Biology of Human Tumors

Clinical Cancer Research

Tumor Genotype Is an Independent Prognostic Factor in Primary Gastrointestinal Stromal Tumors of Gastric Origin: A European Multicenter Analysis Based on ConticaGIST

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Abstract

Purpose: Although the mutational status in gastrointestinal stromal tumors (GIST) can predict the response to treatment with tyrosine kinase inhibitors, the role of tumor genotype as a prognostic factor remains controversial. The ConticaGIST study sought to determine the pathologic and molecular factors associated with disease-free survival (DFS) in patients with operable, imatinib-naive GIST.

Experimental Design: Clinicopathologic and molecular data from 1,056 patients with localized GIST who underwent surgery with curative intention (R0/R1) and were registered in the European ConticaGIST database were prospectively obtained and reviewed. Risk of tumor recurrence was stratified using the modified NIH criteria. The median follow-up was 52 months.

Results: On testing for potential prognostic parameters, the following were associated with inferior DFS on multivariable Cox model analysis: primary nongastric site, size >10 cm, mitotic index >10 mitoses per 50 high power field, and the *KIT* exon 9 duplication [hazard ratio (HR), 1.47; 95% confidence interval (CI), 0.9–2.5; $P = 0.037$] and *KIT* exon 11 deletions involving codons 557 and/or 558 [*KIT*del-inc557/558; HR, 1.45; 95% CI, 1.0–2.2; $P = 0.004$]. Conversely, *PDGFRA* exon 18 mutations were indicators of better prognosis [HR, 0.23; 95% CI, 0.1–0.6; $P = 0.002$]. *KIT*del-inc557/558 were an adverse indicator only in GIST localized in the stomach ($P < 0.001$) but not in tumors with nongastric origin. In gastric GIST, all other mutations presented remarkably superior 5-year DFS.

Conclusions: In conclusion, tumor genotype is an independent molecular prognostic variable associated with gastric GIST and should be used for optimizing tailored adjuvant imatinib treatment. *Clin Cancer Res; 20(23); 6105–16. ©2014 AACR.*

Introduction

Gastrointestinal stromal tumors (GIST) are rare mesenchymal neoplasms of the gastrointestinal tract, characterized by the expression of KIT protein, detectable in about 95% of tumors. Constitutively activating mutations in the genes coding for receptor tyrosine kinase *KIT* (70%–75% of cases) or platelet-derived growth factor receptor alpha (*PDGFRA*; 10%–14% of cases) play a crucial role in the biology of these tumors, and are critical therapeutic targets (for review see ref. 1). The mutations of the *KIT* gene in GIST occur most frequently in *KIT* exon 11 (juxtamembrane domain), followed by *KIT* exon 9 (extracellular domain); less often, primary mutations in the ATP-binding pocket (exon 13) or activation loop (exon 17) are found. Primary *PDGFRA* mutations are usually present in the activation loop (exon 18; 90% of cases); among them, *PDGFRA*

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

In 2008, imatinib was granted accelerated approval by the FDA for adjuvant use in gastrointestinal stromal tumors (GIST). Eligibility for this treatment relies on well-established risk assessment criteria (i.e., tumor site, size and mitotic count, and tumor rupture). Given the costs and potential side effects of imatinib therapy, the identification of additional factors for accurate relapse prediction is important for the proper management of patients. In the present work, the prognostic value of the tumor *KIT*/*PDGFRA* mutational analysis was investigated in a large prospective series of patients with localized GIST. Our findings indicate that evaluation of tumor genotype has prognostic value especially in GIST of gastric origin. The presence of *KIT* exon 11 deletions, including codons 557 and/or 558, or *PDGFRA* exon 18 mutations could be used as a new parameter to estimate the risk of relapse in gastric GIST for an optimal selection of patients for adjuvant therapy.

p.D842V mutation is the most common. Approximately 10% to 15% of adult GIST and 85% of pediatric GIST, defined as *KIT*/*PDGFRA* "wild type" (WT), lack *KIT* or *PDGFRA* mutations. These tumors are highly heterogeneous and profoundly different from *KIT*/*PDGFRA*-mutated GIST in terms of clinical behavior and molecular profiles, and they are now considered as separate pathologic entities (2).

The most common organ site for primary GIST is stomach (50%–60%), followed by small intestine (20%–30%), colon and rectum (10%), and esophagus (5%). Rarely, the tumors appear without attachment to gastrointestinal tract (so-called extragastrointestinal GIST). Complete surgical resection is possible in most localized GIST and constitutes the current standard of care. Although a significant proportion of patients will be cured with surgery alone, approximately 40% to 50% will eventually relapse, usually within the first 5 years (3, 4). The malignant potential in GIST varies from negligible in minute gastric GIST tumorlets to aggressive cancer (5, 6). The treatment of recurrent GIST is the first-line tyrosine kinase inhibitor, imatinib mesylate, if surgery is not an option. Over 80% of patients with advanced GIST benefit from imatinib treatment. Assessment of the risk of recurrence is important in the management of operable GIST, and needs to be estimated also when considering the use of adjuvant imatinib therapy, which is a widely accepted treatment standard in patients at higher risk of relapse (7). Among risk-stratification tools, the most widely used are the Armed Forces Institute of Pathology (AFIP) classification (8) and the modified NIH consensus (9). Primary tumor size, mitotic count, and tumor site are well-established risk factors for disease-free survival (DFS); tumor rupture has later been associated with the higher risk of relapse (9, 10). Moreover, in the last years the potential role of mutational status as a prognostic factor has been examined in a number of retrospective studies. In particular, it has been shown that deletions of *KIT* exon 11, especially those involving codon 557 and/or codon 558 (designated as *KIT*del-inc557/558), are associated with malignant behavior (11–14). Conversely, *KIT*/*PDGFRA* WT GIST and most *PDGFRA*-mutated GIST generally have a lower potential for malignancy (2, 15). However, the available data are still insufficient to be incorporated into routine clinical risk assessment.

