# KIT, PDGFRA, and BRAF Mutational Spectrum Impacts on the Natural History of Imatinib-naive Localized GIST

## *A Population-based Study*

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**Abstract:** The mutation status of *KIT* or *PDGFRA* notoriously affects the response of advanced gastrointestinal stromal tumors (GISTs) to tyrosine kinase inhibitors. Conversely, it is currently still unclear whether mutation status impinges on the prognosis of localized, untreated GISTs. Hence, at present, this variable is not included in decision making for adjuvant therapy. A series of 451 primary localized GISTs were analyzed for *KIT*, *PDGFRA*, and *BRAF* mutations. Univariable and multivariable analyses and a backward selection procedure were used to assess the impact of mutation status on overall survival and to identify prognostically homogenous groups. Mutation was a significant prognostic indicator of overall survival in naive, localized GISTs ($P < 0.001$): *KIT*-mutated patients had a worse outcome than *PDGFRA*-mutated or triple-negative (*KIT, PDGFRA, BRAF* wild-type) cases. Multivariable Cox regression models allowed us to identify 3 molecular risk groups: group I exhibited the best outcome and included *PDGFRA* exon 12, *BRAF*, and *KIT* exon 13-mutated cases; group II, of intermediate clinical phenotype (HR = 3.06), included triple-negative, *KIT* exon 17, *PDGFRA* exon 18 D842V, and *PDGFRA* exon 14-mutated cases; group III displayed the worst outcome (hazard ratio = 4.52), and comprised *KIT* exon 9 and exon 11 and *PDGFRA* exon 18 mutations apart from D842V. This study highlights the prognostic impact of mutation status on the natural course of GIST and suggests that the molecular prognostic grouping may complement the conventional clinicopathologic risk stratification criteria in decision making for adjuvant therapy.

**Key Words:** GIST, *KIT*, *PDGFRA*, *BRAF*, SDH, primary, localized, naive, overall survival, risk assessment, adjuvant therapy, population-based study

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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract, typically exhibiting activating mutations of either *KIT* or *PDGFRA* tyrosine kinases.[1] GISTs display variable clinical behavior, from indolent to highly aggressive, and a variable response to imatinib or other tyrosine kinase inhibitors (TKI).[1] Risk for progression is currently assessed according to tumor size, anatomic site, and mitotic activity.[2–6] Notably, whereas the predictive value of *KIT*/*PDGFRA* mutations toward TKI is well established,



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the role of these mutations in the prognosis of localized, untreated GIST is still unclear. Although activation of the *KIT*/*PDGFRA* pathway is insufficient to confer a "fully malignant phenotype,"[7] several observations suggest that it impacts on the natural course of the disease. Specifically, the clinical behavior of GIST is reported to be influenced by: the genetic target of mutation (with *PDGFRA*-mutated cases pursuing a more indolent course compared with *KIT*-mutated cases), the exon that is mutated (with *KIT* exon 9 mutations, essentially consisting of A502_Y503dup, associated with unfavorable outcome), the specific codon implicated (with *KIT* exon 11 W557_K558del considered to carry a poor prognosis), the nature of the mutation (with *KIT* exon 11 deletions being associated with lower disease-free survival than missense mutations or internal tandem duplications).[8–20]

Very recently, 2 prospective studies,[20,21] one based on the placebo arm of the ACOSOG Z9001 trial and the other on the conticaGIST registry, reached somewhat discordant conclusions on the influence of genotype on the disease-free survival of patients with completely resected GISTs. Whereas the former reported no impact of *KIT*/*PDGFRA* status on patients' outcome,[21] the latter demonstrated the prognostic value of specific types of mutations in the context of gastric GIST.[20]

Hence, whether *KIT*/*PDGFRA* mutations have an impact on the prognosis of localized naive GIST remains controversial, and there is debate about how best to include these data in the portfolio of information available to the clinician for use in decision making for adjuvant therapy. This is particularly relevant in the context of intermediate-risk GIST, a sort of "gray area" for which no consensus exists on whether they should or should not be treated with imatinib.[22]

Bearing this in mind, we interrogated a well-characterized preimatinib population-based series of localized, surgically resected primary GISTs[5] to shed light on the clinical impact of mutation status on the natural history of GIST.

## MATERIALS AND METHODS

### Case Series

This study relies on 451 localized, surgically resected primary GISTs, retrieved from a previously reported series of centrally reviewed GISTs.[5] GIST diagnosis was based on morphology, CD117 and DOG1 expression, and exclusion of the entities within the differential diagnosis. The study inclusion criteria were: GIST $>2$ cm; GIST yielding DNA suitable for molecular analysis; patients of any age, who had been surgically treated; no neoadjuvant or adjuvant therapy; no other malignant tumor beside GIST; no history of neurofibromatosis type 1 or other familial tumors (7 neurofibromatosis type 1 cases from the previous series were excluded from the study). Informed consent was obtained from all living patients in accordance with national legislation. The expression of 2 major components of the SDH complex, SDHB (1:750, pH9 WB, clone 21A11 0; Abcam) and SDHA (1:3000, pH9 WB, clone 2E3GC12FB2AE2; Abcam), was evaluated on tissue microarrays of GIST without *KIT*/*PDGFRA*/*BRAF* mutations. Tumors were considered positive in the presence of granular cytoplasmic staining. No or faint staining was only interpreted as negative when internal positive controls, be they endothelial or inflammatory cells, stained positively. On the basis of these criteria, SDHB and SDHA expression could be assessed in 41 and 38 cases testing negative for *KIT*/*PDGFRA*/*BRAF* mutations, respectively.

