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ORIGINAL PAPER

Frequencies of *KIT* and *PDGFRA* mutations in the MolecGIST prospective population-based study differ from those of advanced GISTs

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Abstract Gastrointestinal stromal tumors (GISTs) are the most common human sarcoma. Most of the data available on GISTs derive from retrospective studies of patients referred to oncology centers. The MolecGIST study sought to determine and correlate clinicopathological and molecular characteristics of GISTs. Tumor samples and clinical records were prospectively obtained and reviewed for patients diagnosed in France during a 24-month period. Five hundred and ninety-six patients were included, of whom 10% had synchronous metastases. GISTs originated from the stomach, small bowel or other site in 56.4, 30.2 and 13.4% of cases, respectively. The main prognostic markers, tumor localization, size and mitotic index were not independent variables ($P < 0.0001$). Mutational status was determined in 492 (83%) patients, and 138 different mutations were identified. *KIT* and *PDGFRA* mutations were detected in 348 (71%) and 74 (15%) patients, respectively, contrasting with 82.8 and 2.1% in patients with advanced GIST (MetaGIST) ($P < 0.0001$). Further comparison of localized GISTs in the MolecGIST cohort with advanced GISTs from previous clinical trials showed

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that the mutations of *PDGFRA* exon18 (D842V and others) as well as *KIT* exon11 substitutions (W557R and V559D) were more likely to be seen in patients with localized GISTs (odds ratio 7.9, 3.1, 2.7 and 2.5, respectively), while *KIT* exon 9 502_503dup and *KIT* exon 11 557_559del were more frequent in metastatic GISTs (odds ratio of 0.3 and 0.5, respectively). These data suggest that *KIT* and *PDGFRA* mutations and standardized mitotic count deserve to be investigated to evaluate the relapse risk of GISTs.

Keywords Epidemiology · Sarcoma · Gastrointestinal tumor · Tyrosine kinase receptor

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common human sarcoma and account for 18% of all sarcomas [1]. GISTs may arise anywhere in the gastrointestinal tract and abdomen. The spectrum of morphology includes spindle cells and epithelioid variants. Most GISTs are KIT- or PDGFRA-driven neoplasms; indeed, more than 95% of GISTs express KIT, and more than 80% have gain of function mutations of *KIT* or *PDGFRA* [2]. The reported frequencies of *KIT* and *PDGFRA* mutations are 80 and 5–8%, respectively [3].

GISTs were rarely diagnosed before the seminal paper by Hirota et al. [4] demonstrated the oncogenic role of KIT and identified KIT immunohistochemistry as an aid in diagnosis. The demonstration of the major therapeutic effects of the KIT inhibitor imatinib on GISTs necessitated that pathologists be able to reliably distinguish this tumor from smooth muscle and peripheral nerve derived tumors [5]. The demonstration of gain-of-function mutations of *PDGFRA* is even more recent [6]. Thus, most of the data on GISTs are based on retrospective studies of patients referred to oncology centers.

At the time of presentation, 11–47% of patients with a GIST are metastatic [1, 7]. After surgical resection of localized GISTs, the risk of relapse varies from 0 to 90% [8], and 5-year survival is 50% [9]. Up to 22% of patients older than age 50 may have a micro-GIST under 10 mm in diameter [10]; however, most of these patients are never diagnosed and do not require any specific therapy. Nevertheless, it is critically important to evaluate the prognosis of primary GISTs, particularly the risk of metastasis after the resection of localized tumors. The first classification of relapse risk, known as NIH consensus, was an expert consensus based on mitotic index and size of the tumor [11]. More recent classifications, based on retrospective series of patients, also include the primary tumor site as well as the presence/absence of tumor rupture [8, 12, 13]. Adjuvant treatment with imatinib was recently shown to improve the disease-free survival of GIST patients with a primary tumor more than 3 cm [14]. Subgroup analysis of this study disclosed that the benefit of imatinib was mainly observed in patients with large tumors and/or tumors with high mitotic index (i.e., high-risk tumors). Accordingly, in Europe, adjuvant treatment with imatinib is restricted to patients with significant risk of relapse. This emphasizes the major impact of risk classification of GISTs in the routine clinical management of patients.