In the present study, we assess the role of the tumor *KIT* and *PDGFRA* genotype along with key prognostic factors for DFS in localized, operable GIST in the so far largest dataset from 1,056 patients registered in the European ConticaGIST database.

Materials and Methods

Patients

We assessed the information from patients that were included in the ConticaGIST registry (https://conticagist.sarcomabcb.org) and diagnosed between January 1985 and April 2012 in 13 contributing institutions from four European countries. The majority of cases, i.e., all those diagnosed from 2001, were studied prospectively (83% of all). Each patient was assigned a registry code prior data transfer to ensure data anonymization. The study was approved by Ethics Boards of participating institutions. A small subset ($n = 127$) of the present cohort has been previously published (16). Only patients with primary GIST diagnosis who underwent surgery with curative intent (R0 or R1) were eligible. Pre- or postoperatively, the patients were not treated with any chemotherapeutic agents, including imatinib, until disease recurrence. From 1,625 patients with data transferred from the pooled series, 559 did not fulfill these eligibility criteria and were excluded from the study. Because of the small number of patients, we have also excluded 10 pediatric GISTs (age of diagnosis <18). Finally, a total of 1,056 patients were included for further analysis. DFS, defined as the duration from surgery to relapse (local recurrence or metastasis) or last follow-up, was used for prognosis evaluation. Relapse was determined based on routine imaging assessment and/or biopsy. The follow-up was updated on January 15, 2014. If patients were relapse free at the time of the last follow-up, they were censored for a disease recurrence.

Histology and mutational analysis

All samples were reviewed in one of the four reference centers [Institut Bergonié, Bordeaux, France ($n = 448$); M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland ($n = 279$); University Hospitals Leuven, Belgium ($n = 221$); and GIST and Sarcoma Registry North Rhine-Westphalia Germany ($n = 108$)] by expert sarcoma pathologists. The diagnosis of GIST was based on histology, immunohistochemistry, and molecular pathology. Eligible patients were required to have tumor morphology compatible with GIST and

positive immunostaining for KIT protein (CD117). Approximately 3% of tumors did not reveal KIT immunopositivity, and in such cases, the diagnosis was considered to be confirmed if a mutation was found in either *KIT* or *PDGFRA* genes. Mitotic index (MI) was evaluated per 50 high power fields (HPF), by examination of hematoxilin and eosin staining (H&E)-stained specimens. Risk stratification was defined according to the modified NIH classification (9).

Mutational analysis of *KIT* and *PDGFRA* was performed based on DNA isolated from formalin-fixed, paraffin-embedded (FFPE) or frozen material. DNA was extracted after histological review, from tumor areas containing at least 80% of tumor cells. Mutation of *KIT* exons 9, 11, 13, and 17 and *PDGFRA* exons 12, 14, and 18 was identified by Sanger sequencing of PCR products, according to the routine protocols available in participating institutions. The institutions, which performed over 95% of mutational analysis, were included in external, international quality control programs (17, 18). Mutation nomenclature followed the recommendations of the Human Genome Variation Society (www.hgvs.org).

Statistical analysis

For univariate analysis, the χ^2 or Fisher exact tests and Mann–Whitney test were used to compare categorical and continuous variables, respectively. Survival analysis was performed with the Kaplan–Meier method, and survival between groups was compared using the log-rank test. Univariate and multivariate Cox proportional hazard models were used to determine associations between variables of interest (tumor size, MI, tumor rupture, and tumor genotype) and DFS. The strengths of associations were summarized with hazard ratio (HR) and corresponding 95% confidence interval (CI).

The value of $P < 0.05$ was interpreted as statistically significant. Analyses were carried out with STATISTICA v.12 software package (StatSoft Inc).

Results

Clinicopathologic characteristics

Information about primary tumor size, MI, anatomical site, and mutational status was available from 996, 974, 1,050, and 1,056 patients, respectively (Table 1). The DFS data were available from 998 patients.

The majority of tumors were localized in the stomach (55.6%), followed by small intestine (28.1%), duodenum (5.8%), and colon and rectum (5.5%). The median tumor size was 6.0 cm (mean, 7.4 ± 5.3 cm; range, 0.2–40 cm), and 20.6% of tumors were >10 cm at initial diagnosis. The median number of mitoses was 4 per 50 HPF (mean, 13 ± 27/50 HPF; range, 0–300/50 HPF), with a mitotic rate >5/50 HPF documented in 38.8% of cases. Perioperative tumor rupture was recorded in 6.3% of patients. Using the modified NIH classification, the risk of relapse could be assessed in 957 cases: 7.7%, 36.4%, 17.5%, and 38.4% were very low, low-, intermediate-, and high-risk tumors, respectively. Patient's demographic and clinicopathologic characteristics are provided in Table 1.

Genotype analysis

Overall, mutations were identified in 899 (85.1%) cases (Table 1). The *KIT* or *PDGFRA* mutations were found in 751 (71.1%) and 148 (14.0%) samples, respectively. Mutations localized within exons 9 and 11 of *KIT* and exon 18 of *PDGFRA* accounted for 861 (96.8%) of all the mutations. All but one *KIT* exon 9 mutations were duplications, whereas *KIT* exon 11 mutations were mainly deletions (34%), followed by substitutions (19.7%) and duplications (6.6%). The *KIT*del-inc557/558 or specific p.W557_K558del were detected in 226 (21.4%) and 66 (6.3%) of all cases, respectively. Among other most frequent *KIT* mutations, six were substitutions (p.V559D, p.V560D, p.W557R, p.L576P, and p.V559D, and p.K642E) and one a deletion (p.V560del). Among *PDGFRA* mutations, the most prevalent was the p.D842V substitution (9.8% of all mutations and 65.2% of the *PDGFRA* exon 18 mutants). The 10 most frequent mutations were correlated with clinicopathologic data (Table 2).

