	F	K	G	N	-	-	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L		
Cases 1, 12, 18	F	G	K	-	-	T	S	A	Y	F	N	F	A	F	K	G	N	-	-	Y	I	D	P	T	Q	L	P	Y	D	H	K	W	E	F	P	R	N	R	L		

-, deleted amino acid residues.
*Includes an insertion of two residues (histidine and asparagine) in codon 550, besides the represented deletion.

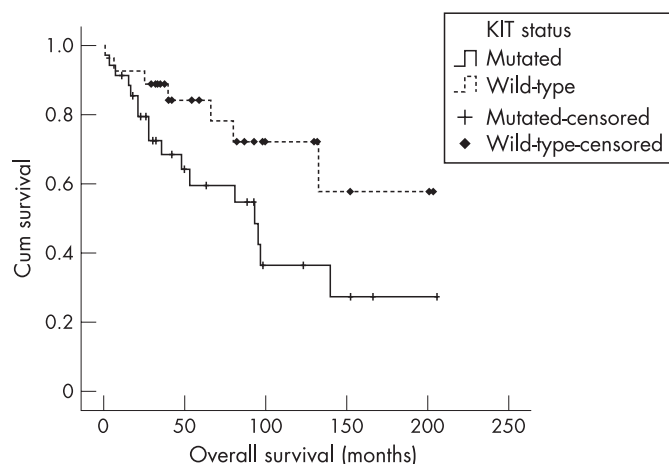


Figure 2 Kaplan–Meier curve for the 63 patients with gastrointestinal stromal tumours, regarding *KIT* alterations. Patients having a wild-type *KIT* ($n = 27$) have a better prognosis than patients having tumours harbouring mutated *KIT* ($n = 36$) ($p = 0.046$).

(table 4). The mutations in exon 12 consisted of a point mutation (D583G) and an in-frame deletion (583del586). Two cases disclosed a point mutation (D842V) in exon 18; there was another case with a point mutation (I843T) together with an in-frame deletion (844del847). In addition, we identified two silent mutations, one in exon 12 (D577D) and another in exon 18 (I834I). No mutation was observed in exon 14 of *PDGFRA*. Furthermore, mutational analysis of exons 12 and 18 showed the presence of a known homozygous substitution A>G (polymorphism R) in the third position of the codon for proline 567 in exon 12, and an insertion in intron 18 (IVS18-50insA).

All GISTs with *PDGFRA* mutations showed CD117 immunoreactive tumour cells.

DISCUSSION

The intensive cancer research in the last decade has highlighted the fundamental role of RTKs, in particular of *KIT* and *PDGFRA* in GIST pathogenesis.¹⁰ These two RTKs are of great value for therapeutic management as a result of the development of RTK inhibitors, such as imatinib and sunitinib.^{22–24} There is, however, insufficient epidemiological data on the frequency and type of mutations in the *KIT* and *PDGFRA* genes in GISTs from countries in southern Europe countries, such as Portugal.

In this study, we have shown that 92% of GISTs express CD117, irrespective of the topography, age or gender, in accordance with previous studies in other populations.^{25–26} No statistically significant correlation was depicted between CD117 expression and presence of *KIT* mutations ($p = 0.3933$). In fact, two of the six CD117-negative GISTs contained a *KIT* mutation (a missense mutation in exon 9, and a three base-pair deletion in exon 11). Other authors have also encountered *KIT* mutations in CD117-negative GIST cases.²⁷ Our molecular study was useful for the definitive diagnosis of GIST in 2/6 CD117 negative cases. The frequency of the CD117-negative wild-type cases for *KIT* and *PDGFRA* mutations found in our series (5%), fits with results described in the literature.²⁷

We showed the presence of *KIT* mutations in 56% of GIST cases, 91% (40/44) being located in exon 11. These frequencies are in accordance with previously published ranges for other populations (30–80%), particularly those of the Iberian Peninsula.^{1–10–28} In 75% (30/40) of these cases, mutations were

Table 3 Correlation of *KIT* mutations with clinical-pathological features of gastrointestinal stromal tumours ($n = 63$)

Parameter	<i>KIT</i> mutation negative (%)	<i>KIT</i> mutation positive (%)	p value
Age, y (SD)	57.2 (16.6)	62.8 (14.2)	0.16
Gender			0.94
Male	43.3	56.7	
Female	42.4	57.6	
Location			0.27
Gastric	51.4	48.6	
Small intestine	35.0	65.0	
Other	25.0	75.0	
Dimension (cm)			0.93
<5	44.0	56.0	
≥5	42.8	57.2	
Mitotic index (50 HPF)			0.80
<5	45.4	54.5	
5–10	44.4	55.6	
>10	33.3	66.7	
Risk grade			0.70
VLR–LR	40.0	60.0	
IR	53.3	46.7	
HR	42.3	57.7	
Histological subtype			0.01
Spindle	41.5	58.5	
Epithelioid	100.0	0.0	
Mixed	25.0	75.0	
CD117 expression			0.39
Positive	40.4	59.6	
Negative	60.0	40.0	
Follow-up, months (SD)	148.7 (17.1)	100.8 (12.1)	0.05

