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KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours

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ABSTRACT

A recent randomized EORTC phase III trial, comparing two doses of imatinib in patients with advanced gastrointestinal stromal tumours (GISTs), reported dose dependency for progression-free survival. The current analysis of that study aimed to assess if tumour mutational status correlates with clinical response to imatinib. Pre-treatment samples of GISTs from 377 patients enrolled in phase III study were analyzed for mutations of *KIT* or *PDGFRA* by combination of D-HPLC and direct sequencing of tumour genomic DNA. Mutation types were correlated with patients' survival data. The presence of exon 9-activating mutations in *KIT* was the strongest adverse prognostic factor for response to imatinib, increasing the relative risk of progression by 171% ($P < 0.0001$) and the relative risk of death by 190% ($P < 0.0001$) when compared with *KIT* exon 11 mutants. Similarly, the relative risk of progression was increased by 108% ($P < 0.0001$) and the relative risk of death by 76% ($P = 0.028$) in patients without detectable *KIT* or *PDGFRA* mutations. In patients whose tumours expressed an exon 9 KIT oncoprotein, treatment with the high-dose regimen resulted in a significantly superior progression-free survival ($P = 0.0013$), with a reduction

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of the relative risk of 61%. We conclude that tumour genotype is of major prognostic significance for progression-free survival and overall survival in patients treated with imatinib for advanced GISTs. Our findings suggest the need for differential treatment of patients with GISTs, with *KIT* exon 9 mutant patients benefiting the most from the 800 mg daily dose of the drug.

1. Introduction

Gastrointestinal stromal tumours (GISTs), the most frequent mesenchymal tumours of the gastrointestinal tract, commonly show oncogenic activating mutations of the KIT tyrosine kinase.[1–5] Imatinib mesylate (Glivec®/Gleevec®, formerly STI571, Novartis Pharma AG, Basel, Switzerland), a small-molecule inhibitor of BCR-ABL, KIT and PDGFR tyrosine kinases, targets the aberrant signalling pathways that are critical for tumour cell proliferation and survival.[5,6] Recent advances in understanding the molecular pathogenesis of GISTs has led to the remarkably successful use of imatinib in the treatment of advanced tumours, inducing high response rates leading to unprecedented improvements in overall survival.

The clinical activity of imatinib has been confirmed in GISTs, both in an EORTC (European Organization for Research and Treatment of Cancer) phase I study,[7] in which the highest feasible dose of imatinib was defined as 400 mg twice a day, and in phase II studies with doses of 400–800 mg daily.[8] Today, imatinib is approved worldwide for treatment of patients with GISTs, with a recommended dose of 400 mg daily.[9]

Two large randomized phase III trials, comparing imatinib 400 mg once a day with 400 mg twice daily have confirmed the high effectiveness of imatinib in terms of progression-free survival and overall survival.[10,11] One of these studies has also documented a significant advantage of the high-dose regimen (400 mg twice daily) in terms of progression-free survival.[9] The second study by Rankin showed a similar result, albeit the difference did not reach statistical significance.[11] Benefits in progression-free survival and response rate were seen following crossover to high-dose imatinib.[12] Clinical characteristics that are prognostic for response and survival are presented elsewhere.[13] Potentially even more important, a variety of biological mechanisms may also be responsible for initial or late drug resistance.[14] The early clinical observations link imatinib sensitivity to the presence and the type of KIT/PDGFRA mutations in the tumour.[15,16] If and how the apparent better rate of progression-free survival with the highest feasible imatinib dose (800 mg daily) is related to the genomic profile of the tumour is currently unknown.

In this report, we aimed to correlate clinical response to imatinib with the mutational status of pretreatment GIST specimens from patients recruited in randomized EORTC-ISG-AGITG phase III study to explore if the response to highest feasible imatinib dose is linked to tumour genotype.

2. Patients and methods

Between February 2001 and February 2002, three cooperative groups (EORTC STBSG, ISG and AGITG) randomized 946 patients with advanced Gastro-Intestinal Stromal Tumours (GIST) to receive imatinib at a daily dose of 400 mg (400 mg o.d.) versus 800 mg (400 mg b.i.d.). Details on eligibility criteria, treatment and follow-up have been published elsewhere.[10] Patients assigned the once a day regimen who had progression were subsequently offered the option of crossover. Patients had regular physical examinations and evaluations of performance status. CT-scans were performed after 2, 4 and 6 months, and 3 monthly thereafter, until progression of disease. Both response and progression have been objectively assessed on the basis of the size of the lesions according to RECIST criteria. Survival was assessed from the day of randomization.

The local institutional review board of each participating institution approved the study, and written informed consent was obtained from each patient.

Genomic DNA was extracted from 10-μm sections of the same paraffin-embedded tumour blocks used for immunohistochemistry, using a microdissection technique to reduce contamination with non-neoplastic tissue. Exons 9, 11, 13 and 17 of the *KIT* gene were amplified by polymerase chain reaction, and amplicons were analyzed for mutations by a combination of D-HPLC prescreening (Transgenomic WAVE DHPLC system; Transgenomic, Ltd., UK) and bidirectional sequencing, as previously described.[16] Specimens that had no detectable *KIT* mutation (*KIT* wild-type) were further tested for *PDGFRA* exons 12 and 18 mutations.

3. Statistical analysis

Patients were classified into subgroups, according to the mutations identified in the analysis, taking into account the presence or absence of any specific type of *KIT* mutation, presence or absence of *PDGFRA* mutation, and absence of any *KIT* or *PDGFRA* mutation (wild-type cases). Distribution of previously identified prognostic factors[10,13] has been tabulated for each group.