As expected, the *PDGFRA* p.D842V mutation was associated mainly with primary gastric localization (93.2%), whereas *KIT* exon 9 duplication was found prevalently in nongastric tumors (90.9%). When compared with *KIT* p.W557_K558del, *KIT* p.A502_Y503dup had a lower median mitotic rate (6 vs. 16/50 HPF; $P = 0.005$), whereas there was no difference in tumor size for these mutants (7 vs. 8 cm; $P = 0.257$). Notably, *KIT* p.W557_K558del mutants equally segregated in gastric and nongastric sites (55% vs. 45%). Moreover, *KIT* p.W557_K558del was more frequently identified in patients ≤ 60 years (59% vs. 42.4%; $P = 0.01$), in tumors >5 cm (84.5% vs. 57.7%; $P = 0.0001$), with MI >5/50 HPF (68.9% vs. 39.4%; $P < 0.0001$), and classified as high risk (70.2% vs. 38.9%; $P < 0.0001$), when compared with other *KIT* exon 11 mutants. The median tumor size was 8.0 cm (mean, 9.1 cm; range, 1–30 cm), and median mitotic rate was 16/50 HPF (mean, 30.6/50 HPF; range, 0–300), and both values were significantly higher than in other *KIT* exon 11 mutants ($P < 0.0001$ for both). Notably, the clinicopathologic characteristics of tumors bearing *KIT* p.W557_K558del were comparable with the group of tumors with *KIT*del-inc557/558, within which tumor size, mitotic rate, and fraction of high-risk tumors were also significantly higher than in tumors with other *KIT* exon 11 mutants ($P < 0.001$; Table 3).

Survival analysis

During a median follow-up period of 52 months (range, 1–304 months), progressive disease was observed in 369 patients (34.9%). The median DFS in this group was 17.6 (range, 1–252) months.

For further analysis, only the most frequent tumor genotypes (*KIT* exon 9, *KIT*del-inc557/558, other *KIT*

Table 1. General characteristics of patients and tumors according to gastric or nongastric tumors' origin

	All cases	Gastric	Nongastric
	n = 1,056 (%)	*n* = 584 (%)	*n* = 466 (%)
Age (years)			
Median (range)	62.3 (19–98)	64.7 (19–98)	59.4 (20–95)
≤60	462 (43.8)	216 (37.0)	242 (51.9)
>60	594 (56.2)	368 (63.0)	224 (48.1)
Gender			
Female	531 (50.3)	293 (50.2)	235 (50.4)
Male	525 (49.7)	291 (49.8)	231 (49.6)
Tumor site			
Stomach	584 (55.6)	584 (100.0)	n/a
Small intestine	295 (28.1)	n/a	295 (63.3)
Duodenum	61 (5.8)	n/a	61 (13.1)
Colon or rectum	58 (5.5)	n/a	58 (12.4)
Others	52 (5.0)	n/a	52 (11.2)
Not available	6	n/a	n/a
Tumor size (cm)			
Median (range)	6.0 (0.2–40)	5.5 (0.2–40)	7 (0.3–30)
≤2.0	87 (8.7)	58 (10.2)	29 (6.8)
2.1–5.0	323 (32.4)	208 (36.9)	114 (26.6)
5.1–10.0	381 (38.3)	209 (37.1)	171 (39.8)
>10.0	205 (20.6)	89 (15.8)	115 (26.8)
Not available	60	20	37
Mitoses (per 50 HPF)			
Median (range)	4 (0–300)	3 (0–300)	5 (0–150)
≤5	596 (61.2)	368 (66.3)	226 (54.2)
6–10	128 (13.1)	68 (12.3)	60 (14.4)
>10	250 (25.7)	119 (21.4)	131 (31.4)
Not available	82	29	49
Tumor rupture			
Yes	54 (6.3)	9 (1.9)	44 (11.5)
No	800 (93.7)	460 (98.1)	337 (88.5)
Not available	202	115	86
Modified NIH classification			
Very low	74 (7.7)	54 (10.0)	20 (4.8)
Low	348 (36.4)	275 (50.7)	73 (17.7)
Intermediate	167 (17.5)	85 (15.7)	82 (19.8)
High	368 (38.4)	128 (23.6)	239 (57.7)
Not available	99	42	52
DFS			
Median (months)	101.4	143.4	47.8
DFS >5 years	267 (45.3)	155 (58.1)	109 (34.2)
DFS >10 years	44 (11.0)	22 (15.3)	22 (8.7)
Mutational status of *KIT*/*PDGFRA*			
KIT exon 9	78 (7.4)	7 (1.2)	69 (14.8)
KIT exon 11	648 (61.3)	350 (59.9)	295 (63.3)
*KIT*del-inc557/558	226 (21.4)	116 (19.9)	110 (23.6)
Deletion outside W557_K558	133 (12.6)	63 (10.8)	69 (14.8)
Substitution	208 (19.7)	109 (18.7)	99 (21.2)
Duplication	70 (6.6)	56 (9.6)	12 (2.6)
Complex	11 (1.0)	6 (1.0)	5 (1.1)
KIT exon 13	19 (1.8)	5 (0.9)	14 (3.0)

(Continued on the following page)

Table 1. General characteristics of patients and tumors according to gastric or nongastric tumors' origin (Cont'd)

	All cases	Gastric	Nongastric
	n = 1,056 (%)	*n* = 584 (%)	*n* = 466 (%)
KIT exon 17	6 (0.6)	2 (0.3)	4 (0.9)
PDGFRA exon 12	10 (0.9)	7 (1.2)	3 (0.6)
PDGFRA exon 14	3 (0.3)	2 (0.3)	1 (0.2)
PDGFRA exon 18	135 (12.8)	125 (21.4)	9 (2.0)
No mutation detected	157 (14.9)	86 (14.7)	71 (15.2)

NOTE: Data are numbers (%), unless otherwise indicated.

