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# Distribution and prognostic value of histopathologic data and immunohistochemical markers in gastrointestinal stromal tumours (GISTs): An analysis of the EORTC phase III trial of treatment of metastatic GISTs with imatinib mesylate

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ABSTRACT

*Rationale:* The 62005 EORTC phase III trial, comparing two doses of imatinib in patients with advanced GIST, reported a median progression-free survival of 25 months with a trend towards dose dependency for progression-free survival. The current analysis of that study aimed to assess whether histological/immunohistochemical parameters correlate with clinical response to imatinib.

*Patients and methods:* Pre-treatment samples of GISTs from 546 patients enroled in phase III study were analysed for immunohistochemical characteristics, correlations with clinico-pathological data, with survival and with tumours' genotype.

*Results:* There was no correlation between immunomorphological or clinical characteristics and response to treatment, PFS or OS. No correlations between immunophenotype of the tumour and PFS or OS in the two dose arms were observed.

*Conclusions:* The results confirm the heterogeneity of GIST in terms of immunophenotypic expression, but indicate that these parameters have no impact on the outcome of the patients under imatinib treatment.



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## 1. Introduction

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal tumours of the gastrointestinal tract.[1] The incidence based upon two national surveys is thought to be around 12.7 per million inhabitants in the western world.[2,3] In the pre-imatinib era, the 5-year survival ranges between 35% and 65%. Reliable predictors of the natural course are lacking but tumour size and mitotic index serve as the best at present.[4] Morphologically, GISTs display a spectrum ranging from epithelioid to spindle cell morphology. This morphological spectrum of GIST is well known and even recognised after changes in morphology during imatinib treatment.[5–7] Immunohistochemically, these tumours express KIT (CD117), CD34, smooth muscle actin (α-SMA), S-100 and rarely desmin as differentiation markers, reflecting their presumed recapitulation of the interstitial cells of Cajal. Differential expression of KIT/PDGFRA-mutant isoforms in epithelioid and mixed variants of gastrointestinal stromal tumours depends predominantly on the tumour site.[8–10] The influence of the expression pattern of phenotypic markers like CD34, α-SMA, S-100 and desmin on survival and/or response to imatinib is yet unknown.

Between February 2001 and 2002, three cooperative groups (EORTC STBSG, ISG and AGITG) have randomised 946 patients with advanced Gastrointestinal 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 previously.[11] Paraffin-embedded tumour blocks were retrospectively collected for 555 patients included in the trial. These blocks were used to review the diagnosis by conventional histological and immunohistochemical analysis, and to identify KIT or PDGFRA mutations in the tumours. These two analyses were independently conducted. Results of the mutation analysis demonstrated a statistically significant lower progression-free and overall survival of patients with tumours bearing *KIT* exon 9 mutations and patients without detectable mutations of *KIT* and *PDGFRA* within the classical hot spots ('wild-type' GISTs), when compared with *KIT* exon 11-mutants.[12] The benefit of imatinib observed in the high-dose arm was significant in the carriers of KIT exon 9 mutations only. It is still not clear whether routine diagnostic immunohistochemical analysis of GIST tumours is useful to distinguish prognostic subsets of GIST patients, and/or differential response to imatinib treatment. To address this issue, we performed an analysis of the pathological data in order to study the distribution and prognostic value of histological and immunohistochemical parameters in a large group of patients with unresectable or metastatic GIST, treated with imatinib.

## 2. Materials and methods

### 2.1. Central pathology review

Performing pathology central review was initially not scheduled as part of the protocol. However, by protocol amendment investigators were invited to submit tumour blocks after the study had been closed for accrual, since at that time data started to emerge on the possible relevance of mutation subtypes and pathology subtypes. The pathology assessment at central review was based on conventional H and E stains and indirect immunoperoxidase stains for CD117 (polyclonal(pc), 1/250; DAKO, Glostrup, Denmark), CD34 (monoclonal (mc), 1/10; Becton Dickinson, San Jose, CA, USA), α-smooth muscle actin (α-SMA) (mc, 1/100; DAKO, Glostrup, Denmark), desmin (mc, 1/20; ICN, Aurora, OH, USA) and S-100 (pc, 1/300; DAKO, Glostrup, Denmark). The CD117 immunostaining was performed without antigen retrieval and the presence of mast cells served as an internal control. Necrosis was reported as none, <50% or >50%, and the mitotic activity was noted as number per10 high power fields (HPF = 0.1734 square millimeters). The immunohistochemical parameters were reported as positive/negative. Pathology was assessed by two members (RS and PH) of the Subcommittee for Pathology and Tumour Biology of the EORTC Soft Tissue and Bone Sarcoma Group.