3.1. *Correlation with treatment outcome*

The primary endpoint of the study was progression-free survival (PFS). Secondary endpoint was overall survival (OS). Patients still alive and progression-free at the time of the analysis have been censored at the date of last reported follow-up.

The Kaplan–Meier method has been used to evaluate progression-free and overall survival in the different groups of patients. Comparisons between groups were performed using the log-rank test. The Cox regression model was used for the multivariate analysis. The cumulative incidence of response

has been analyzed by the competing risk method, considering treatment discontinuation in the absence of response as a competing risk. Comparisons between groups were performed using the Gray test. Response to cross-over was assessed using the growth modulation index (GMI). This index is defined as the ratio between the time to progression after cross-over (TTP2) and the time to progression under standard dose (TTP1). Patients with a growth modulation index superior to 1.25 are considered to be responders.[17]

4. Results

Paraffin-embedded tumour blocks were retrospectively collected for 532 patients included in the trial. After central pathology review, 56 cases were reclassified as non-GISTs and excluded from the study. Amongst the 476 tumours with confirmed GIST diagnosis, 377 yielded DNA of adequate quality and quantity for full *KIT* genotype analysis. Characteristics for the 377 patients included in the current analysis are given in Table 1. The distribution of those factors was similar in those 377 patients and in the 946 patients randomized in the trial.[10]

4.1. *Genotype analysis*

In total, 315 (83.6%) of the 377 analyzed GISTs had activating mutations of *KIT* gene: 248 (65.8%) harboured exon 11, 58 (15.4%) exon 9, six (1.6%) exon 13, and three (0.8%) revealed exon 17 mutations.

The most common type of *KIT* exon 11 mutations was isolated in-frame deletion (137 tumours; 55.2%), followed by complex type mutations, e.g., in-frame deletions coexisting with point mutations, insertions, or both, or insertions coexisting with point mutations (76 tumours in total; 20.5%). In 78 (31.5%) of cases the mutations in exon 11 clustered in the region between codons 550 and 560, with the most common WK 557–558 deletion being present in 26 cases (10.5%). Both isolated deletions and complex mutations were highly heterogeneous and involved one to several codons. Large *KIT* exon 11 deletions (either isolated or associated with other type of mutation), which involved more than 15 codons, and which extended distally, eliminating large portion of the KIT juxtamembrane domain were identified in 39 (10.3%) cases (7 as simple deletions, and 12 as complex mutations).

Single point mutations in *KIT* exon 11 were identified in 29 (7.7%) tumours, the most common being V559D ($n = 9$) and L576P ($n = 8$) substitution. Other comprised W557R ($n = 3$), V560D ($n = 4$), G565S ($n = 2$), W557G ($n = 1$), V559G ($n = 1$) and K558N ($n = 1$) point mutation.

Frequency of involvement of *KIT* exon 11 codons affected by deletion (Del) or point mutation (PM) is shown in Fig. 1. In addition, there were six single in-frame internal tandem duplications of variable length, involving the 3′ part of *KIT* exon 11. Activating mutations in *KIT* exons 9 were the same as previously described in GISTs, e.g., AY501–502 duplications/insertions, and only one of these was hemizygous/homozygous. Mutations in the KIT TK1 domain (exon 13) were missense substitutions, previously reported K642E and a novel E635K, which were identified in five and one tumours, respectively. Mutations involving the kinase activation loop of KIT included N822K, D820Y and N822H point mutations. Seventy-four (19.6%) tumours had no detectable *KIT* mutations (*KIT*-WT), and 62 of those were tested for *PDGFRA* mutations in juxtamembrane (exon 12) and activation loop (exon 18) domains (the lack of sufficient amount of DNA did not allow further genotyping of the remaining 12 tumours). Ten out of 62 (16.1%) of *KIT*-WT GISTs harboured a *PDGFRA* mutant isoforms: four of these mutation isoforms were described previously as imatinib-resistant (three D842V, and one D846V substitution), and five as imatinib-sensitive [three D561V substitutions, and three deletions (IMH843–845, DIM842–844, and DIMH842–845, one tumour each)]. Following final *PDGFRA* mutational screening, 52 tumours of the study were considered to be wild-type (without mutations within examined *KIT* or *PDGFRA* exons) and included into statistical analysis. In 49 (15.0%) of tumours with detectable *KIT* ($n = 315$) or *PDGFRA* ($n = 10$) mutations no wild-type allele was detected, indicating a homozygous/hemizygous genotype.

4.2. *Correlation of tumour genotype with progression-free survival and overall survival*

The current report includes all available patient follow-up data until December 20, 2004. Median follow-up at the time of the present analysis was 33 months; 99%, 81% and 30% of the patients have been followed for 1, 2 and 3 years, respectively.

In total, 131 (34.7%) deaths and 236 (62.6%) treatment failures (progression or deaths) have been reported: 110 (84%) reported deaths were due to progression.

Fig. 2A shows progression-free survival and overall survival curves for the total study population. Progression-free survival (Fig. 2B) and overall survival (Fig. 2C) were also analyzed for the three largest subgroups of kinase genotypes represented in this study: mutation of *KIT* exon 11, mutation of *KIT* exon 9, or no detectable mutation of *KIT* or *PDGFRA*.