MolecGIST is a prospective French population-based study, whose purpose was to evaluate the incidence of GISTs in France and to determine the clinical and pathological characteristics of patients as well as the spectrum and frequency of *KIT* and *PDGFRA* mutations.

Methods

Study design

MolecGIST is a prospective population-based study of GISTs. Inclusion criteria were diagnosis of GIST between May 31, 2006 and May 30, 2008 in a patient living in France. Cases were identified by pathologists, surgeons, gastroenterologists, oncologists or any other physicians or by patients themselves. MolecGIST study was approved by GSF/GETO (sarcoma French Group), FFCD (Digestive Cancer French Group), AFC (Surgeon French Society) and SFP (Pathology French Society) and supported by Ensemble Contre le GIST (GIST Patients French Association). Communication on MolecGIST was performed by these Societies, by the member of the scientific committee and by Internet (http://www.gist-france.org). After the identification of the patients, clinical records were reviewed, and the initial pathologist was contacted to send a tumor sample to one of the 11 reference centers for histology review and *KIT* and *PDGFRA* mutation detection. Signaling of incident cases was performed by pathologists, physicians, surgeons and/or patients themselves. In two regions of France (Aquitaine and Rhone–Alpes), the signaling was completed by systematic review

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of all the cases diagnosed as GISTs or sarcomas in any pathology laboratory of the region.

MolecGIST was approved by French ethical committee (CPPRB Saint Germain en Laye #06029, April 24th 2006). According to this institutional review board, informed consent was not required because it was not an interventional trial, but each patient was informed by its physician and had the possibility to refuse inclusion in this study. Clinical, histological and molecular data were collected in the MolecGIST data center (Ambroise Paré hospital, Boulogne, France). Follow-up of the patients was planned in MolecGIST study and is ongoing, but still needs additional grants to be completed.

Histology and molecular analyses

All samples were reviewed in one of the 11 reference centers. The diagnosis of GIST was based on histology, immunohistochemistry and molecular pathology. KIT (CD117) immunostaining was performed on all cases, and DOG-1 staining was performed on KIT negative cases. Other immunostains were performed at the discretion of the reviewing pathologist. Mitotic index was evaluated on 5 mm^2 of tumor, corresponding to 20–25 high power fields, depending on the microscope used [8].

Mutational analyses were performed when tumor blocks were available. DNA was extracted after histologic review, from tumor areas containing at least 80% of tumor cells. Screening of mutations was performed by LAPP or DHPLC [15, 16]. Mutations of *KIT* exons 9, 11, 13 and 17 and *PDGFRA* exons 12, 14 and 18 were identified by sequencing of PCR products. The three main reference centers (Ambroise Paré Hospital, Bergonié Institut and E-douard Herriot Hospital), who performed 68% of the mutational analyses, were included in an international external quality control program [17].

Statistical analysis

Data were described using percentage for qualitative variables and mean, median and range for numerical variables. Chi-squared test was used to compare the distributions of qualitative variables, while the analysis of variance was used to compare distributions of continuous quantitative variables, ordinal variables being compared by a non-parametric Kruskal–Wallis method. Tests were considered as significant for $P < 0.05$. All statistical analyses were performed using SAS 9.1.

Strength of association between a given mutation and advanced disease was estimated by odds ratio, as localized GISTs of MolecGIST and advanced GISTs of two phase III trials (SWOG S0033 and EORTC 62005) could be considered as a case–control study.

Results

Nine hundred and sixteen cases were reported, corresponding to 879 patients. Cases were identified by pathologists (86.0%), gastroenterologist (7.4%) or patients themselves (2.4%). Samples were sent by 348 pathologists, and 68% of the samples were sent to 3 of the 11 reference centers. Most of the exclusions were due to a date of initial diagnosis out of the inclusion period, and five patients were excluded because they lived outside of France. In total, 596 patients were included in the study.