Abbreviations: *KIT*del-inc557/558, deletion including W557 and/or K558; n/a, not applicable.

exon 11 and *PDGFRA* exon 18 mutants) were taken into account. By the survival analysis, there was a significant difference when patients were grouped according to the tumor size, MI, location of the primary GIST, or mutational status (all $P < 0.001$; Fig. 1A–D). Overall, both *KIT* exon 9 and *KIT*del-inc557/558 mutants were associated with relatively equal and inferior DFS (median DFS in both groups, 45.5 months; 5-year DFS, 37.9% and 33.1%, respectively), whereas *PDGFRA* exon 18 mutation correlated with very favorable disease outcome (median DFS not reached; 5-year DFS, 75%) in comparison with other mutants. There was no significant difference in DFS of *PDGFRA* p.D842V versus other *PDGFRA* exon 18 mutants ($P = 0.21$; data not shown). Of note, however, within the dominant *PDGFRA* mutants of gastric origin, the vast majority that progressed (11 of 14) carried exon 18 *PDGFRA*-D842V substitution.

In univariate analysis, tumor size, MI, primary location, rupture, and mutational status were strongly associated with DFS (Table 4). In the multivariate analysis, all prognostic factors but tumor rupture remained significant for DFS (Table 4).

With regard to tumor genotype, *KIT* exon 9 and *KIT*del-inc557/558 were associated with an increased risk for tumor progression [HR, 1.47; 95% CI, 0.9–2.5 and 1.45 (95% CI, 1.0–2.2), respectively], whereas *PDGFRA* exon 18 mutation (HR, 0.23; 95% CI, 0.1–0.6) was associated with a better DFS.

Given the relatively high frequency of *KIT*del-inc557/558 mutants (overall 21.7%) with the equal distribution in gastric and nongastric sites, we have further explored the possible impact of this genotype on DFS, depending on the anatomical site of tumor origin. Importantly, in clear contrast with other *KIT* exon 11, *KIT* exon 9, and *PDGFRA* exon 18 mutations, the poor prognosticator of *KIT*del-inc557/558 on patients' survival was only significant in GIST localized in the stomach ($P < 0.001$; Fig. 1E), but not in tumors with nongastric origin ($P = 0.26$; Fig. 1F). The same associations were also evident when comparing *KIT*del-inc557/558 mutants with other exon

Table 2. Site, high-risk frequency, size, MI, and 5-year DFS of the 10 most frequent mutations

	All cases *n* (%)	Gastric *n* (%)	High risk *n* (%)	Median size, cm (range)	Median MI, per 50 HPF (range)	DFS >5 years *n* (%)
All cases	1,056	584 (55.3)	368 (38.5)	6 (0.2–40)	4 (0–300)	267 (45.3)
All mutated cases	899 (85.1)	498 (55.4)	323 (39.3)	6 (0.3–30)	4 (0–300)	228 (44.7)
PDGFRA ex. 18, p.D842V	88 (8.3)	82 (93.2)	19 (23.2)	6 (1–30)	2 (0–150)	26 (70.3)
KIT ex. 9, p.A502_Y503dup	77 (7.3)	7 (9.1)	42 (60.9)	7 (1–30)	6 (0–105)	21 (36.8)
KIT ex. 11, p.W557_K558del	66 (6.3)	36 (54.5)	40 (70.2)	8 (1–30)	16 (0–300)	14 (24.6)
KIT ex. 11, p.V559D	45 (4.3)	30 (66.7)	17 (38.6)	4.7 (0.3–20)	5 (0–80)	12 (48.0)
KIT ex. 11, p.V560D	38 (3.6)	17 (44.7)	12 (32.4)	5 (0.5–20)	5 (0–200)	12 (66.7)
KIT ex. 11, p.W557R	35 (3.3)	20 (57.1)	9 (27.3)	6 (2–24)	3 (0–30)	8 (61.5)
KIT ex. 11, p.L576P	20 (1.9)	10 (50.0)	4 (21.1)	5.5 (0.8–11)	3 (0–9)	7 (70.0)
KIT ex. 13, p.K642E	18 (1.7)	4 (22.2)	7 (41.2)	6 (1.5–14)	2 (0–45)	4 (80.0)
KIT ex. 11, p.V559G	18 (1.7)	7 (38.9)	3 (17.6)	3.5 (0.9–18)	2 (0–29)	7 (70.0)
KIT ex. 11, p.V560del	17 (1.6)	9 (52.9)	4 (26.7)	6 (0.7–15)	4 (0–150)	1 (25.0)

Abbreviation: ex, exon.

Table 3. The relationship between *KIT* exon 11 mutation type and clinicopathologic features of GIST