Patients whose tumours expressed an exon 9 mutant KIT protein showed significantly worse progression-free survival and overall survival when compared to patient whose tumours expressed an exon 11 mutant KIT isoform, with a relative risk increase of 171% and 190% ($P < 0.0001$ for both end-points), respectively. There was also a significant difference in progression-free survival and overall survival in favour of the *KIT* exon 11 mutation subgroup compared with those patients without detectable *KIT* or *PDGFRA* mutation, with relative risk increase of 108% and 76% ($P < 0.0001$ and $P = 0.028$), respectively.

There was no significant difference in the rate of progression or overall survival for the subgroup with *KIT* exon 9 mutation compared with WT subgroup ($P = 0.3$). Also there was no statistically significant difference in the progression-free survival or overall survival between the groups of patients with *KIT* exon 11 point mutations and with *KIT* exon 11 deletions, or between the group with heterozygous tumours and the group with tumours homozygous/hemizygous for *KIT* exon 11 mutations (data not shown).

Multivariate analysis was performed to investigate whether the unfavourable prognosis of the *KIT* exon 9 mutation subgroup and the subgroup of patients with wild-type tumour genotype was significantly independent of the already

Table 1 – Distribution of the investigated co-factors for the 377 patients of the current study

	KIT mutants				*PDGFRA* mutants	Wild type	Total
	Exon 9	Exon 11	Exon 13	Exon 17			
Age (years)							
<40	0	17	1	0	0	8	26
	0.00	6.85	16.67	0.00	0.00	15.38	6.90
40–50	10	48	0	0	3	8	69
	17.24	19.35	0.00	0.00	30.00	15.38	18.30
50–60	14	55	1	1	2	11	84
	24.14	22.18	16.67	33.33	20.00	21.15	22.28
60–70	20	79	4	0	4	15	122
	34.48	31.85	66.67	0.00	40.00	28.85	32.36
>70	14	49	0	2	1	10	76
	24.14	19.76	0.00	66.67	10.00	19.23	20.16
Gender							
Male	40	156	4	3	8	24	235
	68.97	62.90	66.67	100.00	80.00	46.15	62.33
Female	18	92	2	0	2	28	142
	31.03	37.10	33.33	0.00	20.00	53.85	37.67
Performance status							
0	27	103	4	2	3	26	165
	46.55	41.53	66.67	66.67	30.00	50.00	43.77
1	21	119	2	1	7	22	172
	36.21	47.98	33.33	33.33	70.00	42.31	45.62
2	6	20	0	0	0	4	30
	10.34	8.06	0.00	0.00	0.00	7.69	7.96
3	4	6	0	0	0	0	10
	6.90	2.42	0.00	0.00	0.00	0.00	2.65
Site of primary disease							
Intraabdominal	9	43	2	0	2	8	64
	15.52	17.34	33.33	0.00	20.00	15.38	16.98
Gastric	4	99	2	1	3	15	124
	6.90	39.92	33.33	33.33	30.00	28.85	32.89
Sm bowel	25	47	1	2	4	14	93
	43.10	18.95	16.67	66.67	40.00	26.92	24.67
Duodenum	8	26	0	0	0	5	39
	13.79	10.48	0.00	0.00	0.00	9.62	10.34
Other	12	33	1	0	1	10	57
	20.69	12.81	16.67	0.00	10.00	16.23	14.12
Size of largest lesion (cm)							
<4	5	51	3	1	4	14	78
	8.62	20.56	50.00	33.33	40.00	26.92	20.69
4–8	20	85	2	0	3	14	124
	34.48	34.27	33.33	0.00	30.00	26.92	32.89
8–12	17	57	1	0	0	15	90
	29.31	22.98	16.67	0.00	0.00	28.85	23.87
>12	16	55	0	2	3	9	85
	27.59	22.18	0.00	66.67	30.00	17.31	22.55
Haemoglobin (g/L)							
<7	17	68	0	1	3	12	101
	29.31	27.42	0.00	33.33	30.00	23.08	26.79
7–8	12	63	1	0	3	16	95
	20.69	25.40	16.67	0.00	30.00	30.77	25.20
8–8.8	13	58	3	1	2	15	92
	22.41	23.39	50.00	33.33	20.00	28.85	24.40
>8.8	16	59	2	1	2	9	89
	27.59	23.79	33.33	33.33	20.00	17.31	23.61
Granulocytes ($\times 10^9$/L)							
<4	14	89	1	1	5	13	123
	24.14	35.89	16.67	33.33	50.00	25.00	32.63
4–5	17	46	2	2	2	14	83
	29.31	18.55	33.33	66.67	20.00	26.92	22.02

Table 1 – *continued*

	KIT mutants				*PDGFRA* mutants	Wild type	Total
	Exon 9	Exon 11	Exon 13	Exon 17			
5–6.5	11	56	1	0	3	11	82
	18.97	22.58	16.67	0.00	30.00	21.15	21.75
>6.5	16	57	2	0	0	14	89
	27.59	22.98	33.33	0.00	0.00	26.92	23.61
Total	58	248	6	3	10	52	377

