KIT and *PDGFRa* mutational patterns in Sardinian patients with gastrointestinal stromal tumors

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Gastrointestinal stromal tumor (GIST) is the most common mesenchymal malignancy of the gastrointestinal tract. We provide in the present article the molecular characterization of a series of primary GISTs in a cohort of Sardinian patients (Italy), with the aim to describe the patterns of *KIT* and *PDGFRa* mutations and the corresponding clinical features. Ninety-nine Sardinian patients with histologically-proven diagnosis of GIST were included in the study. Medical records and pathology reports were used to assess the demographic and clinical features of the patients and the disease at the time of the diagnosis. Formalin-fixed, paraffin-embedded tissue samples were retrieved for each case, and mutation analysis of the *KIT* and *PDGFRa* genes was performed. *KIT* and *PDGFRa* mutations were detected in 81.8% and 5% of the cases, respectively. The most common *KIT* mutation was W557_K558del in exon 11, while D842V in exon 18 was the most common *PDGFRa* genetic alteration; V561D was the only *PDGFRa* mutation found in exon 12. The global "wild-type" cases, with no mutations in either the *KIT* or *PDGFRa* genes, were 13 (13.1%). The mean survival of those patients was approximately 46.9 (±43.9) months. Globally, 86.9% of Sardinian patients with GIST had a *KIT* or *PDGFRa* mutation; the former were more frequent in comparison with other Italian cohorts, while *PDGFRa* mutations were rare. No statistical differences in survival between mutated and wild-type cases, and between *KIT* and *PDGFRa* mutated cases were detected in our study. *European Journal of Cancer Prevention* 30: 53–58

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Introduction

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal malignancy of the gastrointestinal tract, which can occasionally affect also the mesentery or the retroperitoneum (Doyle and Hornick, 2014). GISTs with little or no malignant features smaller than 1 cm are very frequent in subjects younger than 50. Nevertheless, larger clinically manifested GISTs are rare, especially when compared with the epithelial counterpart of digestive tract tumors like colorectal carcinomas, representing the 1% of all the gastrointestinal malignancies, with an annual incidence of about 1/100 000 individuals (Ridolfini *et al*., 2011; Palmieri *et al*., 2013; Metaxas *et al*., 2016).

Surgery is the first choice for treatment in patients with clinically manifested lesions, but approximately 40% of them develop recurrence or metastasis, after radical resection (Joensuu *et al*., 2012). Moreover, locally advanced or metastatic GISTs are refractory to conventional chemotherapy or radiation. On the other hand, it has been demonstrated that the development and progression of GISTs depends on specific mutations of genes that activate receptors for tyrosine kinases like *KIT* and *platelet-derived growth factor A* (*PDGFRa*) in about 82–87% of the cases (Szucs *et al*., 2017). This leaded to the development of targeted agents, called tyrosine kinase inhibitors (TKIs), which revolutionized not only the treatment of GISTs, but all the modern thinking in oncological pharmacology and therapy. Imatinib was introduced in clinical practice more than one and a half decades ago, due to its pronounced clinical efficacy in GISTs harboring *KIT* and *PDGFRa* mutations.

Therefore, the identification of *KIT* and *PDGFRa* mutations is currently required in clinical practice for the accurate selection of the patients to treat with TKIs.

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Unfortunately, a consistent percentage of GISTs do not harbor these mutations, representing the most challenging cases from a therapeutic point of view (Keung and Raut, 2017). Furthermore, non-response or development of resistance to TKIs have also been describe (Antonescu, 2011). Secondary resistance to imatinib occurs in 40-50% of treated patients with a median time to progression of approximately 24 months (Szucs *et al.*, 2017). Therefore, knowledge of the epidemiology of *KIT* and *PDGFRa* mutational patterns in specific populations is essential in order to optimally interpret their impact on the clinical management and therapeutic outcomes. We provide in the present article the molecular characterization of a series of primary GISTs in a cohort of Sardinian patients (Italy), with the aim to describe the patterns of *KIT* and *PDGFRa* mutations, their correlations with the clinical features of the disease, and their impact on prognosis.

Methods

Patients

Ninety-nine Sardinian patients with histologically proven diagnosis of GIST were included in the study. They were consecutively collected from January 2000 to December 2018, regardless of age at diagnosis and disease characteristics of the primary tumor. Medical records and pathology reports were used to assess the demographic, clinical and pathological features of the patients and the disease at the time of the diagnosis. Sardinian origin was ascertained in all cases through verification of the place of birth; cases with uncertain origin were excluded. The local cancer registry was used for further epidemiological and prognostic information. This registry was created in 1992 by the local health agency for the epidemiological surveillance of tumors in the province, and in 1999, it became part of the Italian Association for Tumor Registries (Associazione Italiana Registri Tumori). It collects data on tumoral diseases through the local hospitals, health care services and other registries (e.g. death registries). The study was carried out in accordance with the principles of the Declaration of Helsinki, and was approved by the Committee for the Ethics of the Research and Bioethics of the National Research Council.