The median age at the time of diagnosis was 64.0 year (range, [56–75]). Female and male patients accounted for 47.3 and 52.7%, respectively (Fig. 1a). Primary tumors were localized within stomach (56%), small intestine (30%), rectum (3%) or were in other organs (11%) (Fig. 1b). The median tumor size was 50 mm (mean, 67.3 ± 57.7 mm; range, [2–350]) (Fig. 1c). One hundred and forty-one (27.4%) of the GISTs had a mitotic index higher than 5/5 mm^2 (Fig. 1d).

At the time of diagnosis, 10.0% of GISTs had synchronous metastases, 1.6% were locally advanced and 88.4% were resectable, of whom 15 (3%) were not resected. Among localized GISTs ($n = 491$), 270 (55.0%) of GISTs were intermediate to high risk according to NIH consensus risk classification (Fig. 2a) [11], while 182 (37.1%) were moderate to high risk according to AFIP classification (Fig. 2b) ($P < 0.0001$) [8]. Thirty (6.1%) of localized GISTs were localized outside the gastrointestinal tract and could therefore not be classified with AFIP classification [8]. Twenty-five (83%) of these latter cases were intermediate or high risk according to NIH consensus classification.

Identification of GIST cases was exhaustive in two regions (Aquitaine and Rhone–Alpes) because of specific organization, but was not exhaustive in the other regions of France. Comparison of clinical with pathological data was carried out between exhaustive and other regions. The mean age, sex, size of tumor, the localization (gastric/extra-gastric), status at diagnosis and mitotic index were not different (Table S1), suggesting that both populations were identical.

Mutation detection has been completed in 492 patients, corresponding to 82.6% of MolecGIST series. In 11.9% of cases, tumor DNA quality was not sufficient for analysis, and in 5.5%, only slides were available. *KIT* and *PDGFRA* mutations were detected in 70.7 and 15.0% of tumors, respectively, while 14.2% GISTs were not mutated. Mutations localized within exon 9 and 11 of *KIT* and exon 18 of *PDGFRA* accounted for 396 (94.1%) of all the mutations (Fig. 3a). All *KIT* exon 9 mutations were duplications, while *KIT* exon 11 mutations were deletions 152 (48.9%), substitutions 106 (34.1%), duplications/