### Molecular Analyses

DNA was extracted from representative blocks of formalin-fixed/paraffin-embedded tissues with tumor cellularity $>80\%$. Sections of 10 μm thickness were deparaffinized by serial xylene/ethanol washings. DNA was extracted using the EZ1 Biorobot (Qiagen GmbH) and amplified by polymerase chain reaction, according to previously described polymerase chain reaction conditions.[7,23] Mutation analysis was performed by Sanger sequencing using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The different exons were sequentially screened in the following order until mutation was detected: *KIT* exon 11, *KIT* exon 9, *PDGFRA* exon 18, *PDGFRA* exons 12 and 14, *KIT* exons 13 and 17. Cases devoid of *KIT*/*PDGFRA* mutation were further investigated for *BRAF* exon 15 mutations.[7]

### In Silico Analysis

Three in silico predictors (SIFT, PolyPhen2, Mutation taster) were used to estimate the possible pathogenetic effect of novel missense mutations.

### Statistical Analyses

The main endpoint of the study was overall survival (OS), which was calculated as the time from the date of diagnosis until the date of death or last contact, in the case of survivors. OS curves were estimated using the Kaplan-Meier method and compared by the log-rank test.

Multivariable Cox regression models were used to investigate the potential prognostic value of mutation variables, in addition to the conventional clinical/pathologic variables (patients' age, tumor site and size, and mitotic index) included in our previously developed nomogram.[5] To this end, an ordinal variable was generated whose levels were the different exons ordered according to the corresponding 10-year OS subgroup. The variable was then coded using a dummy scheme demonstrated to be useful when the objective is to identify contrasts in the dependent variable between successive levels of an independent ordinal variable.[24] We used a backward selection procedure based on the Akaike information criterion[25] to select the mutation variable dummies. By repeatedly eliminating the dummies, contiguous levels are merged at each step. At the end of the process, the selected dummies can classify patients into prognostically heterogenous groups. The performance of the final model and—for the sake of comparison—that of

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the model including the clinical/pathological variables only were quantified using Harrell et al's[26] *C* statistics. This is a measure of discriminative ability, assuming values between 0.5 and 1.0, where a value of 1.0 indicates perfect performance. A bootstrap procedure[27] was adopted to adjust the *C* statistic estimate for the optimism implicit in the use of sample data for model fitting and variable selection. The final model was also used to estimate the adjusted OS curves in the identified prognostic groups. Given that an infinite number of curves could, ideally, be estimated—as the model contained continuous covariates such as patients' age, tumor size, or mitotic index—the adjusted curves were obtained for each group by averaging the curves estimated according to the observed combinations of the clinical variables.

A nomogram for OS prediction was obtained from the final Cox model including mutation variable prognostic groups together with all the variables already considered in our previously developed nomogram.[5] In accordance with the previous nomogram, age was modeled as a stratification factor ($\leq 65$, $> 65$ y).

The analyses were performed using SAS and R software. We considered a statistical test to be significant when the corresponding 2-sided *P* value was $< 0.05$.

Categorical variables were compared between groups with a $\chi^2$ or Fisher exact test where appropriate. The Kruskal-Wallis test was used to compare continuous variable distributions.

## RESULTS

### Molecular Subgroups

In this series of 451 analyzed GISTs, 392 (86.9%) carried mutations in *KIT* (292 cases; 64.7%), *PDGFRA* (95 cases; 21.1%), or *BRAF* (5 cases; 1.1%). The remaining 59 (13.1%) were negative for *KIT, PDGFRA,* and *BRAF* mutations (hereafter named triple-negative GIST). (Table 1 and Supplementary Table S1, Supplemental Digital Content 1, http://links.lww.com/PAS/A270).

Specifically, *KIT* mutations affected exon 11 in 253/292 cases (86.6%), 21 of which were W557_K558del, and exon 9 in 32 cases (11.0%), 30 of which (94.0%) were A502_Y503dup. No solitary deletion was detected at either 557 or 558. *PDGFRA* mutations involved exon 18 in 78/95 cases (82.1%), 60 of which were D842V. The *BRAF* mutation detected in 5 cases was the canonical V600E substitution.

The mutation was clearly in homozygosity/hemizygosity in 13 *KIT*-mutated and 2 *PDGFRA*-mutated cases.