### 2.2. Statistical analysis

Cross-tabulation has been performed for all the pairs of pathology/immunohistochemistry parameters ($\chi^2$-test for two by two tables, Mantel–Haenzel $\chi^2$-test for trend for tables including necrosis or mitoses). Correlations between pathology/immunohistochemistry parameters and the previously reported prognostic factors have been explored.[13] The $\chi^2$-test was used for two by two tables, and the Mantel–Haenzel $\chi^2$-test for trend was used for tables including categorical variables that were all ordered. Correlation with disease origin and with the mutations status was studied for all the parameters. As those two parameters are categorical (and not ordered), only the overall $\chi^2$-test could be performed. Overall survival (OS) has been used as the primary end-point in this analysis. This parameter has been computed from the date of randomisation to the date of death; patients still alive at the time of the analysis were censored at the date of last reported follow-up. Progression-free survival (PFS) was the principal end-point for the main analysis of the trial's results, and for the analysis of mutation data.[12] Analysis of this end-point is also provided in the present report. PFS has been defined from the date of randomisation to the date of first progression or death from any cause, whichever occurred first. 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 PFS and overall survival in the different groups of patients. Comparisons between groups were performed using the logrank test for binary variables. For variables with more than two (ordered) categories, the Wald tests are provided as follows: overall test, binary comparison of categories 2 and up *versus* category 1, and test for trend. Corresponding hazard ratios are also provided with their 95% confidence interval. The clinical database was frozen for analysis in December 2005. Additional follow-up had been obtained after the analysis of the main study results and of the prognostic factors.

## 3. Results

### 3.1. Patients/pathology and correlations

Of the 946 patients entered in the study, 555 tumour blocks could be collected.

For the current analysis, the median follow-up for patients included in this analysis is 42 months; 99%, 92% and 86% of the patients have been followed for, respectively, 1, 2 and 3 years (Kaplan–Meier estimates).

Nine blocks of 555 were inadequate for pathology review (non-tumour tissue or not enough tissue for analysis). The remaining 546 cases were reviewed, 498 of which were classified as GIST.

The baseline clinicopathological characteristics of the 498 patients classified as GIST indicated a prevalence of male over female patients (62.7% male *versus* 37.3%, respectively). One hundred and eighty eight (40%) patients had a growth of the primary lesion (incomplete resection or recurrence) at the entry to the study, whilst 308 (60%) patients were presented with metastatic disease. One hundred and forty six (29.4%) patients have entered the study more than 24 months after the initial diagnosis; 228 (91.6%) had prior surgery, 66 (26.5%) prior chemotherapy and 12 (4.8%) prior radiotherapy. By the anatomic site, 35.2% of the tumours were localised in the stomach wall, 33.3% in the small bowel, 13.8% in the large bowel/rectum/oesophagus (other GI) and 17.3% outside the GI tract (intra-abdominal and other location). One hundred and eighteen (23.7%) patients were presented with a lesion greater than 12 cm in maximal diameter.

The majority of tumours corresponded to the spindle cell type, only a minority was purely epithelioid. This is, however, based on an overall impression, since the phenotype was not registered systematically. CD34 expression was present in 72% of cases, α-SMA in 40%, S100 protein in 27% and desmin in 5.5%. The immunohistochemical data did not correlate with the independent prognostic factors previously reported in this population, nor with an overall survival or PFS.

A high percentage of necrosis was correlated to high mitotic rate and α-SMA immunonegativity (for both $p < 0.001$). The mitotic index was significantly lower in primary/recurrent lesions than in metastatic lesions ($p < 0.01\%$). The mitotic count or amount of necrosis did not correlate with prognosis (data not shown). When comparing the CD34-immunopositive group with the CD34-immunonegative group, it appeared that the CD34-immunonegative GISTs had significantly less desmin ($p < 0.01$), and these tumours were more frequently localised in the small intestine than in the other locations (51.5% small intestine *versus* 16.9% gastric *versus* 13.0% other GI *versus* 17.7% intraabdominal tumours; $p < 0.001$). Patients with CD34-immunonegative tumours had significantly less liver lesions (24.8% *versus*75.2%; $p < 0.01$), but no different survival (data not shown).