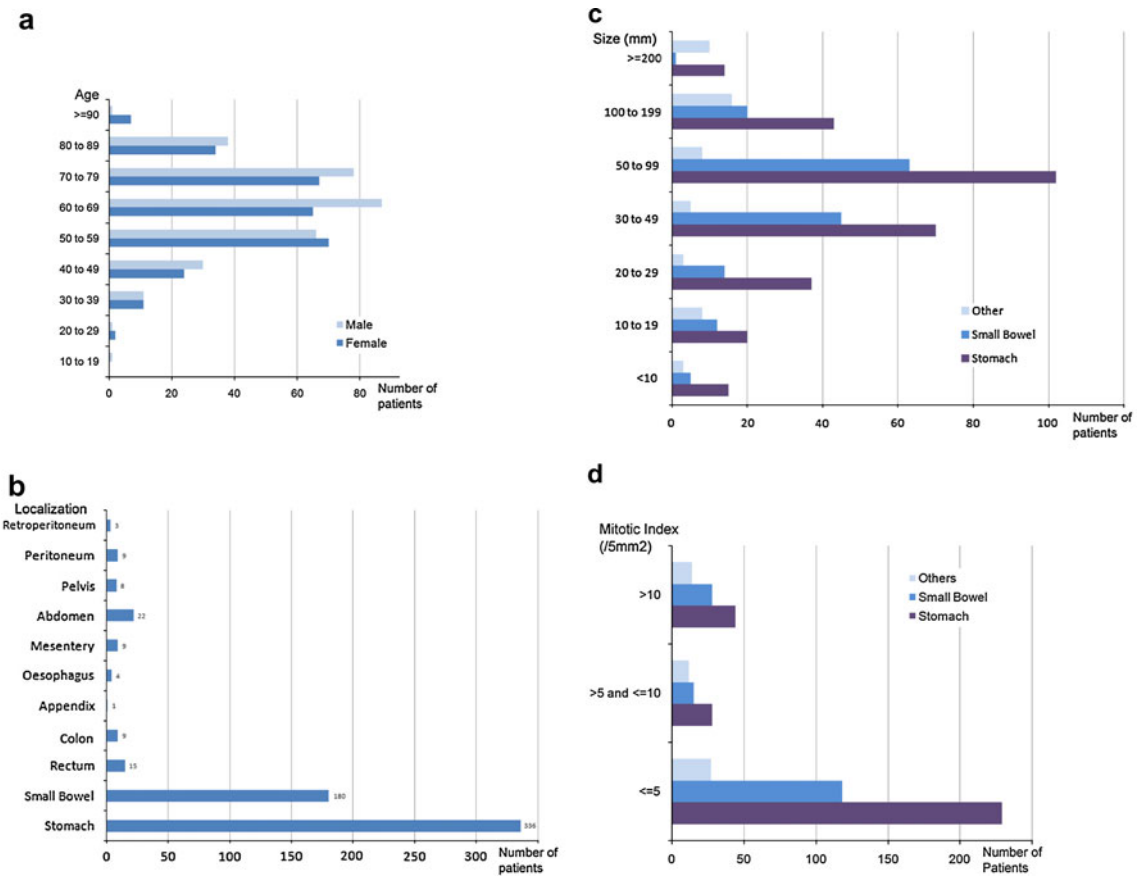

Fig. 1 Characteristics of the patients included in MolecGIST at the time of diagnosis. **a** Age and sex of the patients. **b** Primary localization of GISTs. **c** Size of GISTs (mm). **d** Mitotic index of GISTs (/5 mm^2)

insertions 18 (5.8%) or more complex mutations 35 (11.3%). Altogether, 138 different mutations were detected in MolecGIST series (not shown). Among them, seven mutations were present in more than 3.5% of mutated GISTs (Fig. 3b) and could therefore be specifically correlated with clinical and pathological data. Additionally, some other mutations have been grouped: *PDGFRA* exon 18 other than D842V, *KIT* exon 11 duplications/insertions, *KIT* exon 11 deletions involving Tyr568 and/or Tyr570, because specific analyses of such groups have already been published in other series [18–21]. These 10 most frequent mutations or groups of mutations accounted for 238 (56.2%) of all mutated GISTs.

Patients with small bowel GISTs were significantly younger than the others ($P < 0.0001$) (Table S2). The mean size of gastric, small intestine and other tumors was 64.9, 59.1 and 105.7 mm, respectively ($P < 0.0001$). GISTs with high mitotic index were significantly larger than the others ($P < 0.0001$). Primary localizations of GISTs with available mutational status were similar to those of the whole MolecGIST series, suggesting that both populations were identical. *PDGFRA* exon 18 mutations were associated with primary gastric localization, while *KIT* exon 9 mutations were almost exclusively extra-gastric (Table 1).

The frequency of mutations in population-based MolecGIST was compared with that of 772 patients with advanced GIST included in the SWOG S0033 and EORTC62005 phase III studies and reported in MetaGIST study [22]. The frequencies of *KIT* mutant, *PDGFRA* mutant and non-mutated patients were 82.3, 2.1 and 15.6 in MetaGIST and 70.7, 15.0 and 14.2% in MolecGIST ($P < 0.0001$). We then sought to determine more precisely which mutations were more frequent in localized GISTs. We compared the frequency of the 10 most frequent mutations of the patients with localized GISTs of our series with patients with advanced GISTs of updated SWOG S0033 and EORTC 92005 series (Table 2). Frequencies of six of these mutations were significantly different. Mutations of *PDGFRA* exon 18 (both D842V and others exon 18 mutations) were associated with local disease by an odds ratio of 7.9 and 3.1, respectively. Two substitutions in *KIT* exon 11 (W557R and V559D) were also more likely to be associated with local disease by an odds ratio of 2.7 and 2.5, respectively. By contrast, duplication in *KIT* exon 9 (502_503dup) and deletion in *KIT* exon 11 (557_558del)