Eight novel *KIT* mutations were identified: 7 in exon 11 (2 deletions, 1 insertion, and 4 deletion-insertions) and 1 P456L substitution in exon 9. Three novel *PDGFRA* point mutations were also identified: 2 (R822C, M844R) in exon 18 and 1 in exon 14 (G652R). All 4 novel amino acid substitutions were classified as likely to be affecting protein function according to 3 in silico predictors.

A fraction of GISTs devoid of *KIT*/*PDGFRA* mutation are reported to display reduced SDH complex activity, which can be revealed by loss of SDHB staining.[1,28,29] Among the triple-negative GISTs of our series (59 cases), we were able to assess the expression of SDHB in 41 cases and SDHA in 38 cases, respectively. Loss of SDHB expression was observed in 9/41 cases (24%); loss of SDHA was observed only in 1 case (1/38, 2.6%) and was associated with concomitant loss of SDHB. Notably, all SDH-deficient cases were gastric tumors, in agreement with literature data,[1,28,29] and represented one third of triple-negative gastric GISTs.

### Comparison of Overt GIST Versus Small GIST

The rates and spectrum of mutations of this series were compared with those of a previously characterized series of small-GISTs ($< 2$ cm), including micro-GIST ($< 1$ cm).[7] Compared with the small-GIST set, this series of overt GISTs displayed a higher overall rate of *KIT*/*PDGFRA* mutations (85.8% vs. 74.1%, $P < 0.01$), especially *KIT* exon 11 mutations (56.1% vs. 45.9%, $P < 0.05$), suggesting that these mutations most likely sustain a rapid malignant progression. Conversely, the relative *KIT* versus *PDGFRA* mutation ratio and frequency of prognostically relevant *KIT* mutations (A502_Y503dup and W557_K558del) were similar between the 2 groups.

### Correlation of Mutation Status and SDH Immunoreactivity With Clinicopathologic Parameters

Mutations and SDH immunoreactivity were then analyzed in relation to conventional clinicopathologic parameters, namely size, mitotic index, and site (Table 2).

#### Size

*PDGFRA* exon 12 and 14 mutations tended to be associated with large tumor size, suggesting that these mutations have a more indolent behavior.

#### Mitotic Index

GISTs carrying the *KIT* W557_K558 deletion displayed a higher median mitotic index than the whole series (8 mitoses/50 HPF vs. 3 mitoses/50 HPF, $P = 0.02$).

#### Site

The proportion of *KIT* W557_K558del was higher in the rectum subgroup compared with other locations ($P < 0.05$), and rectal GISTs are notoriously aggressive tumors.[30] In agreement with the literature, most *PDGFRA* mutations (91/95) clustered in the stomach ($P < 0.0001$), with the sole exception of 3 small intestinal GISTs carrying exon 12 mutations (P581S, D591N) and 1 duodenal tumor carrying the common D842 substitution. The canonical *KIT* exon 9 A502_Y503dup (22/30, 73%) ($P < 0.0001$) and the *BRAF* V600E substitution (5/5) clustered in the small intestine. The 2 *KIT* exon 9 point mutations (P456L, S476N) were found in the stomach.


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**TABLE 1.** Frequencies of *KIT, PDGFRA,* and *BRAF* Mutations

| Gene | Exon | Cases (%) | Mutation Subgroup | Cases (%) |
|---|---|---|---|---|
| *KIT* | 9 | 32 (7.1) | A502_Y503dup | 30 (6.6) |
| | | | Others | 2 (0.4) |
| | 11 | 253 (56.1) | W557_K558del | 21 (4.7) |
| | | | Point mutations 559/560 | 60 (13.3) |
| | | | Point mutations 557/558 | 18 (4.0) |
| | | | Internal tandem duplication | 14 (3.1) |
| | | | Others | 140 (31.0) |
| | 13 | 4 (0.9) | | |
| | 17 | 3 (0.6) | | |
| *PDGFRA* | 12 | 10 (2.2) | | |
| | 14 | 7 (1.6) | | |
| | 18 | 78 (17.3) | D842V | 60 (13.3) |
| | | | Others | 18 (4.0) |
| *BRAF* | 15 | 5 (1.1) | V600E | 5 (1.1) |
| *KIT*/*PDGFRA*/*BRAF* | — | — | Triple-negative | 59 (13.1) |

SDH-deficient tumors were all gastric (9/9), diagnosed at a younger age (median age at diagnosis: 54 y for SDH-deficient tumors vs. 66 y for the whole series), with a predominance in female individuals (6 female vs. 3 male). No patient had a history of other tumors, including paraganglioma.

## Association Between Mutational Status and OS

We have previously proposed a nomogram to predict OS on the basis of patients' age, tumor location, size, and mitotic index. In our series, OS represented a valid surrogate of disease-specific survival in the below-65 age stratum.[5]

We compared the OS of the 5 major genotypes (*KIT* vs. *PDGFRA* vs. *BRAF* vs. triple-negative/SDH-positive, and triple-negative/SDH-negative). As there was no significant difference in the OS of triple-negative/SDHB-proficient and triple-negative/SDHB-deficient cases in our series ($P = 0.56$), these cases were taken as a whole (triple-negative tumors) in subsequent analyses.