### 3.2. *KIT-immunonegative GIST*

In seven GISTs (1.4%) no KIT expression was seen. Of these CD117-immunonegative GISTs, five arose from the stomach and one from the retroperitoneum. CD34 expression was present in 5 of 7 cases and in one case focal desmin expression was seen. The diagnosis of GIST was made because of exclusion of any differential diagnosis by histology and additional immunohistochemistry. Amongst five genotyped CD117-immunonegative tumours, one harboured a *PDGFRA* V561A single substitution, whilst three others harboured *KIT* exon 11 deletions. Interestingly, one of the latter was a K550–K558 delinsQ homozygous mutation, whilst the second was an 11 bp deletion in *KIT* intron 10 that affected the splicing site and resulted in the deletion of codons 550–558. Although KIT-immunonegative GISTs showed worse PFS and OS than KIT-immunopositive GISTs, remarkably 4 of 7 patients benefited from the treatment in terms of showing late progression rather than primary resistance (Fig. 1).

### 3.3. *Non-GIST tumours*

Forty eight (8.8%) tumours were classified as non-GISTs. The distribution of the locations (clearly different from the GISTs) was 12.2% in the stomach wall, 16.7% in the small intestine, 32.6% in large bowel/rectum/oesophagus and 27.3% outside the GI tract. The final diagnosis in these cases was leiomyosarcoma, melanoma and carcinoma. Patients with non-GISTs had more frequently lung metastases (32%) than patient with a GIST (8.5%), whilst the opposite was true for liver metastases (72% of GIST *versus* 36.7% of non-GIST). Notably, two of 48 tumours classified as non-GIST still had *KIT*/*PDGFRA* mutations. Both tumours were considered to be leiomyosarcomas and were clinically progressive. One, which had spindle appearance and diffuse desmin expression, carried a frameshift mutation (deletion of one nucleotide of codon 642 of *KIT* exon 13). The predicted protein resulting from this mutation would lack part of the kinase domain. The second case carried a frequently described *PDGFRA* D842V mutation, and it was a gastric tumour of epithelioid appearance that would resemble in fact KIT-immunonegative GIST except for the diffuse desmin immunopositivity. The best overall response rate, overall survival and PFS were all significantly worse in non-GIST patients (Table 1; Fig. 1).

### 3.4. *Mutation of KIT/PDGFRA and immunohistochemical analysis*

In total, 378 (76%) tumours classified as GISTs were screened for both, *KIT* and *PDGFRA*, mutations. The vast majority of GISTs (316; 83.6%) had activating mutations of *KIT* gene, i.e. 249 (65.9%) harboured exon 11, 58 (15.3%) exon 9, six (1.9%) exon 13 and three (0.9%) exon 17 mutations. The *PDGFRA* mutations were detected in 3.2% (12 of 62 tested *KIT* wild-type cases).

Analysis of the distribution of tumours with different genotypes according to tumours anatomic site was published previously.[12] Notably, although there was a prevalence of *KIT* exon 9-mutants that originated from the small intestine (56.9%), a high fraction of tumours with this genotype was also registered in other sites (6.9% stomach, 18.9% colon/rectum and 17.4% intraabdominal/other). The *KIT* exon 11-mutants were predominantly of stomach origin (99 of 248; 39.9%) and this comprised the large proportion of gastric tumours under study (99 of 124; 79.8%). Interestingly, only two of 10 (20.0%) of *PDGFRA*-mutant GISTs originated from the stomach.

In general, the immunohistochemical data did not correlate with the mutational status of GISTs under study. The largest tumour group with a specific genotype (tumours that carried *KIT* exon 11 or *KIT* exon 9 mutations, and wild-type