	***KIT*del-inc557/558**	**Other *KIT* exon 11 mutation**	
	***n* (%)**	***n* (%)**	***P* value**
Age (years)			
Median (range)	60 (19–93)	64 (21–98)	<0.0001[a]
≤60	113 (50.0)	173 (41.0)	0.028[b]
>60	113 (50.0)	249 (59.0)	
Gender			
Female	109 (48.2)	227 (53.8)	n.s.
Male	117 (51.8)	195 (46.2)	
Tumor site			
Gastric	116 (51.3)	234 (55.8)	n.s.
Nongastric	110 (48.7)	185 (44.2)	
n/a	0	3	
Tumor size (cm)			
Median (range)	7.0 (0.8–30)	5.8 (0.3–25)	<0.0001[a]
≤2.0	10 (4.9)	32 (8.0)	0.010[b]
2.1–5.0	54 (26.2)	145 (36.4)	
5.1–10.0	98 (47.6)	153 (38.4)	
>10.0	47 (22.8)	68 (17.1)	
n/a	17	24	
Mitoses (per 50 HPF)			
Median (range)	6 (0–300)	4 (0–200)	<0.0001[a]
≤5	95 (46.1)	250 (63.6)	<0.0001[b]
6–10	30 (14.6)	58 (14.8)	
>10	81 (39.3)	85 (21.6)	
n/a	20	29	
Tumor rupture			
Yes	18 (9.6)	19 (5.7)	n.s.
No	170 (90.4)	315 (94.3)	
n/a	38	88	
Modified NIH classification			
Very low	9 (4.3)	27 (6.9)	0.001[b]
Low	47 (23.6)	162 (41.6)	
Intermediate	33 (16.6)	69 (17.7)	
High	110 (55.3)	131 (33.7)	
n/a	27	33	
DFS			
Median (months)	45.5	118.5	<0.0001[c]
PFS >5 years	53 (33.1)	104 (47.5)	0.0051[b]
PFS >10 years	7 (5.5)	17 (12.1)	n.s.

NOTE: Data are numbers (%), unless otherwise indicated.

Abbreviations: *KIT*del-inc557/558, deletion including W557 and/or K558; n/a, not available; n.s., not significant.

[a]Mann–Whitney *U* test; [b]χ^2 test; [c]log-rank test.

11 *KIT* mutations, overall ($P < 0.0001$; Supplementary Fig. S1A) or comparing gastric ($P < 0.0001$; Supplementary Fig. S1B) and nongastric ($P = 0.599$; Supplementary Fig. S1C) GIST.

This phenomenon might be related to the observation that gastric GIST with *KIT*del-inc557/558 had a larger size (7 vs. 5.8 cm; $P < 0.0001$) and a higher mitotic rate (6 vs. 4/50 HPF; $P < 0.0001$) when compared with other *KIT* exon 11 mutants. Consequently, the fraction of patients with gastric GIST harboring *KIT*del-inc557/558 who relapsed 5 years after surgery was twice as high as patients with other *KIT* exon 11 mutations (61% vs. 29%, respectively; $P < 0.0001$).

Remarkably, even in tumors classified as non–high risk [(very)low and intermediate] according to modified NIH criteria, and originating from the stomach ($n = 320$), the