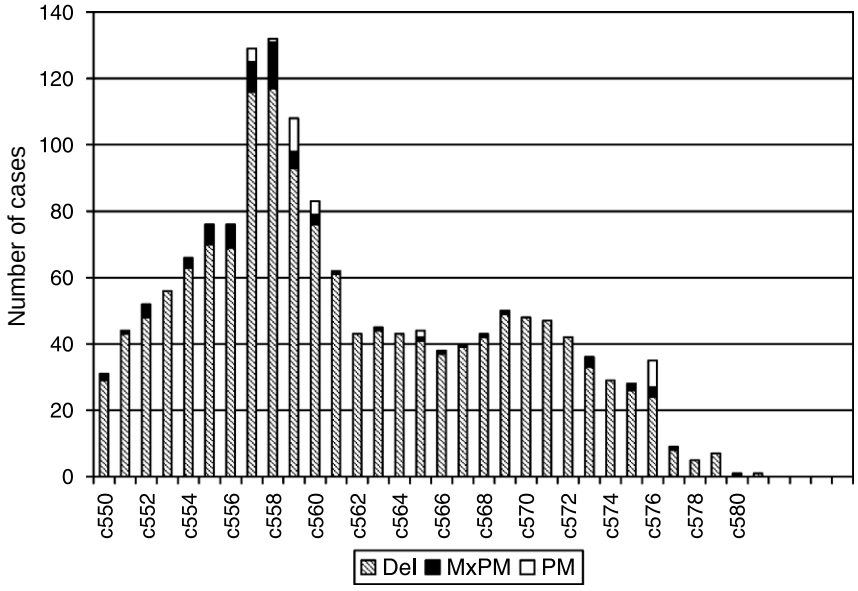

Fig. 1 – Frequency of involvement of *KIT* exon 11 codons affected by deletions (Del), mixed type (MxPM) or point mutations (PM) in 242 malignant GISTs identified in the EORTC phase III GIST study.

previously documented clinical prognostic factors for progression-free survival, e.g., baseline haemoglobin level and granulocyte count, lesion size, small bowel origin, and randomized treatment. The baseline haemoglobin level and granulocyte count kept their independent prognostic value in the multivariate model (Table 3). The size of the lesion (correlated to the presence of *KIT* exon 9 mutation) lost part of its significance, but remained in the model. Small bowel origin completely lost significance, which suggests that the adverse prognosis of patients with GIST of small bowel origin is due to a large proportion of *KIT* exon 9 mutants (27% as compared with 3% in GIST of stomach origin, see Table 1).

4.3. *Correlation of tumour genotype with clinical response data*

Response data for the total study population and the subgroups of patients with various tumour genotypes are listed in Table 2. Best overall response to imatinib therapy evaluated according to RECIST is not a very relevant end-point, because response to therapy may not immediately translate into a reduction in the size of the lesions, and "objective responses" could be first documented more than one year after start of therapy. However, this efficacy parameter has been used in prior publications of mutation data,[15,16] and response rates are therefore provided in this report for comparison with those published data.

The cumulative incidence of response is an estimate of the proportion of patients who have achieved an objective response (complete response [CR] or partial response [PR]) as a function of the time elapsed since randomization. Patients who discontinue protocol therapy in the absence of response (in most cases, because of disease progression) are no longer candidates for response to protocol therapy and this factor has therefore been considered as a competing risk in the analysis.

Fig. 3 shows the cumulative incidence of response observed in the three largest subgroups of kinase genotypes analyzed in this study, which after 2 years was 69% in *KIT* exon 11 versus 34% in *KIT* exon 9 versus 25% in WT subgroup.

The number of *KIT* exon 13-, *KIT* exon 17-, and *PDGFRA*-mutant GISTs in the study was too small to perform a formal analysis. Notably, however, none of the *KIT* exon 13-mutant and *KIT* exon17-mutant tumours (three each) showed primary resistance to the drug. In the *PDGFRA*-mutants subgroup, none of the four patients with the