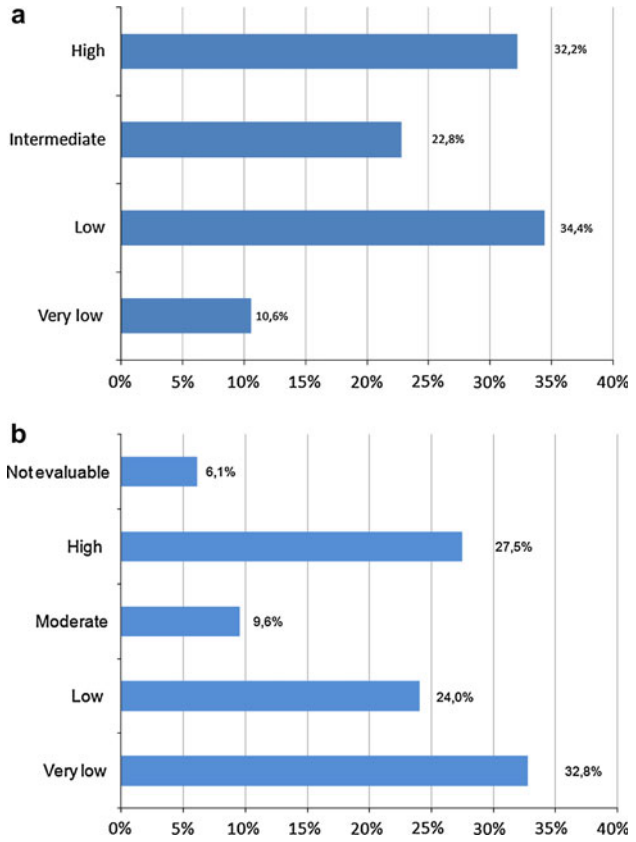

Fig. 2 Stage and risk evaluation of the patients included in MolecGIST. **a** Risk assessment, according NIH consensus [11]. **b** Risk assessment, according to AFIP classification [8]

were more likely to be associated with advanced GISTs by an odds ratio of 0.3 and 0.5, respectively.

The frequencies of *KIT* mutant, *PDGFRA* mutant and non-mutated patients were 38 (66.7%), 5 (8.8%) and 9 (15.8%) in the 57 patients of MolecGIST with advanced GISTs.

Discussion

We report, herein, a large prospective population-based study of GISTs conducted in France between May 31, 2006 and May 30, 2008. During this 24-month period, 596 patients were included, while the other referred cases were excluded mainly because the date of initial diagnosis was outside of the inclusion period. Pathology review and molecular analyses were not centralized, but concentrated from 348 initial pathologists to 11 centers, three of which having received 68% of the samples. Characteristics of the patients living in two regions where inclusion was exhaustive were similar to those of patients in the other French regions.

GISTs were originating from stomach, small intestine, rectum and other localizations in 56, 30, 2.5 and 11%, respectively.