HPF, high power field ($\times 400$); VLR, very low risk; LR, low risk; IR, intermediate risk; HR, high risk.

clustered in the region between codons 550 and 561, known to be the most frequently altered section of exon 11, with 57% (17/30) affecting codon 557 or 558. These two codons are reported to be associated with the metastatic behaviour of GISTs.^{10–29} However, of these 17 GIST cases, only four recurred (4/17, 24%). Even though it has been previously reported that all point mutations occur exclusively in codons 557, 559, 560 and 576, we have additionally encountered a novel point mutation in codon 570 (Y570F).¹ Mutations in *KIT* exon 9 have been correlated with a small intestinal topography, but only one of our four GIST cases harbouring a mutation in this exon was located in the small intestine.^{1–10} In the present study, and in agreement with previous reports, *KIT* mutation positive status was shown to be associated with worse GIST prognosis, translated into shorter patient survival.¹⁰

Table 4 Amino acid sequence of exons 12 and 18 of wild-type and mutated *PDGFRA* protein

Exon 12	580									
Wild type	L	P	Y	D	S	R	W	E	F	P
Case 4	L	P	Y	G	S	R	W	E	F	P
Case 71	L	P	Y	–	–	–	–	E	F	P
Exon 18	840									
Wild type	A	R	D	I	M	H	D	S	N	Y
Cases 41, 54	A	R	V	I	M	H	D	S	N	Y
Case 34	A	R	D	T	–	–	–	–	N	Y

–, deleted amino acid residues.

Take-home messages

- ▶ The frequency of *KIT* and *PDGFRA* activating mutations has been described in a series of Portuguese gastrointestinal stromal tumours (GISTs).
- ▶ Of the Portuguese patients with GISTs, 56% harboured *KIT* mutations and 6% exhibited *PDGFRA* mutations.
- ▶ The presence of *KIT* mutations in GISTs was associated with a worse patient prognosis; however, these mutations are indicative of favourable response to imatinib-based therapy.

Concerning *PDGFRA*, mutations were detected in 6% (5/78) of our cases, corresponding to 15% of *KIT* wild-type GISTs. Two of these mutations are known to be imatinib-resistant (D842V).²² An association between gastric location and presence of *PDGFRA* mutation has been reported.³⁰ In our series, although the number of cases with mutations in *PDGFRA* is low for statistical evaluation (n = 5), 80% (4/5) of mutations occurred in the stomach.

It is now well established that the response of GIST patients to imatinib-based therapy is dependent not only on the presence, but also on the type of *KIT* and *PDGFRA* mutation exhibited.^{10 16 22} Specifically, mutations affecting the juxtamembrane domain (exon 11, partial response in up to 84% of cases) or the extracellular domain (exon 9, partial response in up to 48%) predict objective response to imatinib.^{10 16 22} On the other hand, it is also known that some mutations are responsible for imatinib resistance, namely in *KIT* V654A and W670I (exon 13), D816V and T823D (exon 17), and *PDGFRA* D842V (exon 18).^{16 31 32} Of these resistant mutations, only D842V mutation was detected in two GISTs in our series. Recently, the US Food and Drug Administration approved a new RTK inhibitor, sunitinib (Sutent, Pfizer, New York, USA) as a second-line therapy for GIST patients who experience disease progression in spite of increased doses of imatinib, mainly due to primary or acquired secondary imatinib-resistant mutations, or who are unable to tolerate treatment with imatinib.^{24 33} Therefore, with these two RTK inhibitors available, there is an imperative need to redefine GIST pathological (diagnosis/prognostic) evaluation, as well as to consider molecular characterisation of both *KIT* and *PDGFRA*, in order to achieve an efficient and predictive tailored therapeutic management for each individual patient.

In conclusion, we have reported for the first time the frequency of *KIT* and *PDGFRA* mutations in a large series of Portuguese GIST patients. We have shown the presence of *KIT* mutations in 56% of cases and *PDGFRA* mutations in 6% of cases. In addition, the presence of mutated *KIT* was associated with a shorter patient survival. The great majority of *KIT* activating mutations (91%) were located in exon 11, indicative of a favourable response to imatinib-based therapy in the management of these patients. Finally, our results might be useful to integrate a multi-institutional consortium database for the clarification of the epidemiology, biology and management of GIST patients.

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