Significant heterogeneity ($P < 0.001$) was observed at univariable Kaplan-Meier analysis: *KIT*-mutated patients displayed a lower OS than did *PDGFRA*-mutated and triple-negative patients, who showed comparable rates. Specifically, 120-month OS (95% confidence interval [CI]) was: 100% for *BRAF*, 67.5% (58.1%-78.3%) for *PDGFRA*, 62.5% (50.5%-77.5%) for triple-negative, and 46.3% (40.6%-52.9%) for *KIT*. Among the *KIT*-mutated cases, *KIT* W557_K558del displayed a trend toward worse survival.

The inclusion of mutation data in a multivariable Cox model, together with conventional clinicopathologic variables (size, mitotic index, site), led to the identification of mutation patterns with similar OS through a backward selection procedure.

This approach enabled us to distinguish 3 molecular prognostic groups (Table 3, Fig. 1A): group I, the group with the best outcome, included a limited number of patients carrying either *PDGFRA* exon 12, *BRAF*, or *KIT*

**TABLE 2.** Distribution of *KIT, PDGFRA, BRAF* Mutation and SDHB Loss of Expression According to Mitotic Index, Tumor Size, and Site

| | ***KIT* (Exon/Mutation)** | | | | | ***PDGFRA* (Exon/Mutation)** | | | | ***BRAF*** | **Triple Negative*** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **9** | **11 W557_ K558del** | **11 Other** | **13** | **17** | **12** | **14** | **18 D842V** | **18 Other** | **15 V600E** | **All** | **SDH⁻** | **SDH+** |
| MI | | | | | | | | | | | | | |
| Median | 3.5 | 8 | 3.5 | 4 | 5 | 2.5 | 4 | 2 | 3 | 3 | 3 | 4 | 3 |
| Mean | 8.9 | 20.4 | 14.6 | 3.5 | 6 | 3.9 | 3.9 | 2.5 | 9.7 | 5 | 11.9 | 7.4 | 12.1 |
| Size (cm) | | | | | | | | | | | | | |
| Median | 6 | 6 | 6 | 5.3 | 7.5 | 8.5 | 8 | 6 | 6.5 | 5.4 | 6 | 4 | 6 |
| Mean | 7.4 | 7 | 8.2 | 4.9 | 5.9 | 8.3 | 8 | 6.5 | 8.2 | 6.7 | 7.4 | 4.7 | 7.4 |
| Site | | | | | | | | | | | | | |
| Stomach | 4 | 17 | 134 | 0 | 1 | 7 | 7 | 59 | 18 | 0 | 38 | 9 | 18 |
| Small intestine | 22 | 0 | 67 | 2 | 0 | 3 | 0 | 0 | 0 | 5 | 16 | 0 | 11 |
| Rectum | 3 | 3 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Duodenum | 1 | 0 | 8 | 2 | 1 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 1 |
| Colon | 1 | 1 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Omentum | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Retroperit. | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*SDHB expression could be assessed in 41/59 triple-negative GISTs.
MI indicates mitotic index.

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**TABLE 3.** Multivariable Cox Model Results

| | Current Model (451 pts) | | | Previous Model (526 pts; Rossi et al[5]) | | |
|---|---|---|---|---|---|---|
| | HR | CI | Wald Test *P* | HR | CI | Wald Test *P* |
| Age at diagnosis (y)* | | | < 0.0001 | | | < 0.0001 |
| 72 vs. 54 | 2.91 | (2.32-3.65) | | 2.82 | (2.29-3.46) | |
| Tumor site | | | 0.016 | | | 0.013 |
| SI vs. Stom | 1.10 | (0.81-1.51) | | 1.13 | (0.86-1.50) | |
| Colon/Rect vs. Stom | 1.34 | (0.80-2.24) | | 1.58 | (0.99-2.51) | |
| Other vs. Stom | 3.05 | (1.50-6.21) | | 2.62 | (1.32-5.21) | |
| Tumor size (cm)* | | | 0.010 | | | 0.047 |
| 10 vs. 4 | 1.56 | (1.15-2.12) | | 1.37 | (1.05-1.80) | |
| MI (/50 HPF)* | | | < 0.0001 | | | < 0.0001 |
| 8 vs. 1 | 2.06 | (1.59-2.68) | | 2.09 | (1.64-2.67) | |
| Molecular groups | | | 0.001 | | | |
| Group II vs. group I | 3.06 | (1.09-8.58) | | | | |
| Group III vs. group I | 4.52 | (1.65-12.37) | | | | |

Group I: BRAF exon 15, *KIT* exon 13, *PDGFRA* exon 12.
Group II: triple-negative, *KIT* exon 17, *PDGFRA* exon 14, *PDGFRA* exon 18 D842V.
Group III: *KIT* exon 9, *KIT* exon 11, *PDGFRA* 18-non842.
*The 2 values are the third and first quartile, respectively.
HR indicates hazard ratio; MI, mitotic index; Rect, rectum; SI, small intestine; Stom, stomach.