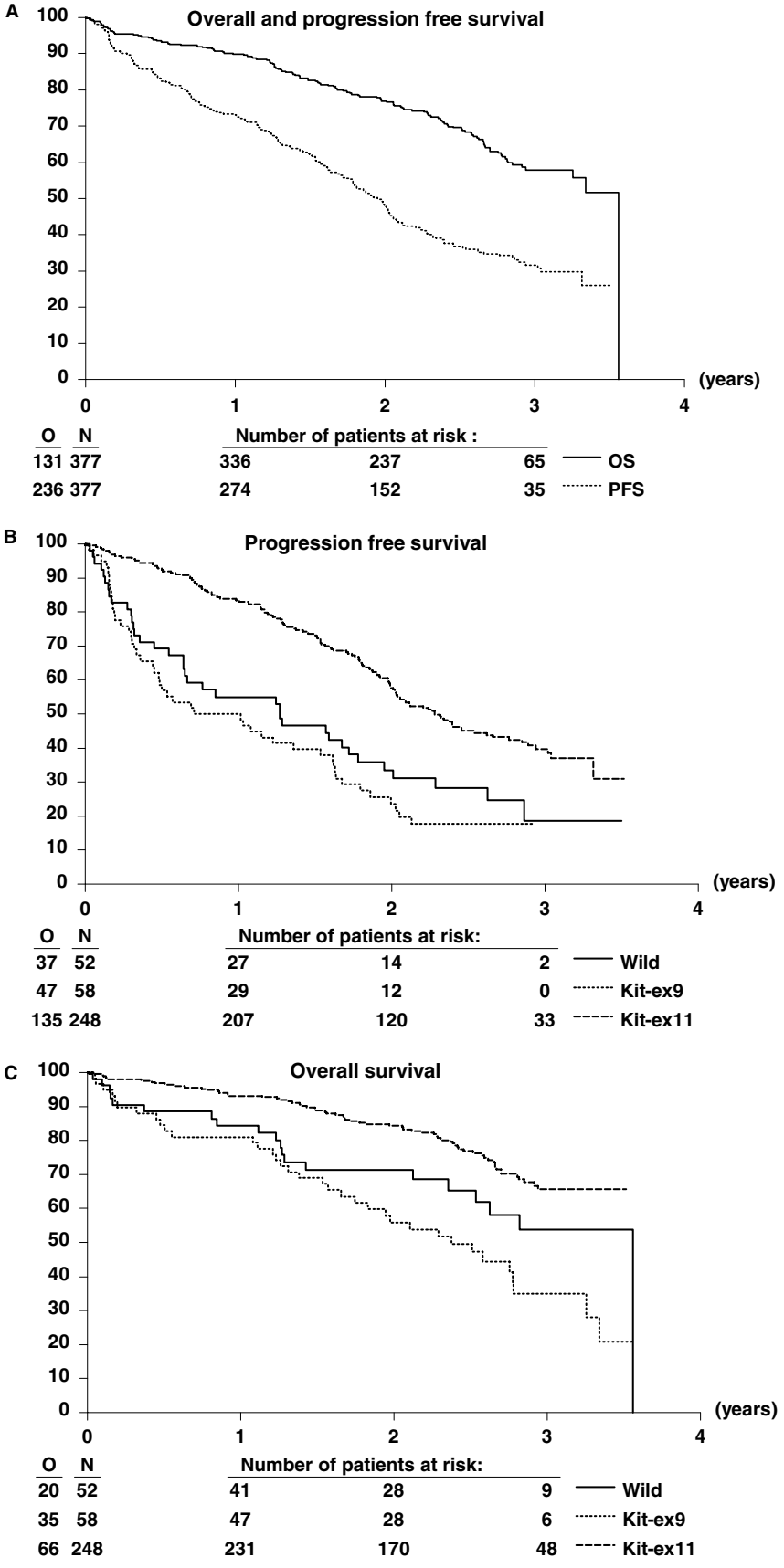

Fig. 2 – (A) Kaplan–Meier estimate of the probability of progression-free survival (PFS) and overall survival (OS) for all 377 patients under the study. (B) Kaplan–Meier estimate of the probability of progression-free survival and (C) overall survival for patients with *KIT* exon 11 mutations versus *KIT* exon 9 mutations versus no detectable mutations for *KIT* or *PDGFRA*.

Table 2 – Response data for the total study population and the subgroups of patients with various tumour genotypes

Variable	Univariate analysis		Multivariate model	
	P-value	Hazard ratio	*P*-value	Hazard ratio
KIT exon 9 mutations	<0.0001	2.225	<0.0001	2.464
Wild-type	0.0070	1.623	0.0006	1.890
Baseline granulocytes	<0.0001	1.061	0.0014	1.052
Baseline hemoglobin	0.0020	0.836	0.0185	0.874
Max. diameter of lesions	<0.0001	1.035	0.0221	1.022
Bowel origin	0.0096	1.456	ns	

Table 3 – Prognostic factors for progression-free survival

Response	*KIT* mutants				*PDGFRA* mutants	Wild type	Total
	Exon 9	Exon 11	Exon 13	Exon 17			
CR	3	16	0	0	0	0	19
	5.17%	6.45%	–	–	–	–	5.04%
PR	17	152	4	2	3	12	190
	29.31%	61.29%	66.67%	66.67%	30.00%	23.08%	50.40%
NC	27	63	2	1	3	26	122
	46.55%	25.40%	33.33%	33.33%	30.00%	50.00%	32.36%
PD	10	8	0	0	4	10	32
	17.24%	3.23%	–	–	40.00%	19.23%	8.49%
Uneval.	1	9	0	0	0	4	14
	1.72%	3.63%	–	–	–	7.69%	3.71%
Total	58	248	6	3	10	52	377

CR, complete remission; PR, partial response; NC, stable disease; PD, progressive disease; Uneval., not evaluated (all according to RECIST criteria).

imatinib-resistant mutation (D842V or D846V) responded to the drug, whereas five out of six patients with imatinib-sensitive PDGFRA oncoproteins achieved an objective response to imatinib therapy.

4.4. *Correlation of dose response with tumour genotype*

The impact of the randomly allocated initial dose of imatinib (400 mg once versus twice daily) on time to progression has been evaluated in all cases (Fig. 4A), and in the three largest prognostic groups of kinase genotypes identified in this analysis, e.g., in patients whose tumours carried mutation of *KIT* exon 11 (Fig. 4B), patients whose tumours revealed mutation of *KIT* exon 9 (Fig. 4C), or those who did not show a detectable mutation of *KIT* or *PDGFRA* (Fig. 4D). Table 4 shows, for each subgroup, the estimate of the hazard ratio between the two arms, taking the standard dose arm (400 mg once daily) as a reference.

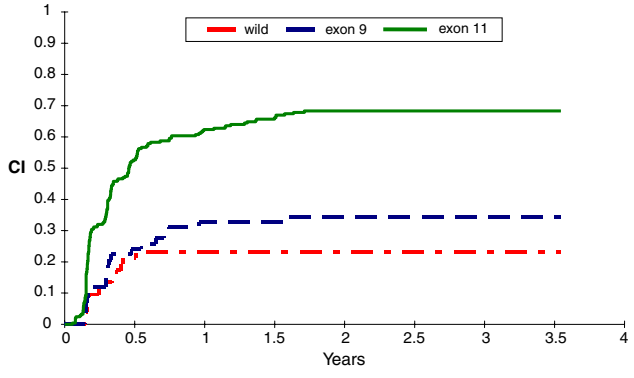

Fig. 3 – Cumulative incidence of response observed in the three largest subgroups of kinase genotypes analyzed in this study.