Specimens and molecular analyses

Formalin-fixed, paraffin-embedded tissue samples from the 99 GISTs were obtained from the archives of the Units of Pathology participating in the study, and all the cases were reviewed by a dedicated pathologist (A.C.), to confirm the initial diagnosis and the morphological and immunohistochemical features of the tumors. Tissue sections were estimated to contain at least 70% neoplastic cells by light microscopy.

Genomic DNA was isolated from tissue sections using standard protocols, as previously described (Palomba *et al.*, 2016). Briefly, somatic DNA was purified using the QIAamp DNA FFPE Tissue kit (Qiagen Inc., Valencia, California, USA) and the DNA quality assessed for each specimen. Mutation analysis was conducted in the coding sequence of the *KIT* (exons 9, 11, 13 and 17; where almost all of the oncogenic mutations responsive to KIT inhibitors are located) and *PDGFRa* (exons 12, 14 and 18; which carry the most frequent mutations involved in resistance to KIT inhibitors) genes by direct sequencing, using an automated fluorescence-based cycle sequencer (ABIPRISM 3130; Life Technologies–Thermo Fisher Scientific, Waltham, Massachusetts, USA), as previously described by our group (Palomba *et al.*, 2012). Protocols for PCR-based assays will be available upon request.

Statistical analysis

Molecular and clinical data were registered in a dedicated digital database, and statistical analysis was performed. Variables were expressed as mean and SD values or median and interquartile ranges (IQRs). Statistical differences were assessed using the unpaired Student's *t*-test or Mann–Whitney rank sum test, the chi-square test or Fisher's exact test as appropriate. Statistical tests were considered significant when the corresponding two-sided *P*-values were <0.05. Mean survival was calculated as the time from the date of diagnosis until the time of death or last contact, in the case of survivors. The analyses were performed using MedCalc for Windows, version 15.4 64 bit (MedCalc Software, Ostend, Belgium).

Results

The mean (±SD) age of the 99 patients included in the molecular analysis was 65.2 (±12.9) years, and 56 (56.6%) were males. The main demographic and clinical features, as well as the general *KIT* and *PDGFRa* mutational frequencies are summarized in Table 1. Globally, 86 (86.9%) of the patients had a *KIT* (81, 81.8%) or *PDGFRa* (5, 5%) mutation. Detailed information about the types of the single mutations encountered are shown in Table 2. The most common KIT mutation was W557_K558del in exon 11 (25.9% of all *KIT* mutations), while D842V in exon 18 was the most common *PDGFRa* alteration (four cases, 80%); V561D was the only *PDGFRa* mutation found in exon 12. The global 'wild-type' cases, with no mutations in either the *KIT* or *PDGFRa* genes, were 13 (13.1%) (Fig. 1). A novel *KIT* mutation was detected in exon 11 (Supplementary Table, Supplemental digital content 1, *http://links.lww.com/EJCP/A282*).

Among the 81 *KIT* mutations encountered, 48 (59.2%) were found in males, while only one (20%) out of the five *PDGFRa* mutations was found in males ($P = 0.156$); the only PDGFR mutation found in exon 12 occurred in a female patient. The mean (±SD) age of the patients with *KIT* and *PDGFRa* mutations at the time of diagnosis was, respectively, 66.2 (±12.7) and 64.8 (±17.9); the difference was not statistically significant ($P = 0.861$).

Table 1 Main demographic, clinical and molecular features of the patients included in the study