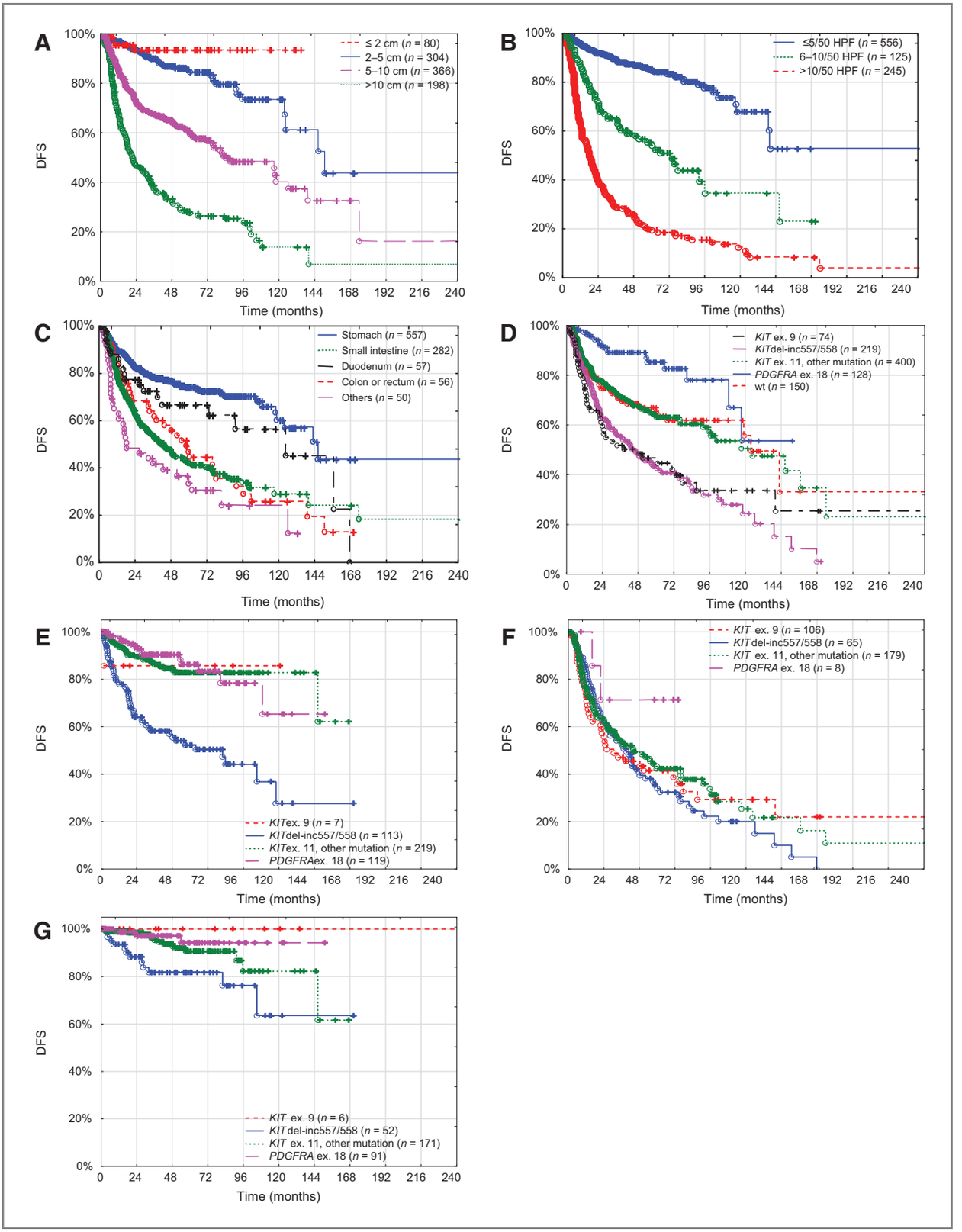

Figure 1. Disease-free survival (DFS) by tumor size (A), mitotic count per 50 HPF (B), tumor site (C), and mutational status (D); $P < 0.0001$ for all. DFS grouped according to the presence of *KIT* exon 9 vs. *KIT*del-inc557/558 vs. other *KIT* 11 mutations vs. *PDGFRA* exon 18 mutations in gastric GIST ($P < 0.0001$; E) and nongastric ($P = 0.454$; F) tumors, and in tumors localized in the stomach, classified as non–high risk ($P = 0.002$; G). Complete observations are marked with circle (o) and censored ones with a plus (+).

Table 4. Impact of tumor size, MI, location, and mutational status on DFS in univariate and multivariate analysis

	Univariate		Multivariate	
Category	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Tumor size (cm)				
≤2	1.0 (reference)		1.0 (reference)	
2–5	1.47 (0.6–3.4)	**0.003**	0.93 (0.4–2.4)	**0.003**
5–10	7.47 (2.8–20.2)	<**0.001**	3.13 (1.0–10.0)	0.061
>10.0	17.18 (6.4–46.4)	<**0.001**	6.75 (2.1–21.6)	<**0.001**
Mitoses (per 50 HPF)				
≤5	1.0 (reference)		1.0 (reference)	
6–10	3.59 (2.6–5.1)	0.187	2.74 (1.9–4.0)	0.297
>10	8.76 (6.7–11.5)	<**0.001**	5.27 (3.9–7.2)	<**0.001**
Tumor site				
Gastric	1.0 (reference)		1.0 (reference)	
Nongastric	2.56 (2.1–3.2)	<**0.001**	1.64 (1.2–2.2)	<**0.001**
Tumor rupture				
No	1.0 (reference)		1.0 (reference)	
Yes	2.30 (1.6–3.2)	<**0.001**	0.87 (0.6–1.3)	0.502
Mutational status of *KIT*/*PDGFRA*				
No mutation detected	1.0 (reference)		1.0 (reference)	
KIT exon 9	1.82 (1.2–2.8)	<**0.001**	1.47 (0.9–2.5)	**0.037**
KIT exon 11				
*KIT*del-inc557/558	1.94 (1.4–2.7)	<**0.001**	1.45 (1.0–2.2)	**0.004**
Other *KIT* exon 11 mutation	0.62 (0.4–0.9)	0.617	0.80 (0.5–1.3)	0.324
PDGFRA exon 18	0.14 (0.1–0.3)	<**0.001**	0.23 (0.1–0.6)	**0.002**

NOTE: In bold, statistically significant *P* values.

Abbreviation: *KIT*del-inc557/558, deletion including W557 and/or K558.

presence of *KIT*del-inc557/558 remained an important prognosticator for poor outcome in comparison with other *KIT* exon 11 mutations, *KIT* exon 9 and *PDGFRA* exon 18 mutations ($P = 0.002$; Fig. 1G). As indicated on Supplementary Fig. S1D, the same was true when comparing *KIT*del-inc557/558 with other exon 11 *KIT* mutations in non–high-risk tumors from the stomach ($P = 0.002$). This relationship was not observed in nongastric tumors ($P = 0.45$; data not shown).

Interestingly, when considering nontypical primary tumor locations, only one of the seven gastric GIST with *KIT* exon 9 mutation relapsed during the median 56.9 months of follow-up. Similarly, the frequency of relapse was lower in gastric versus nongastric *PDGFRA* exon 18 mutants [11.8% (14 of 119) vs. 25% (2 of 8), respectively]. However, the number of the tumors with *KIT* exon 9 and *PDGFRA* exon 18 mutations originating from nontypical anatomical sites was too low for a conclusive analysis.

Discussion

In the present work, the prognostic value of the genotype in localized GIST, along with the already known prognostic factors for DFS, was investigated in a large prospective series of patients with GIST, diagnosed in four different European countries with the longest follow-up time published to date. On multivariate analysis, relapse was predicted by MI >10 mitoses/50 HPF, tumor size >10 cm, primary nongastric tumor location, and independently by a specific tumor genotype. In particular, the effect of tumor size (HR, 6.75) and mitotic count (HR, 5.7) was remarkable, and also evident for tumor location (HR 1.64). Surprisingly, tumor rupture has not been shown to be an independent predictor of outcome on multivariate analysis. The reasons for the latter remain speculative, but the completeness of reporting of tumor rupture might have had a role, because this parameter was frequently not reported in the older cases and was lacking in nearly 20% of patients. In addition, the intrinsic characteristics of our cohort, which included relatively lower number of tumors ≤2 cm in size than other pooled series (only 7.8% vs. 12.3–18.2%, respectively; ref. 4), could play a role.