exon 13 mutations; group II, with an intermediate prognosis, included triple-negative GISTs, *KIT* exon 17, *PDGFRA* exon 14-mutated cases, and *PDGFRA* D842V cases (group II vs. group I: hazard ratio = 3.06; 95% CI: 1.09-8.58); the worst outcome was observed in group III, comprising the canonical *KIT* mutations (exons 9 and 11) and *PDGFRA* exon 18 mutations other than D842V, hereafter named exon 18-non842 (group III vs. group I: hazard ratio = 4.52; 95% CI: 1.65-12.37). The mutation status was an independent significant prognostic factor ($P = 0.001$), and its inclusion resulted in a slight improvement in the discriminative ability ($C = 0.732$ vs. 0.725) of our previously proposed model.[5] Notably, ex-post analysis of our tumor series highlighted the potential prognostic ability of the molecular signature to further stratify patients within the low-moderate or high categories according to AFIP/Miettinen criteria[3] (Figs. 1B, C; compare with fig. 1C in Rossi et al[5]).

The correlations between clinical course and mutation status were further confirmed within anatomic GIST subgroups. OS in *KIT* exon 9 and *KIT* exon 11-mutated GISTs was similar, even among small intestinal GISTs ($P = 0.742$; Fig. 2A). This is in contrast with the alleged unfavorable effect exerted by exon 9 mutations.[8,13,14,16] In addition, within the gastric group, the *KIT* mutation conveyed an adverse effect compared with the *PDGFRA* exon 18 mutation: 120-month OS was 47.3% and 66.2%, respectively ($P = 0.027$; Fig. 2B).

In agreement with literature data, the majority of GISTs (56.1%) in the present series carried a *KIT* exon 11 mutation. In an attempt to further stratify this large group, we compared the clinical behavior of exon 11 point mutation versus deletion. No significant difference was observed in the 120-month OS, although mutations seemed to perform slightly better (mutations 48.2%, 95% CI: 37.9%-61.4%; deletions: 43.4%, 95% CI: 35.6%-52.9%; $P = 0.766$).

*KIT* exon 11 encodes for the protein juxtamembrane domain, which can be essentially divided into a proximal region (11-PR, codons 550-561), including the "allosteric binding site," and a distal region (11-DR, codons 562-591), comprising 2 tyrosine residues that are key for KIT activation.[31] We then hypothesized that mutations involving different parts of exon 11 might have different effects on KIT-driven tumorigenesis. On these grounds, we partitioned the *KIT* exon 11-mutated cases into 2 subgroups: a first subgroup (209 cases) with gene alterations encompassing 11-PR; a second subgroup (44 cases) mutated in 11-DR. Although there was no significant difference in the OS curves at 10-year follow-up ($P = 0.341$), there was a clear-cut trend toward poorer prognosis for the 11-PR subgroup within the first 5 years after surgery (Fig. 2C).

On comparing cases with point mutations versus structural variants (deletions/duplications/insertions) at 11-PR, a trend toward lower, albeit not statistically significant, OS was observed for the latter group (Fig. 2D). Similarly, cases with internal tandem duplications at 11-DR (14 cases) tended to perform better within the first years after surgery compared with other alterations in the same region (47 cases) (Fig. 2E).

The outcome of homozygous/hemizygous mutated patients was strikingly worse in the first years after surgery, indicating that loss of the wild-type allele conveys an aggressive phenotype, in agreement with previous studies.[32–34] Of the 15 cases with homozygous/hemizygous mutations, 8 patients did in fact die within the 52-month follow-up, 1 after 153 months.

In an effort to translate this body of information in the clinical arena, we tentatively integrated the molecular categorization into a nomogram developed from the multivariable Cox model through a backward selection procedure. In this nomogram (Supplementary Fig. 1, Supplemental Digital Content 2, http://links.lww.com/PAS/A271), the


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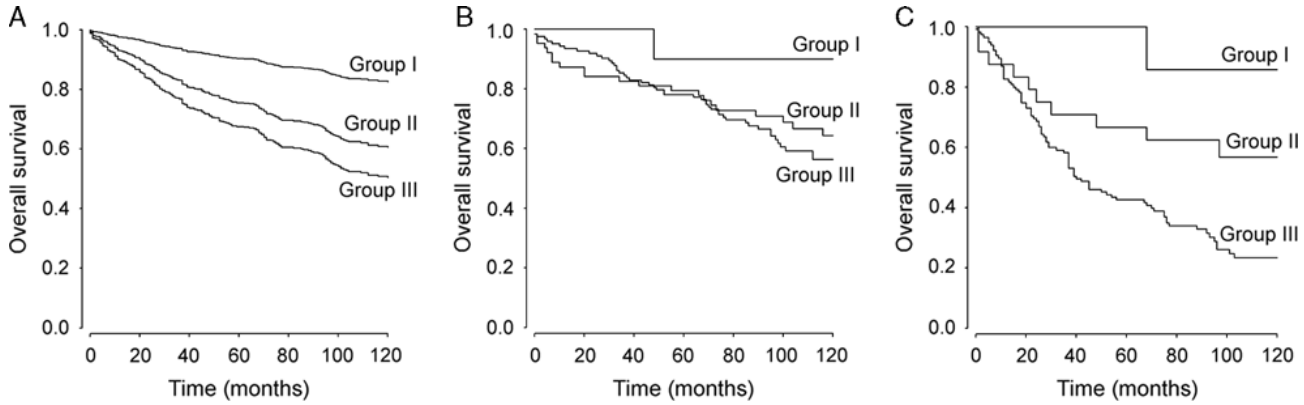