In patients whose tumours expressed an exon 9 mutant KIT protein, the use of the high-dose regimen resulted in a significantly superior progression-free survival ($P = 0.0013$), with a 61% reduction in the relative risk. On the contrary, time to progression was not affected by the initial dose in patients whose tumours showed an exon 11 *KIT* mutation or patients with tumours of wild-type genotype. In the latter group of patients, the non-significant long-term trend is in favour of the standard dose arm. No significant dose-effect was observed in the incidence of responses or in overall survival in any of those subgroups.

In patients randomized to the standard dose arm, cross-over to high-dose (800 mg daily) was recommended at the time of progression. Response to cross-over (assessed using the growth modulation index) occurred significantly more often in wild-type cases (83%) compared to *KIT* exon 11 mutants (7%) ($P = 0.0012$, Fisher exact test), and in *KIT* exon 9 mutants (57%) compared to *KIT* exon 11 mutants ($P = 0.0017$, Fisher exact test).

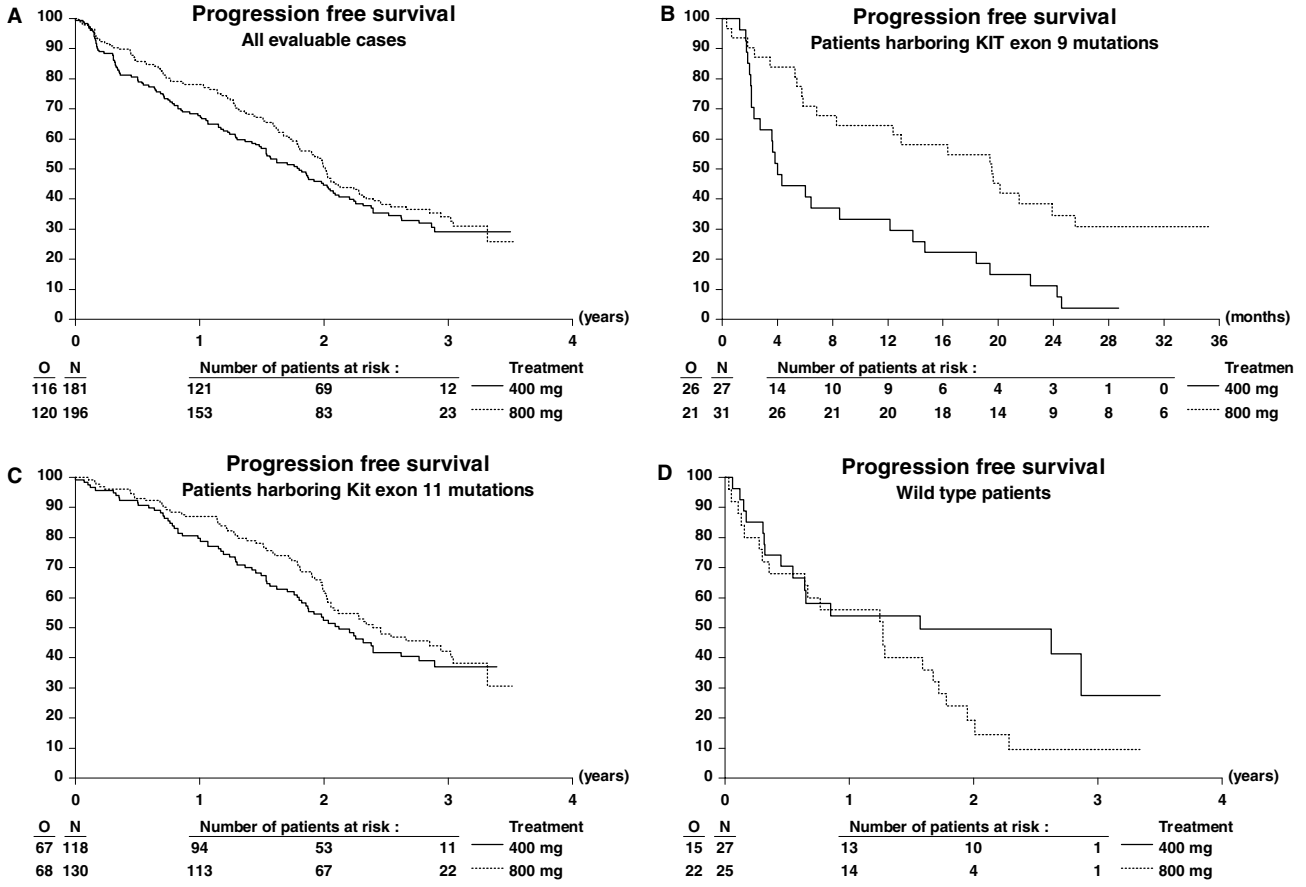

Fig. 4 – The impact of the randomly allocated initial dose of imatinib on time to progression: (A) Kaplan–Meier estimate of the probability of progression-free survival for all 377 patients in the study, (B) for patients with tumours bearing *KIT* exon 9 mutations, (C) for patients with tumours carrying *KIT* exon 11 mutations, (D) and for patients with tumours with wild-type genotype.