Most importantly, our results demonstrate the independent significance of the *KIT*del-inc557/558 mutation, which represents an adverse relapse indicator for gastric GIST, while confronting this factor with the site of tumor origin and other commonly used risk classification criteria (modified NIH classification). Several previous reports

have documented the increased risk of recurrence in GIST harboring a *KIT* exon 11 deletion as compared with another type of exon 11 mutation, *KIT* exon 9 mutation, or *KIT*/*PDGFRA* WT genotype (14, 16, 19–21). In particular, the *KIT* exon 11 deletions involving codons 557 and/or 558 were associated with metastasis and aggressive disease (12, 13, 22). Nevertheless, the association of *KIT*del-inc557/558 mutation as poor prognosticator specifically in GIST of gastric origin was hitherto not reported. Most likely, previous studies were underpowered to reveal this correlation. Interestingly, although the *KIT*del-inc557/558 mutations were equally common in tumors of gastric and nongastric origin in our cohort, they were significantly more frequently identified in patients $\leq$60 years, in tumors >5 cm, with mitotic rate >5/50 HPF, and consequently in high-risk GIST when compared with other *KIT* mutations. Furthermore, patients with *KIT*del-inc557/558 had significantly shorter DFS as compared with those with other *KIT* exon 11 mutations. These characteristics are most likely related to the inherently higher aggressive potential (possibly more robust KIT signaling) of *KIT*del-inc557/558 mutants. Importantly, the presence of a *KIT*del-inc557/558 mutation was associated with poorer outcomes both in high-risk and non–high-risk patients with gastric GIST. This indicates its additional prognostic value for patient selection for adjuvant therapy, especially because this mutation is known to be sensitive to imatinib (23).

Markedly, *KIT* exon 9 duplication was also associated with inferior DFS in the multivariate analysis of our study. In our cohort, *KIT* exon 9 mutation was observed mostly in nongastric GIST, as previously reported, and is known to have poor clinical outcome (24, 25). However, it is well known that compared with gastric tumors, small intestinal tumors with similar size and mitotic activity have a markedly worse prognosis (8). In our series, GISTs localized outside of the stomach were larger, had a higher mitotic rate, and had worse 5-year DFS (34.2% vs. 58.1%) in comparison with gastric tumors. Nonetheless, comparison between tumors with *KIT* exon 9 and *KIT* exon 11 mutations (both, *KIT*del-inc557/558 and other *KIT* exon 11) of nongastric origin did not show differences in tumor clinical behavior as assessed by survival analysis. Thus, we conclude that in extragastric sites, the worse prognosis of *KIT* exon 9 mutants is related to the tumor location rather than to an intrinsic aggressive biologic nature of this mutation, similarly to what was suggested by others (26, 27). In support of this notion, the vast majority of gastric *KIT* exon 9 mutants in our study (6 of 7) belonged to the non–high-risk category, and only one of them progressed with relatively long DFS (56 months).

PDGFRA mutations are reported in 1.6% to 2.7% of advanced GIST treated in phase III clinical trials (27, 28) and up to 12.9% to 16% of primary tumors in population studies (15, 16, 30). In our study, *PDGFRA* was mutated in 14% of cases, and these mutants were almost exclusively (90.5%) of gastric origin, as previously reported (15, 26, 31, 32). Also in agreement with earlier reports, the most prevalent genotype was the p.D842V substitution (59.5% of all *PDGFRA* mutants). A better outcome and a lower chance of metastasis of GIST carrying *PDGFRA* mutations were suggested before (15, 31). In our study, the identification of *PDGFRA* mutations in GIST was a strong predictor of good clinical outcome on its own by multivariate analysis, and this molecular factor could therefore add a significant value to the current consensus risk criteria used for GIST stratification. In addition, given that these mutations comprise the majority of cases with the imatinib-resistant p.D842V subtype (28, 29, 33), mutational testing is highly relevant, particularly to avoid overtreatment of gastric tumors with imatinib in the adjuvant setting. Recent prospective ACOSOG Z9001 adjuvant imatinib GIST trial reports an excellent survival of patients with *PDGFRA* mutations in placebo group, providing a good argument that these patients may not need the adjuvant treatment at all (33). This issue warrants further studies, possibly using pooled cohorts stratified for the mutational status from the available prospective clinical trials in the adjuvant setting.

The main limitation of this study is that it is not a population-based analysis. Yet, the main clinicopathologic and molecular characteristics, and the frequency of most common types of *KIT* or *PDGFRA* mutations were comparable with what has been reported in the MolecGIST prospective population-based study of 492 patients (30). Particularly, the proportion of patients with intermediate/high-risk tumors (in our study 52.1% vs. 55.0% in MolecGIST) and the frequencies of *KIT* exon 11, *KIT* exon 9, and *PDGFRA* mutations (our study 71.1%, 7.4%, and 14.0% vs. MolecGIST 62.3%, 5.5%, and 15%, respectively) were reasonably similar. In addition, pathologic and molecular features of our cohort matched the data from the placebo arm of the ACOSOG Z9001 adjuvant imatinib GIST trial, including tumor primary location (55.5% vs. 62.4% of gastric GIST, respectively), median tumors size (6.0 cm vs. 6.5 cm, respectively), and MI (4/50 HPF vs. 3/50 HPF, respectively; ref. 21). Likewise, the *KIT* and *PDGFRA* mutations frequency in ACOSOG Z9001 trial was similar to what we found in our series (8.5% vs. 7.4% *KIT* exon 9, 66.3% vs. 61.3% *KIT* exon 11, 10.5% vs. 14% *PDGFRA* mutants; ref. 21). The ACOSOG trial design excluded patients with tumors $\leq$3 cm, however, which may explain the slightly higher prevalence of *KIT* exon 11 mutations and lower frequency of *PDGFRA* mutants as compared with our study.