**FIGURE 1.** Patient stratification according to the molecular signature. A, Kaplan-Meier OS curves, estimated by multivariable Cox regression analyses, according to the 3 molecular prognostic groups: group I (*BRAF* exon 15, *KIT* exon 13, *PDGFRA* exon 12); group II (triple-negative, *KIT* exon 17, *PDGFRA* exon 14, *PDGFRA* exon 18 D842V); group III (*KIT* 9, *KIT* 11, *PDGFRA* 18-non842). B, OS according to the proposed molecular prognostic groups in the set of patients classified as low-moderate risk on the basis of the AFIP/Miettinen criteria. C, OS according to the proposed molecular prognostic groups in the set of patients classified as high risk on the basis of the AFIP/Miettinen criteria.

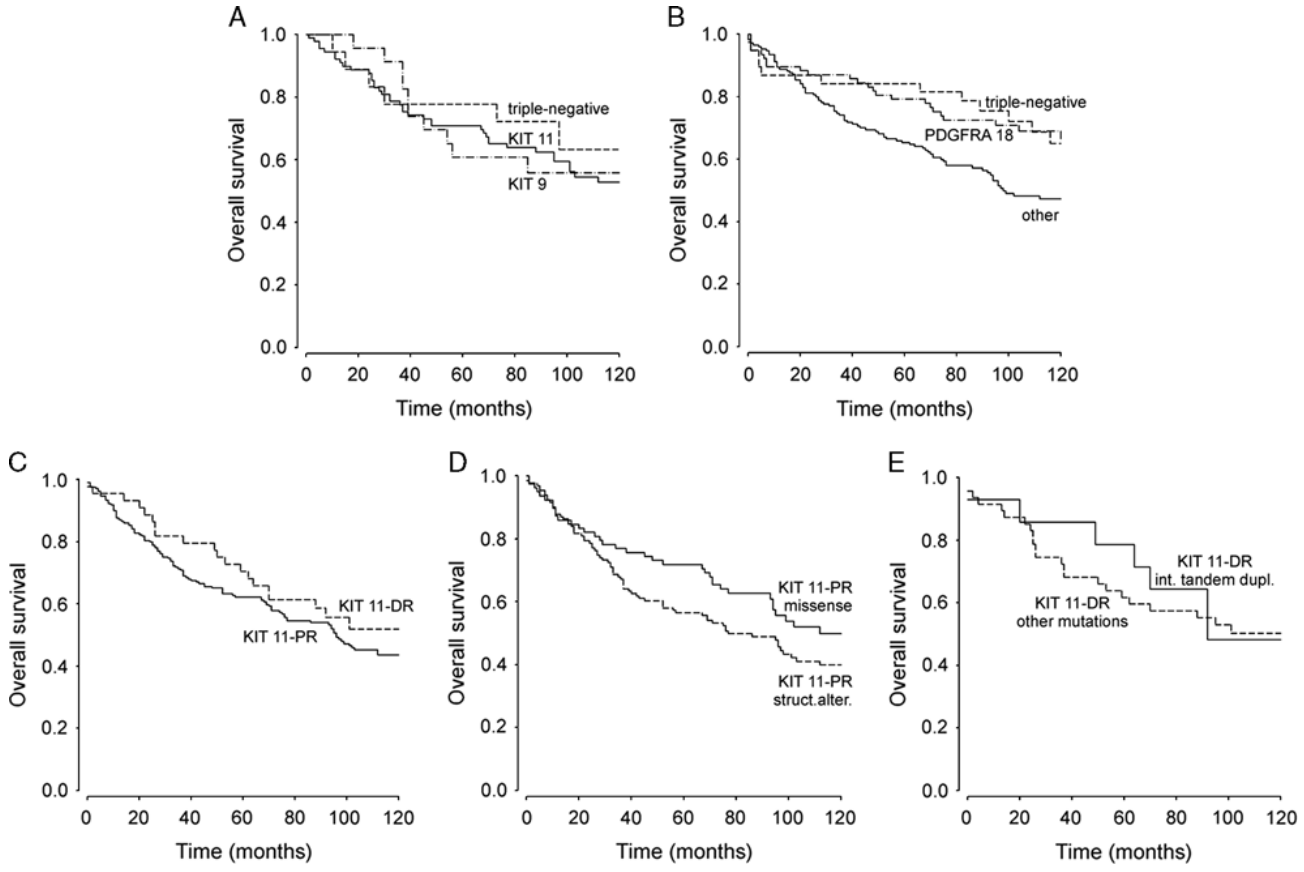


**FIGURE 2.** Association between specific tumor genotypes and OS. A, OS in patients with small intestinal GIST by *KIT* status. Dashed line: triple-negative; dash-dot line: *KIT* exon 9-mutated GIST; solid line: *KIT* exon 11-mutated GIST. B, OS in patients with gastric GIST by *KIT*/*PDGFRA* status. Dashed line: triple-negative; dash-dot line: *PDGFRA* exon 18-mutated GIST; solid line: other mutations. C, OS in patients mutated in *KIT* exon 11: proximal region (11-PR, solid line) versus distal region (11-DR, dashed line). D, OS in patients with *KIT* exon 11 proximal region (11-PR) mutations, according to the type of mutation: missense mutation (solid line) versus structural alterations (dashed line). E, OS in patients with *KIT* exon 11 distal region (11-DR) mutations, according to the type of mutation: internal tandem duplication (solid line) versus other mutations (dashed line).

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mitotic index appeared to make the greatest contribution to survival prediction, followed by molecular risk group, tumor size, and tumor site. Of course, these inferences are preliminary and need to be validated in an independent tumor series of primary imatinib-naive GISTs to draw definitive conclusions.

## DISCUSSION

This paper focuses on the relevance of *KIT*, *PDGFRA*, and *BRAF* mutation status on the natural clinical course of naive, localized GISTs. Although the role of *KIT*/*PDGFRA* mutations in predicting the response to TKI in the context of advanced GIST is widely documented,[1] the impact of mutation status on the natural course of GIST is less defined. In the untreated setting, imatinib-sensitizing mutations do not necessarily convey a good prognosis, and, vice versa, so-called "resistance mutations" may correlate with relatively favorable outcome in the absence of any treatment.[1,8] The prognostic value of tumor genotype, integrated with clinical and pathologic information, might assist the clinician in decision making for adjuvant therapy, especially in the setting of intermediate-risk GIST.