Table 4 – Correlation of dose response with tumour genotype – hazard ratio and CI between two arms

	400 mg		800 mg		Hazard ratio		*P*-value log-rank
	Patients	Events	Patients	Events	Estimate	95% CI	
All evaluated patients	181	116	196	120	0.845	0.654–1.091	0.20
KIT exon 9 mutants	27	26	31	21	0.392	0.218–0.706	0.0013
KIT exon 11 mutants	118	67	130	68	0.821	0.585–1.151	0.25
Wild-type patients	27	15	25	22	1.823	0.938–3.543	0.07

4.5. *Prognostic factors for KIT exon 11-mutant subgroup*

In the current series, 248 patients had tumours that expressed an exon 11 KIT oncoprotein. In this subgroup, an additional exploratory prognostic factor analysis has been conducted, aiming at generating hypotheses on the mechanisms of response and resistance to imatinib. The following factors were considered in the analysis: occurrence of insertions, point mutations and deletions, the presence of complex mutations composed of more than one mutation type, total number of codons deleted, and point mutation or deletion of each individual codon.

The number of codons deleted and mutation/deletion of codons 562, 565, 566, 567 and 579 had a significant prognostic value ($P < 0.01$) in the univariate analysis. Mutation/deletion of codon 579 (the most significant factor in the univariate analysis) had an independent prognostic value ($P < 0.0013$; hazard ratio, 3.37; 95% CI, 1.56–7.26). A second independent adverse prognostic factor could be identified amongst mutations or deletions of codons 562, 565, 566 or 567, or long deletions (>15 codons). Since involvement of codons 562, 565, 566 and 567 was highly associated, and these factors were also associated with the "total number of codons deleted" (very long deletions necessarily include the deletions of codon 565) it was impossible to find out which of those factors had the most significant prognostic value. We have arbitrarily selected codon 565 to show one of the possible multivariate models (Fig. 5).

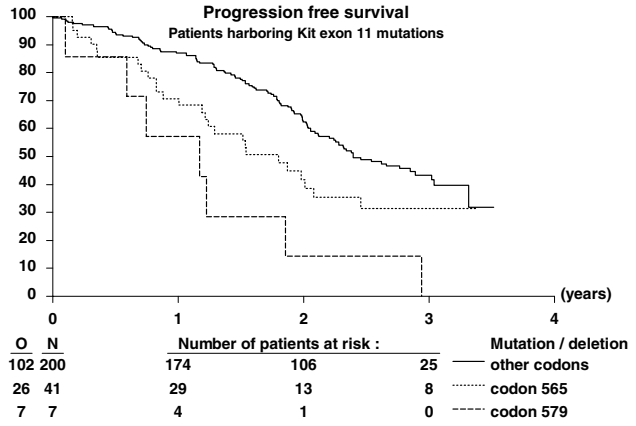

Fig. 5 – The progression-free survival in patients harbouring KIT exon 11 mutations: without mutation/deletion of codon 579 or 565 versus with mutation/deletion of codon 565 but not 579 versus with mutation/deletion of codon 579.

5. Discussion

Our series of 377 patients is the largest available series with advanced malignant GISTs treated with imatinib that has been analyzed for the importance of tumour genotype as an independent prognostic factor in predicting progression and survival. Moreover, it is the largest series currently available to also assess the relevance of initial dose of treatment with imatinib.

We have identified *KIT*-activating mutations in 83.6% of analyzed cases, *PDGFRA*-activating mutations in 2.6% of cases, and 15.4% of the cases were considered as "wild-type". Among GISTs with activating *KIT* mutations, most (64%) had mutations in exon 11, which encodes the intracellular juxtamembrane region of the protein. A second most frequent auto-regulatory mutation, in the extracellular membrane region encoded by exon 9, occurred in 15% of cases. *KIT* mutations located in exons 13 and 17 were identified in a minority of tumours (<2% and <1%, respectively). This distribution is similar to the one reported by Heinrich and co-workers,[15] which was based on a series of 127 patients enrolled in a phase II study [88% of cases with *KIT* mutations (67% in exon 11, 18% in exon 9), and 5% of cases with *PDGFRA* mutations).

Based on the large sample size in the current study, we aimed to identify the genomic profiles of metastatic GISTs that could have an independent prognostic value, particularly in terms of the impact of the highest feasible imatinib dose regimen (400 mg twice daily) on progression-free survival and overall survival. Consistent with the findings published by Heinrich and co-workers,[15] the likelihood of a clinical response to imatinib in our trial correlated with tumour *KIT* mutational status. Patients with GISTs harbouring exon 11 *KIT* mutants who received imatinib had higher response rate, a substantially lower likelihood of progression, and longer median survival than those with tumours expressing exon 9 mutant or wild-type KIT. In patients with GISTs expressing KIT exon 9 mutant proteins or without detectable *KIT*/*PDGFRA* mutations, the cumulative incidence of response after two years of treatment was 34% and 25%, respectively, as compared with 69% in *KIT* exon 11-mutants subgroup. However, in contrast to the results of the previously mentioned phase II study there was no significant difference in the rate of progression or overall survival for the subgroup with *KIT* exon 9 mutations compared with the wild-type subgroup.

A multivariate analysis including the previously reported independent prognostic factors confirmed that the type of mutation is the most important prognostic factor for progression-free survival and that the adverse prognosis of GIST of small bowel origin can be explained by the presence of exon 9 *KIT* mutations in a large proportion of those patients.