Adjuvant treatment with imatinib has recently been shown to delay significantly GIST relapse [14]. In our series, 77% of patients underwent surgical resection of a GIST of at least 3 cm, as those included in this adjuvant pivotal study [14]. Evaluation of risk of GIST recurrence is of main importance to determine which patients could benefit from adjuvant treatment. Most of the published data are based on retrospective series [8, 12], of which only few were population based. In most of the series, the mitotic index was shown to be the main prognostic factor. Widely used risk classifications [8, 11, 12], as well as international expert recommendations [23], indicate that mitotic count should be evaluated on 50 high power field (HPF). Unfortunately, "50 HPF" may correspond to a surface of approximately 5–12 mm^2, depending on the microscope used. AFIP classification is based on the count on 5 mm^2 [8], and Miettinen and Lasota indicated that so-called 50 HPF correspond to 20–25 HPF on modern microscopes. In a recently published large trial on patients with localized GISTs of at least 3 cm [14], the mitotic count was performed on 50 "real" HPF corresponding to 11.8 mm^2. The proportion of patients with more than 5 mitosis/11.8 mm^2 was 38.7% [24], while 28.3% of patients with non-metastatic tumor included in MolecGIST had more than 5 mitosis/5 mm^2. Thus, the literature is confusing and probably interferes with risk evaluation and adjuvant decision in daily practice. In our series, 33% of the patients with very low/low risk could have been classified as intermediate/high risk, by a pathologist counting 50 "real" HPF instead of 5 mm^2. AFIP classification gives a sharper evaluation of risk than NIH consensus. Indeed, the proportion of patients in MolecGIST with moderate/high risk was 45% and 37% with NIH consensus and AFIP classifications, respectively ($P < 0.0001$). However, AFIP classification was not applicable in 6.1% of the patients, because the primary origin of the GISTs, which is not integrated in this classification [8]. Most of these tumors, involving abdomen, pelvis, peritoneum or mesentery were very large, and 83% were intermediate/high risk according to NIH consensus classification. In another population-based series, AFIP risk could not be calculated in 14% of cases [1]. Finally, the prognostic markers of GISTs were not independent. Indeed, we showed here that tumor size and mitotic index were correlated with primary localization ($P < 0.0001$). By contrast, tumor size and mitotic index were correlated with mutations.

Mutational status of GISTs was completed in 492 (83%) patients of MolecGIST. This result obtained with tumor samples from more than 200 different pathology laboratories is similar to 87% obtained in another