To this end, a large series of localized, surgically resected primary GIST with long follow-up (median 10 y) were analyzed for *KIT*, *PDGFRA*, and *BRAF* gene alterations. Mutation data were then included in a multivariable Cox model, together with conventional clinicopathologic variables, and mutation patterns with similar OS were uncovered through a backward selection procedure.

This approach identified 3 distinct molecular prognostic groups with increasing aggressiveness: group I (*PDGFRA* exon 12, *BRAF*, *KIT* exon 13), group II (triple-negative, *KIT* exon 17, *PDGFRA* exon 14, and *PDGFRA* D842V), group III (*KIT* exon 9, *KIT* exon 11, *PDGFRA* exon 18-non842).

Some of the mutations (eg, *BRAF, KIT* exon 13/17, *PDGFRA* ex 12/14) were represented by a limited number of cases, preventing firm conclusions from being drawn about these genotypes. Nonetheless, several interesting observations did emerge from our analysis. First of all, *KIT*-mutated cases clustered in the group with the worst prognosis (group III), whereas the vast majority of *PDGFRA*-mutated tumors fell either in group I or II. Gastric location is known to be a favorable prognostic factor.[3] Yet the expression of a *PDGFRA*-mutated allele had a favorable effect in this setting too, corroborating the added value of *PDGFRA* status on disease outcome over anatomic location per se.

D842V is the most common *PDGFRA* mutation and is also the major cause of primary resistance to imatinib therapy.[1] Hypothesizing that this mutation may underlay a distinctive pathogenetic effect, we sought to analyze D842V-mutated cases separately from the other exon 18-mutated cases. Interestingly, we found that the D842V-resistant mutation, along with *PDGFRA* exon 12 and exon 14 mutations, belonged to the favorable groups (I and II), whereas exon 18-non842 abnormalities belonged to the aggressive group (III). The opportunity of stratifying patient risk according to the *PDGFRA* mutation spectrum may provide an additional rationale for excluding from adjuvant therapies GIST patients with a low probability of relapse and/or those unlikely to respond to imatinib because of the D842V-resistant mutation (groups I and II). Conversely, adjuvant therapy could be contemplated for those cases that, ceteris paribus, exhibit *PDGFRA* exon 18 imatinib-sensitive mutations (group III).