We have compared the progression-free survival between randomized dose regimens (400 versus 800 mg imatinib daily) in the different prognostic subgroups identified in the current study, to investigate whether there could be a correlation between the dose response and tumour genotype. In patients bearing tumours with *KIT* exon 11 mutations progression-free survival was independent of dose. In contrast, patients whose tumours expressed an exon 9 KIT oncoprotein, treatment with the high-dose regimen resulted in a significantly superior progression-free survival ($P = 0.0013$), with a reduction of the relative risk of 61%. This suggests that these patients should be treated with a dose of 800 mg upfront.

The higher progression rate on the standard 400 mg dose in the patients with exon 9 KIT-mutant tumours was mainly observed during the first six months of imatinib treatment. The basis for the differences in the progression induction rates in patients bearing exon 9 KIT-mutant GISTs is unclear, and our findings illustrate the importance of understanding the mechanisms of KIT activation in mediating GIST response to imatinib in this subgroup. These mechanisms have not yet been determined. It is hypothesized that exon 9 AY501-502 duplications/insertions disrupt an antidimerization motif in the extracellular KIT domain, leading to spontaneous receptor homodimerization.[15] These mutations seem to support diverse intracellular signalling compared with exon 11 KIT-mutant tumours,[18,19] and there is a lack of selective pressure against the presence of wild-type allele in these mutants.[20] The in vivo imatinib dose dependency of tumours expressing KIT exon 9 compared to exon 11 isoforms is even more intriguing bearing in mind in vitro data that showed equal sensitivity to inhibition by the drug of all GIST-associated KIT mutant isoforms, as well as GIST cells expressing wild-type KIT protein.[15] The antitumour efficacy of imatinib might be dependent not only on KIT inhibition, but also on the blockade of other kinases that support the growth of a tumour. It is interesting to hypothesize that the exon 9 mutant KIT isoform might have a higher capacity for heterodimerization and activation of the related kinase receptors, the activity of which might be more effectively modulated by the higher imatinib dose. Alternatively, the effect of higher dose may be indirect and associated with the inherent biologic differences of GISTs with *KIT* exon 9 versus 11 mutations.

In the group of patients having tumours with wild-type genotype, treatment with the higher dose did not significantly change the progression-free survival, with a trend even toward the benefit for the lower dose. The interpretation of the response to treatment within this group is difficult since some of the tumours defined as "wild-type" could still harbour *KIT* or *PDGFRA* mutations within exons

not analyzed in the current study. Secondly, GISTs without detectable mutations comprehend a heterogeneous group that may include GISTs arising in children or young adults (pediatric GISTs), patients with neurofibromatosis type 1, patients with Carney's triad, and remaining those for whom the underlying ethiopathologic factors for tumourigenesis are still unknown. The response of each individual category of patients within this heterogeneous group to imatinib warrants further study.

The largest genotype subgroup among the analyzed malignant GIST cases of the current clinical trial involved 248 tumours expressing an exon 11 KIT mutant protein. The sample size of this subgroup allowed for a more detailed statistical analysis. The spectrum of mutation types identified in our series is similar to that reported by others,[20] but the pattern in our series reflects the higher frequency of cases with deletions in the more distal end of the exon 11 and long deletions (>15 codons).

The subsequent statistical analysis of this group of patients aimed to answer the question whether the type of KIT exon 11 mutations or the involvement of particular codons could have an independent prognostic value for progression-free survival during treatment with imatinib. Interestingly, our results suggest that involvement of codons in the distal part of KIT exon 11 translates to the worse response to the therapy. The hazard ratio for progression was significantly higher in tumours with codons 565–567, and particularly high in tumours with codons 577–579 involvement in comparison with tumours bearing mutations in the proximal part of the exon. It is possible that mutations inducing conformational changes, such as large deletions or insertions (the latter being reported mainly in the distal 3′ end of exon 11) may reduce the affinity of KIT for imatinib and moderate the efficacy of the drug. On the other hand, phosphorylated tyrosine residues at positions 567 and 569 of the activated KIT bind SH2 signalling proteins that serve as a docking station for SRC family kinases.[21,22] The alteration of KIT signalling due to the changes in these residues may add to secondary resistance to the drug requires further investigation.

In conclusion, we have confirmed that the molecular subclassification of GISTs is crucial in identifying patients who are at high risk for early progression during imatinib treatment. The presence of *KIT* exon 11-activating mutations in GISTs is the strongest prognostic factor for response and progression in patients treated with imatinib. Remarkably, patients whose tumours expressed an exon 9 mutant KIT protein show significant imatinib dose dependency for progression-free survival compared to patients whose tumours harboured mutant exon 11 or wild-type KIT isoforms. These results suggest that imatinib should be dosed at 400 mg twice a day in patients with tumours bearing *KIT* exon 9 mutations. Other patients could safely start at an initial imatinib dose of 400 mg once daily, and increase to 800 mg when there is evidence of disease progression.

Conflict of interest statement

M.D.-R. and R.S. have received from Novartis travel reimbursement for participation in symposia. A.v.O., J.Y.B. and J.V. have received honoraria from Novartis for consultancy. PC has received honoraria from Novartis for lectures, written contributions, and participation in advisory boards, has received travel reimbursement for meetings, and research or educational grants for his institution. J.Z. has received honoraria for lectures from Novartis and a study grant for the Australasian Gastrointestinal Trials Group. M.V.G. has received a study Grant for EORTC from Novartis. I.J. has received honoraria for consultancy and participation in symposia from Novartis. A.H. has received from Novartis a research grant for her institution. A.L.C. and M.S. declare that they have no conflict of interest.

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