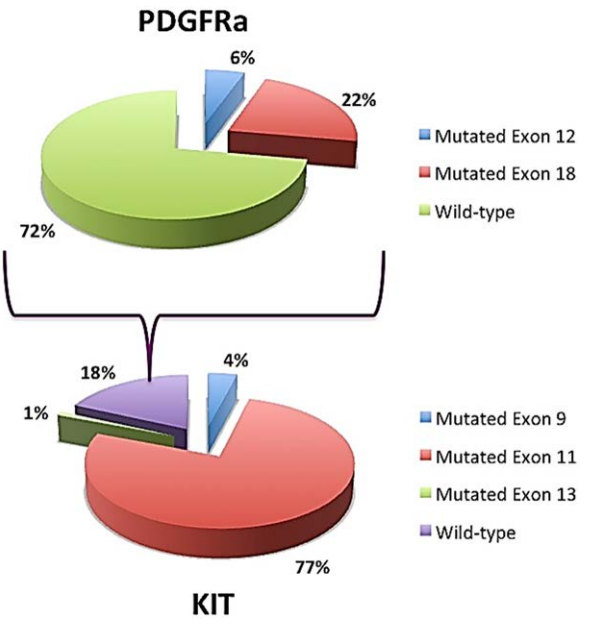
Variables			*P* value
Male sex, n (%)		56 (56.6)	
Age, mean (±SD), years		65.2 (±12.9)	
Tumor localization, n (%)	Stomach	49 (49.5)	
	Jejunum – ileum	31 (31.3)	
	Rectum	7 (7.1)	
	Duodenum	7 (7.1)	
	Colon	5 (5)	
T stage, n (%)	T2	44 (44.4)	
	T3	47 (47.5)	
	T4	8 (8.1)	
N stage, n (%)	N0	61 (61.6)	
	N1	38 (38.4)	
Metastasis (M1), n (%)		38 (38.4)	
C-KIT mutations, n (%)		81 (81.9)	
C-KIT exons, n (%)	Exon 9	4 (4.9)	
	Exon 11	76 (93.8)	
	Exon 13	1 (1.2%)	
PDGFRa mutation, n (%)		5 (5)	
PDGFRa exons, n (%)	Exon 12	1 (20)	
	Exon 18	4 (80)	
Survival, median (IQR), months		31.0 (17.25–67.0)	
Mutated cases survival, median (IQR), months		30.5 (16–66.5)	0.308
Wild-type cases survival, median (IQR), months		39 (21.25–80.25)	
cKIT mutated survival, median (IQR), months		32 (15.5–67.0)	0.353
PDGFRa mutated survival, median (IQR), months		27 (16–30.25)	
Deaths, n (%)		39/79 (49.4)	

IQR, interquartile range.

Table 2 *KIT* mutations detected in our series

Mutation	N° (%)
W557_K558del	21 (25.9)
V559D	10 (12.3)
K558_V559del	4 (4.9)
V555_V559del	4 (4.9)
V569_Y578del	4 (4,9)
Y503_F504ins	4 (4.9)
P551_E554del	2 (2.5)
P551_V555del	2 (2.5)
V559_G565del	2 (2.5)
V560G	2 (2.5)
W557_V559del	2 (2.5)
W557R	3 (3.7)
Other	21 (25.9)
Exon	
11	76 (93.8)
9	4 (4.9)
13	1 (1.2)

Fig. 1



Graphical illustration of the incidence of *KIT* and *PDGFRa* mutations in our cohort.

The most common anatomical site of mutated tumors was the stomach (43, 50%), followed by small intestine (27, 31.4%) and rectum (seven, 8.1%). Four (80%) of the lesions with *PDGFRa* mutation were found in the intestine (three on the small intestine and one on the rectum), including the single exon 12 mutated case, and only one (20%) on the stomach. Differences in anatomical localization between cases with *KIT* and *PDGFRa* mutations were not statistically significant ($P = 0.362$ for the stomach and $P = 0.545$ for small intestine).

KIT and *PDGFRa* mutations were found globally in 36 (94.7%) out of the 38 patients with lymph node involvement at the time of diagnosis (32 *KIT* and four *PDGFRa* mutations, $P = 0.077$). Furthermore, among the 38 patients with metastasis, 33 (86.8%) had at least one mutation (30 *KIT* and three *PDGFRa* mutations, $P = 0.349$).

Among the 99 patients globally included, prognostic data were available in 79 cases and 39 (49.4%) of them were dead at the time of follow-up. The median (IQR) survival of those patients was approximately 31.0 (17.25–67.0) months. Thirty-two (82%) out of the 39 deaths occurred in patients with a *KIT* mutation; three (7.7%) further

deaths occurred in patients with a *PDGFRa* mutation. Globally, the 89.7% of the dead patients had at least one mutation. Among the 40 alive patients, 31 (77.5%) had a *KIT* mutation, and two (5%) had a *PDGFRa* mutation; globally, 33 (82.5%) of the alive patients had at least one mutation. The median survival time in mutated and 'wild-type' cases was 30.5 (16–66.5) and 39 (21.25–80.25) months, respectively ($P = 0.308$). In particular, the median survival time in *KIT* mutated cases was 32 (15.5–67.0) and in *PDGFRa* mutated cases was 27 (16–30.25) without any statistical difference ($P = 0.353$). Furthermore, no statistically significant differences were found in median survival times between patients with and without the W557_K558del mutation in exon 11 ($P = 0.918$), and in patients with and without the V559D mutation in the same exon ($P = 0.529$).