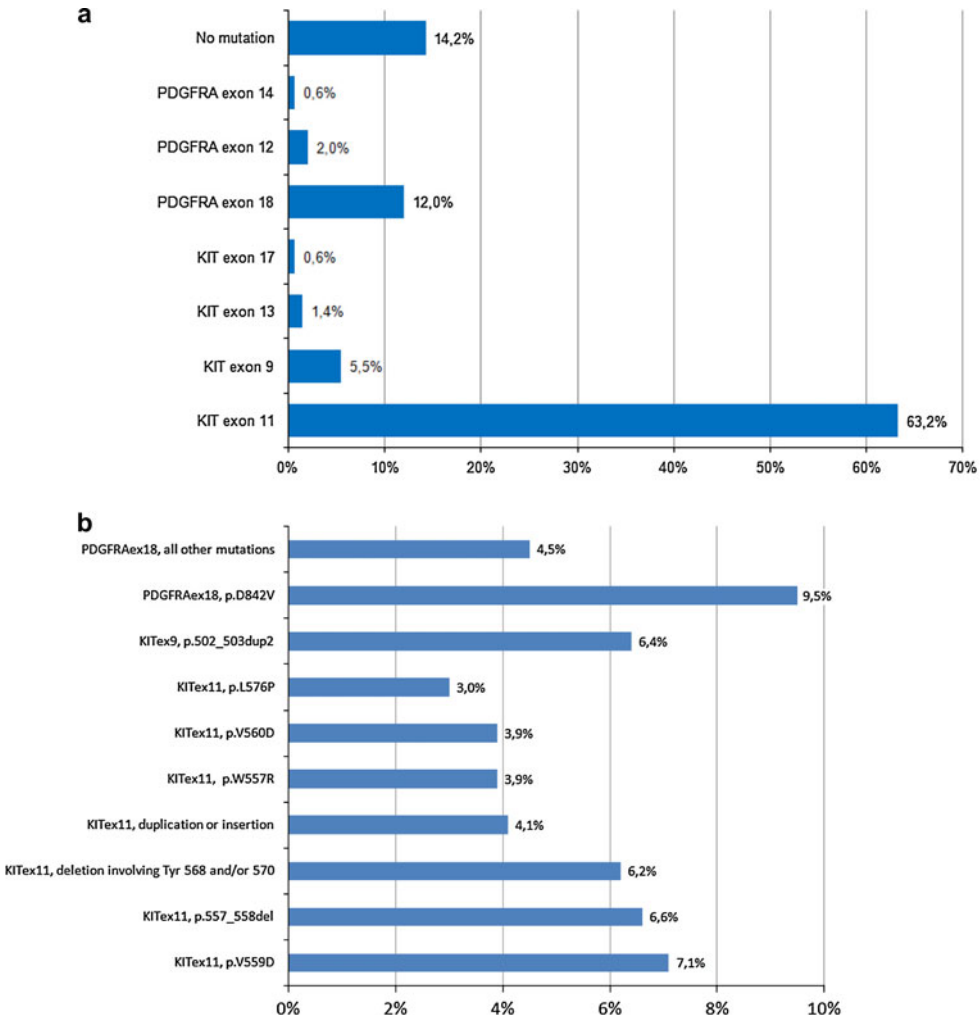

Fig. 3 Mutations of *KIT* and *PDGFRA* in GISTs. **a** Frequency of mutations in MolecGIST. **b** The 10 most frequent mutations in GISTs

Table 1 Primary localizations of the most frequent mutations

	N	Localization Stomach/small bowel/others	% Stomach	*P**
All cases	596	336/180/80	56.4	
All mutated GISTs	422	236/130/56	55.9	
KITex11, p. V559D	31	21/6/4	67.7	NS
KITex11, p.557_558del	29	18/7/4	62.1	NS
KITex11, deletion involving Tyr 568 and/or 570	27	12/12/3	44.4	NS
KITex11, duplication or insertion	18	15/3/0	83.3	0.03
KITex11, p.W557R	17	10/5/2	58.8	NS
KITex11, p. V560D	17	9/6/2	52.9	NS
KITex11, p. L576P	13	7/6/0	53.8	NS
KITex9, p.502_503dup2	27	1/22/4	3.7	<0.0001
PDGFRAex18, p. D842V	40	31/1/8	77.5	0.004
PDGFRAex18, all other mutations	19	14/1/4	73.7	NS

* Comparison between gastric and extra-gastric localization

population-based study from a single center in Northern Norway [25]. Frequencies of *KIT* exon 11, *KIT* exon 9 and *PDGFRA* mutations were 311 (63.2%), 27 (5.5%) and 74 (15%), respectively. The frequency of *KIT* and *PDGFRA* mutations in our large prospective population-based series was different from that of patients with advanced GISTs prospectively included in phase III studies EORTC 62005 and SWOG S0033 ($P < 0.0001$).

Table 2 The most frequent mutations in patients with localized GIST of MolecGIST (population based) compared to patients with advanced GIST in SWOGS0033 and EORTC92005

Mutation	Localized MolecGIST (%)	Advanced SWOG S0033 (%)	Advanced EORTC92005 (%)	All advanced (%)	*P*	Odds ratio [CI]
KITex11, p. V559D	27 (6.2)	13 (2.4)	9 (2.4)	22 (2.5)	0.001	2.5 [1.4–4.5]
KITex11, p. 557_558del	23 (5.3)	57 (10.5)	26 (6.9)	83 (9.6)	0.007	0.5 [0.3–0.8]
KITex11, deletion involving Tyr 568 and/or 570	22 (5.0)	20 (3.7)	4* (6.0)	24* (3.9*)	NS	1.4 [0.8–2.5]
KITex11, duplication or insertion	17 (3.9)	15 (2.8)	6 (1.6)	21 (2.4)	NS	1.6 [0.9–3.1]
KITex11, p. W557R	16 (3.7)	9 (1.7)	3 (0.8)	12 (1.4)	0.007	2.7 [1.3–5.8]
KITex11, p. V560D	16 (3.7)	15 (2.8)	4 (1.1)	19 (2.2)	NS	1.7 [0.9–3.3]
KITex11, p. L576P	13 (3.0)	13 (2.4)	8 (2.1)	21 (2.4)	NS	1.2 [0.6–2.5]
KITex9, p. 502_503dup2	21 (4.8)	56 (10.3)	58 (15.4)	114 (13.1)	<0.0001	0.3 [0.2–0.5]
PDGFRAex18, p. D842V	37 (8.5)	7 (1.3)	3 (0.8)	10 (1.2)	<0.0001	7.9 [3.9–16.1]
PDGFRAex18, all other mutations	17 (3.9)	7 (1.3)	4 (1.1)	11 (1.3)	0.002	3.1 [1.5–6.8]
All genotyped	437	542	377	867		