Our findings on the favorable prognostic value of most *PDGFRA* mutations are in line with the population-based studies from the Polish and French groups.[10,14] However, in the former study, *PDGFRA* point mutations were reported to show relapse-free survival comparable to *KIT* mutations other than A502_Y503dup and W557_K558del.[10] In our series, however, with the exception of those clustering in group III (*PDGFRA* exon 18-non842), *PDGFRA* mutations displayed a more favorable prognosis compared with *KIT* mutations considered as a whole. Moreover, differently from the Polish study, our study does not include metastatic cases and is also strengthened by a significantly longer follow-up period (33 vs. 120 mo), as it is well known that, in the context of GIST, certain mutations may have a "time-dependent" prognostic value.[15]

Only 5 tumors in our series (7.8%), all small intestinal, bore the V600E *BRAF* mutation, supporting the notion that BRAF activation has a marginal role in GIST pathogenesis.[1] The clinical impact of this mutation in the context of GIST has not yet been established. The small number of cases with this alteration does not allow us to draw definitive conclusions but the fact that *BRAF* mutations clustered in group I may suggest a positive prognostic effect.

The triple-negative category included 9 SDH-deficient GISTs, all gastric. Again, the limited number of cases prevents us from assessing whether SDH deficiency impacts clinical outcome. Notably, there was no significant difference between the OS of SDH-proficient and SDH-deficient patients in our series.

Triple-negative GIST and *PDGFRA*-mutated GIST displayed similar OS, confirming their indolent behavior[1,28] and strengthening the rationale for the exclusion of these patients from adjuvant therapy.[21]

Another important message arising from our study is that the clinical course of tumors carrying *KIT* exon 11 mutations does not significantly differ from that of GIST with exon 9 mutation, essentially consisting of A502_Y503dup. This finding somehow "goes against the flow" of common belief that the exon 9 mutation, typically detected in small intestinal GIST, is prognostically worse than the exon 11 mutation. The exon 9 mutation has been demonstrated to occur more frequently in GISTs that were metastatic at diagnosis.[10,14] We believe this information represents a snapshot of the disease status at a given stage of evolution and does not take into account other tumor characteristics that may significantly


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impinge on the clinical course of the disease over time. Moreover, exon 9 A502_Y503dup was claimed to have an unfavorable effect compared with exon 11 mutations before it was acknowledged that tumor site plays a major prognostic role.[16] Hence, the conclusions were probably biased by the prevalence of this mutation in intestinal GIST, notoriously characterized by a poor outcome.[3] When we restricted the analysis to small intestinal GIST, we failed to observe a significant difference in the clinical behavior of *KIT* exon 9 versus exon 11-mutated tumors, thereby corroborating the major prognostic role of anatomic location over exon 9 mutation. Accordingly, both the Polish team[10] and the conticaGIST study[20] reported no difference in relapse-free survival for exon 9 versus exon 11-mutated GISTs, and DeMatteo and coworkers provided evidence that the association of exon 9 with aggressive evolution loses value in multivariable analysis.[13]

The present study confirms our previous observation that exon 11 mutations are more common in overt GIST than in micro-GIST.[7] This further argues against the "favorable" nature of these mutations and, we believe, should caution clinicians about excluding patients from adjuvant therapy on the basis of the assumption that *KIT* exon 11 point mutations are associated with indolent behavior.

Although the unfavorable prognostic value of *KIT* exon 11 mutations overall was striking, no statistically significant differences across the different types of genetic alterations of this exon were observed. Some previous studies linked W557_K558del to a more aggressive phenotype, compared with missense mutations.[9–14] Exon 11 internal tandem duplications, typically located at the distal end of the exon, have instead been associated with a relatively good prognosis,[8] intermediate between deletions and missense mutations.[10] Given the high complexity of the exon 11 mutation spectrum, we separately analyzed the cases carrying mutation in the proximal region (11-PR)—including the "allosteric binding site" that stabilizes KIT in the autoinhibitory state—from those with mutations in the distal region (11-DR), comprising tyrosines Y568 and Y570 responsible for KIT activation.[31] Interestingly, mutations at 11-PR tended to be associated with shorter OS, emphasizing the role of negative allosteric regulation of KIT in the tumor phenotype. Moreover, a trend toward reduced OS was observed for deletions/duplications/insertions at 11-PR, compared with missense mutations, hinting that disruptive alterations are more likely to perturb allosteric regulation and unleash KIT activity. An apparently better course was instead observed for tandem duplications at the 11-DR, in agreement with previous studies.[8,10] Notably, the trends observed in OS were generally more evident in the first years after surgery, consistent with the recently reported prognostic time dependence of deletions at codons 557-558.[15]

In summary, this study attempted to assess the role of the mutation spectrum as an additional risk stratification parameter for naive localized GIST. Overall, our data highlight the prognostic impact of mutation status on the natural course of GIST and confirm that triple-negative and most *PDGFRA*-mutated GISTs are relatively indolent tumors. Conversely, in contrast to common belief, our results indicate that both *KIT* exon 9 and *KIT* exon 11 mutations, as well as *PDGFRA* exon 18-non842 mutations, are unfavorable prognostic factors.

We consider that the molecular prognostic stratification may complement the portfolio of clinicopathologic information and support the clinician in decision making for adjuvant therapy. This may be particularly important for the gray area of GIST classified as intermediate risk according to conventional clinicopathologic parameters.

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