Discussion

Gastrointestinal stromal tumors are the most common mesenchymal tumors of the gastrointestinal tract, arising probably on the mesenchymal pluripotent stem cells or the Cajal cells (Ridolfini *et al*., 2011; Paliogiannis *et al*., 2011). Clinically manifested GISTs commonly involve patients aged between 66 and 69, with only the 3% occurring under the 20 years of age (Ridolfini *et al*., 2011; Tran *et al*., 2005; Miettinen *et al*., 2005); in our series, the mean age was 65.2 years, and we did not detect any case in patients younger than 28. Furthermore, no consistent differences in the incidence between sexes have been described, and this was confirmed also in our series where a slight predilection for males has been observed. The most common anatomical localization of GISTs is the stomach, including generally 40–60% of the cases described in literature (Tran *et al*., 2005; Emory *et al*.,1999); in our series, approximately half of the cases involved the stomach, with the remaining cases involving mainly tracts of the intestine. We did not encounter any lesions in the esophagus or extra-gastrointestinal sites, which represent approximately the 17% of the GISTs (Tran *et al*., 2005; Emory *et al*.,1999).

The global incidence of *KIT*/*PDGFRa* mutations in our series was 86.9%. This percentage is similar with those published in other recent large cohort studies, varying between 82 and 93% (Debiec-Rychter *et al*., 2006; Heinrich *et al*., 2008; Wozniak *et al*., 2012; Wozniak *et al*., 2014; Corless *et al*., 2014; Joensuu *et al*., 2017). According to these studies *KIT* and *PDGFRa* mutations occur respectively in 69.3–87.4% and 1.6–14% of the cases; percentages vary in relation with the number of exons of the genes analyzed and the type of the study (population studies report higher percentages than clinical trials). Patients harboring such mutations can benefit of targeted therapeutic agents, like TKIs. Imatinib was the first TKI introduced in clinical practice in 2002 for the treatment of unresected or metastatic GISTs; since then other TKIs (i.e. sunitinib, regorafenib) have been introduced in clinical practice, and numerous others are currently under evaluation (i.e. masitinib, crenolanib, AZD2171, vatalanib, OSI-930, TKI258, DCC-2618, etc.) (Lim and Tan, 2017).

The percentage of *KIT* mutations found in our series is similar to that reported in these studies (81.9%), while the percentage of *PDGFRa* was relatively low (5%). *KIT* mutations involve generally the exons 11 (61–71%), 9 (7–15%) and 13 (0.5–1.8%) (Szucs *et al*., 2017); the corresponding figures in our study were 93.8%, 4.9% and 1.2% evidencing that in Sardinian patients mutations in the exon 11 are consistently more common than in other populations, while exon 9 mutations are rarer. This may have implications in clinical practice, other than the selection of appropriate targeted therapies; a recent meta-analysis evidenced that GIST patients with *KIT* exon 9 mutations have higher risk of progression than those with exon 11 mutations, and that the 557–558 deletion of *KIT* exon 11, was a valuable predictor of prognosis for patients with GISTs. In our study, as in other older studies (Emile *et al*., 2004; Emile *et al*., 2006), these implications were not confirmed, but it may be due to the relatively small number of cases enrolled.

PDGFRa mutations have been reported to occur mainly in exon 18 (1.2–12.8%) and rarely in exons 12 (0.2–0.9%) and 14 (0.3–0.7%) (Szucs *et al*., 2017). In our study, we found four mutations in exon 18 (4%) and one (1%) in exon 12. These mutations have been correlated with an indolent course of the disease and better prognostic outcomes in several studies. In the ConticaGIST study and in the Polish registry study, pD842V in exon 18 (which represents the most common mutation), showed better survival outcomes when compared with other mutations, including KIT mutations in codon 11 (Debiec-Rychter *et al*., 2006; Wozniak *et al*., 2014). In our study, the pD842V mutation as the only one detected in exon 18, but it did not show any prognostic advantage in terms of mean survival time when compared with patients without this mutation or with patients harboring the W557_K558del mutation in exon 11. Furthermore, the indolent course of PDGFR has been linked to their prognostically favorable origin in the stomach, but in our study most of them involved the small intestine.