* Data available for only 67 patients of the EORTC study

Indeed, the frequency of *PDGFRA* mutations was 74 (15%) and 2.1% in MolecGIST and MetaGIST, respectively. Retrospective studies suggested that GISTs with *KIT* exon 9 mutation may have a bad prognosis [18], while GISTs with *PDGFRA* mutations may have a better prognosis [19]. Furthermore, *KIT* exon 11 557_558del was reported to be of poor prognosis [20]. However, these studies were retrospective [26]. Furthermore, most included a few patients or included patients referred to oncology center, which may result in a statistical bias. Recently, a prognostic study was reported on a series of patients treated in 17 Polish Hospitals [27]. It showed that deletions involving W557 or K558 or exon 9 mutations had a worse relapse-free survival (RFS) than those with PDGFRA and KIT mutations. Unfortunately, the survival data were obtained from only 68% of the patients included in this later study, and survival analysis did not distinguish patients with initially localized or metastatic GIST. Altogether, the prognostic value of *KIT*/*PDGFRA* mutations was reported in several retrospective and one prospective series, but still needs to be confirmed. Thus, we sought to determine more precisely which mutations were more frequent in localized than in advanced GISTs. However, 138 different mutations were detected in MolecGIST. Grouping some of the numerous mutations may impact the results. Thus, grouping should not be performed or should be decided before statistical tests and/or be sustained by biology of tyrosine kinase receptors. We therefore focused on the 10 most frequent mutations, accounting for 56% of the mutated GISTs. The frequencies of 6/10 mutations were different in localized and advanced GISTs. *PDGFRA* exon 18 mutations and *KIT* exon 11 substitutions W557R and V559D were more frequent in localized GISTs by an odds ratio ranging from 2.5 to 7.9, while *KIT* exon 9 502_503dup and *KIT* exon 11 557_558del were more frequent in metastatic GISTs by an odds ratio of 0.3 and 0.5, respectively. However, these odds ratios cannot be considered as relapse risk and only show which mutations are more likely to be present in localized versus metastatic GISTs. Geographical differences might also be responsible for the difference of frequencies of *KIT*/*PDGFRA* mutations. Indeed, the frequency of *PDGFRA* mutations was 2.8, 5, 7.3, 11, 12.9 and 18% in a Chinese [28], US [2], Brasilian [29], Italian [30], Polish [27], and French [1] series, respectively. Interestingly, 71% of patients were metastatic in the Chinese series, while only 11.5 and 12% in the Polish and French series, respectively. Thus, the frequency of *KIT*/*PDGFRA* mutations is more likely related to the number of metastatic patients than to their ethnic origins.

Evaluation of GIST prognosis after surgical resection is mandatory to determine which patients could benefit from adjuvant treatment. The most widely used classifications are based on expert consensus or retrospective studies and do not integrate the mutational status. Furthermore, the lack of standardized evaluation of mitotic count is responsible for bad reproducibility of this major prognostic factor. Forthcoming methods for evaluation of GIST prognostic should integrate the molecular status and be validated on prospective series of patients.

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