The ability to predict GIST outcome constitutes a key element for the proper counseling and management of patients. Particularly, accurate prognostication is essential to identify high-risk tumors, for which an efficient adjuvant systemic treatment is available. The most commonly used routine risk-stratification schemes categorize tumor size and MI, which might cause incorrect risk estimation when both of these variables are close to a cutoff value. Mitotic count is a strong prognostic factor in GIST, but its reliability is controversial. The recent report on 506 patients who underwent surgery for a localized GIST (34) revealed that the risk of relapse (evaluated according to

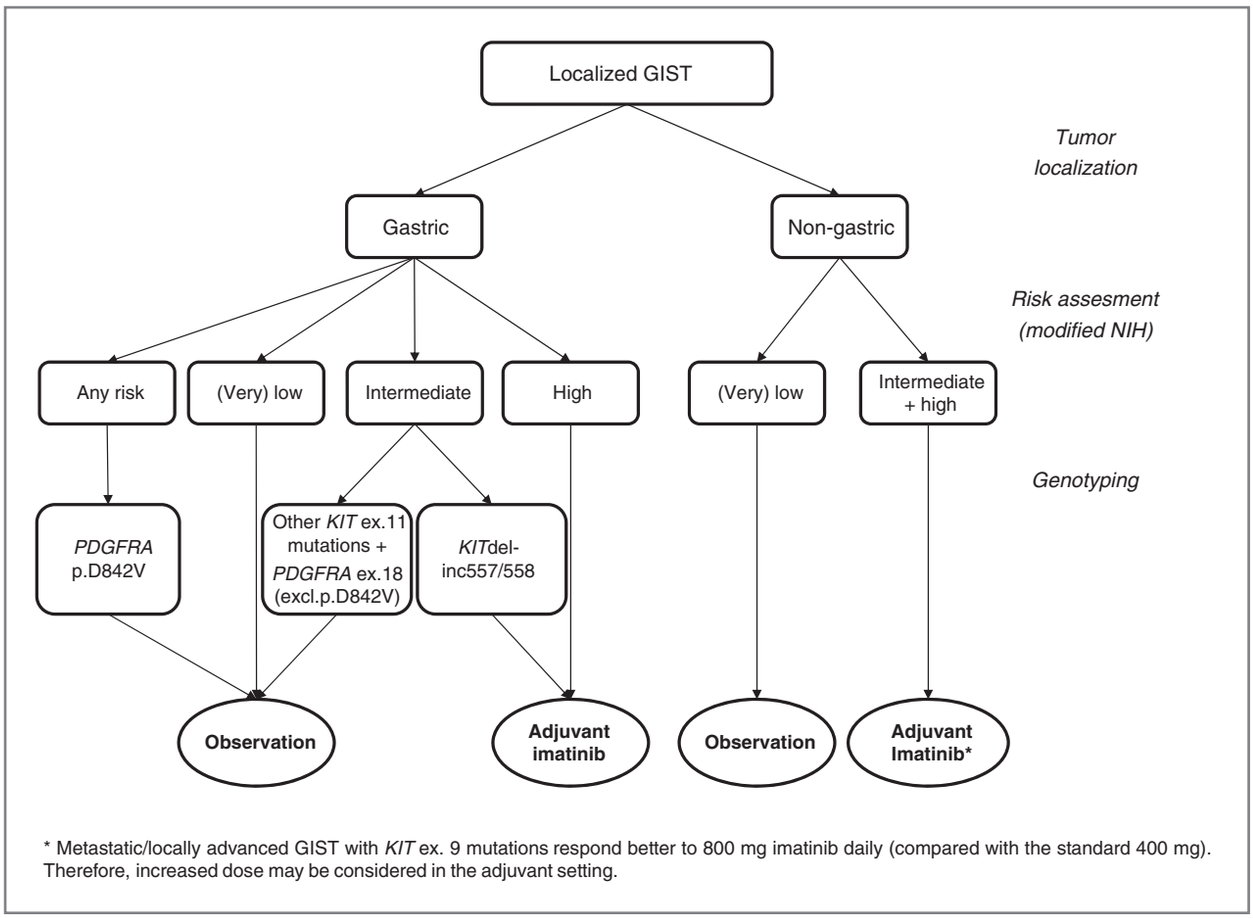

Figure 2. The recommendations for adjuvant imatinib therapy by integration of the risk assessment (based on modified NIH classification) and tumor genotype [*KIT* ex. 9 p.A502_Y503dup, *KIT* ex. 11 (*KIT*del-inc557/558 and other), and *PDGFRA* ex. 18 (p.D842V and other)] in patients with localized GIST. *KIT*/*PDGFRA* WT GIST and tumors with rare mutations (non-p.A502_Y503dup *KIT* ex. 9 as well as *KIT* mutations in exons 8, 13, and 17) are not included due to insufficient data.

AFIP classification) was underestimated in more than 30% of the cases. Subsequently, these patients were undertreated and had a significant rate of relapse compared with patients that benefited from an accurate estimation of the risk of relapse. The U.S. National Comprehensive Cancer Network now recommends consideration of adjuvant imatinib for at least 3 years for patients with a high risk of GIST recurrence (35). Thus, intermediate-risk patients are ineligible for adjuvant imatinib, although 15% to 20% of these patients will progress. Our findings have an important consequence for the selection of patients with intermediate gastric GIST as candidates for adjuvant treatment. Our practical proposal of using mutational analysis when considering adjuvant therapy is presented in Fig. 2. It remains to be shown whether patients with GIST with *KIT*del-inc557/558 genotype should not be kept longer (or even lifelong) on adjuvant imatinib treatment than standard 3 years.

In conclusion, our results underscore the importance of routine genotyping for an optimal management of GIST, either in the adjuvant or advanced disease setting. Evaluation of tumor genotype has especially prognostic value in GIST of gastric origin. Our findings contribute to the identification of a subset of patients with localized gastric GIST with higher risk of relapse irrespectively of standard clinical prognostic factors. The presence of a *KIT*del-inc557/558 or *PDGFRA* exon 18 mutations could be used as an additional parameter for more accurate selection of patients for adjuvant therapy.

Disclosure of Potential Conflicts of Interest

A. Wozniak and E. Wardelmann report receiving speakers bureau honoraria from Novartis. P. Rutkowski reports receiving speakers bureau honoraria from Novartis and Pfizer and is a consultant/advisory board member for Bayer and Novartis. No potential conflicts of interest were disclosed by the other authors.

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