In our series, 13 patients had not any *cKIT* or *PDGFRa* mutations, representing the 'wild-type' subgroup of cases. Nevertheless, recent studies performed with advanced sequencing technologies like next-generation sequencing, showed that the term 'wild-type' is not exact as these tumors harbor a heterogeneous bouquet of genetic alterations dictating clinical and prognostic differences. In particular, 0–40% of all *KIT*/*PDGFRa* wild-type GISTs are succinate dehydrogenase complex-deficient GIST, 4–13% carry a *BRAF* V600E mutation, and some others have *ETV6-NTRK3* and *FGFR1* gene fusions, as well as NF1, TP53, MEN1, MAX, CHD4, FGFR1, CTDNN2,

CBL, ARID1A, BCOR and APC mutations (Nannini *et al.*, 2013; Nannini *et al.*, 2017). The genetic landscape of 'wild-type' GISTs is constantly updated, and will eventually lead to novel molecular classes of these tumors.

In Italy, Origone *et al.* (2013) reported a series of 115 GISTs in 2013, with the patients showing demographic characteristics (age, sex) similar to those of other Caucasian populations and with those of our series; the anatomical distribution of the lesions was also similar, with approximately half of the cases involving the stomach. Among the 80 tumors available for mutational analysis, 64 (80%) presented a *KIT* or *PDGFRA* mutation; in particular, 55 (69%) *KIT* and nine (11%) *PDGFRA* mutations were detected. Thus, the percentage of *KIT* mutations was lower than that of Sardinian patients, and, as opposed, the percentage of *PDGFRA* mutations was higher. Nevertheless, the exon distribution of the KIT mutations was similar to that of our series, while all the nine PDGFRA mutations found involved the exon 18, as opposed to our series were a mutation occurring in the exon 12 was detected. The authors also tested 81 samples for *KRAS* and *BRAF* mutations, but all were negative for these alterations (Origone *et al.*, 2013).

Rossi et al. (2011, 2015) published in recent years reports on the mutational epidemiology of GISTs in Italy, including data mainly from the central and northern regions of the country, excluding Sardinia. In 2015, they published a series of 451 GISTs analyzed; among them 392 (86.9%) carried mutations in *KIT* (292 cases; 64.7%), *PDGFRa* (95 cases; 21.1%), or *BRAF* (five cases; 1.1%). The percentage of *KIT* mutations is lower and the percentage of *PDGFRa* mutations was four-fold higher than that of Sardinians; the distribution of the *KIT* mutations was similar to that reported by Origone *et al.* (2013), and once again, all the *PDGFRa* mutations were detected in the exon 18. These findings suggest different mutational patterns in GISTs occurring in Sardinians in comparison to other Italian populations. Sardinian population is characterized by high levels of genetic homogeneity due to geographical reasons, and have long been recognized as forming a distinct outlier within contemporary European genetic diversity, experienced an immigration of individuals from the Asian areas (mainly the Middle East) into southeastern Europe during the early Neolithic transition (Chiang *et al.*, 2018). Rossi *et al.* (2015) confirmed that mutation was a significant prognostic indicator of overall survival, with *KIT*-mutated patients having a worse outcome than *PDGFRa*-mutated or wild-type cases. This finding was not confirmed in our series, probably a cause of the small number of cases and *PDGFRa* mutations, The pattern of mutations in relation to the anatomical site of origin in our study seems similar to that of other Italian series, with the exception of the higher number of *PDGFRa* mutations occurring in the intestine (four out of the five cases) (Rossi *et al.*, 2015).

Our study has some limitations, especially the relatively low number of cases and, as a consequence, of *PDGFRa* mutations or 'wild type' cases. Furthermore, we did not search for *KIT* mutations in exons 8, 14 and 17 or *PDGFRa* mutations in exon 14, and data regarding the surgical and or clinical treatment of the patients were not available in the registry used for the study. On the other hand, our study was performed in a genetically homogenous Caucasic population, and this allowed to evidence some differences in the mutational patterns in comparison to other populations.

In conclusion, the global incidence of *KIT*/*PDGFRa* mutations in Sardinians with GIST is similar to that of other Caucasian and Italian populations. Nevertheless, *KIT* mutations in exon 11 are significantly more common than those in exons 9 or 13 which are rare. Similarly, *PDGFRa* mutations are relatively rare, and frequently detected in intestinal rather than in gastric tumors. No prognostic advantage of one mutation subtype on another was evidenced in our study.

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Conflicts of interest

G.P. has/had an advisory role for Bristol Myers Squibb, Incyte, Merck Sharp & Dohme, Novartis, Pierre Fabre and Roche Genetech. For the remaining authors, there are no conflicts of interests.

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