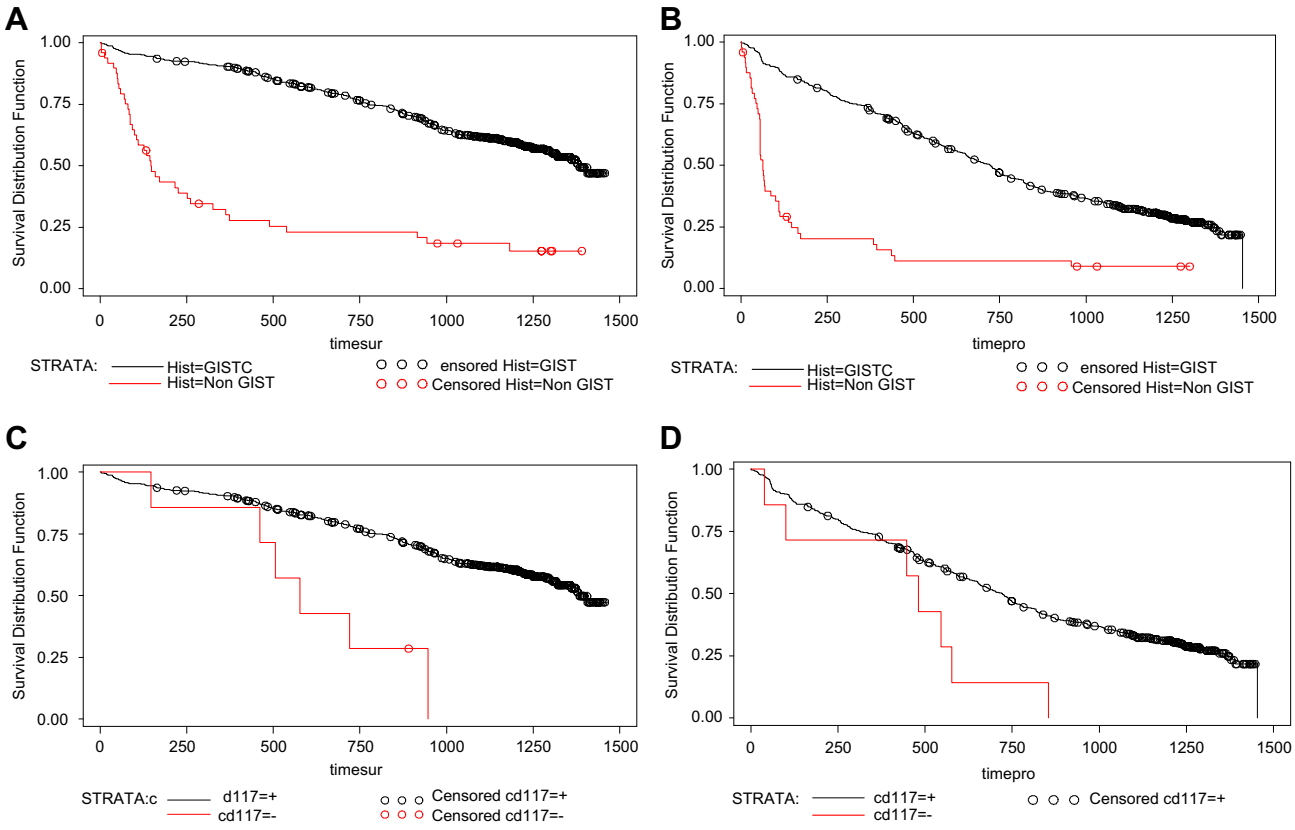


**Fig. 1 – Kaplan–Meier estimate of the probability of (A) overall survival and (B) progression-free survival for misdiagnosed patients *versus* GISTs patients under the study ($p < 0.00001$ for all end-points). Kaplan–Meier estimate of the probability of overall survival (C) and progression-free survival (D) for patients with CD117-immunonegative *versus* CD117-immunopositive GISTs ($p < 0.001$).**

**Table 1 – Best overall response to therapy in non-GIST versus GIST patients**

| | Non-GIST | GIST | |
|---|---|---|---|
| CR | 1 | 29 | 30 |
| | 2.04 | 5.84 | |
| PR | 3 | 252 | 255 |
| | 6.12 | 50.70 | |
| NC | 9 | 161 | 170 |
| | 18.37 | 32.39 | |
| PD | 25 | 26 | 51 |
| | 51.02 | 5.23 | |
| Early death – PD | 4 | 6 | 10 |
| | 8.16 | 1.21 | |
| Unevaluable | 7 | 23 | 30 |
| | 14.29 | 4.62 | |
| Total | 49 | 497 | 546 |

GISTs) showed the same distribution of cases that express CD34, desmin and α-SMA by immunostaining.

## 4. Discussion

Identification of the role of constitutively activated KIT in the pathogenesis of GIST had critically important implications for therapy. Imatinib (Glivec®, Gleevec®, formerly STI571; Novartis Pharma AG, Basel, Switzerland) inhibits the tyrosine-kinase activity of KIT as well as PDGFRA and B.[14] Treatment with imatinib has induced objective tumour responses or halted disease progression in more than 80% of patients with *KIT* (CD117)-positive metastatic or unresectable GIST in clinical trials.[11,15,16] It is now clear that the tumour genotype influences the response to the drug.[12,17,18] In this paper, the role of the phenotype has been assessed in a large group of unresectable or metastatic GIST, treated with imatinib. Of the 546 reviewed cases 498 were classified as GIST and 48 (8.8%) as non-GIST. When focusing on the 498 GIST cases, it is clear that the distribution of the location differs from what is classically described. Most GISTs are reported to occur in the stomach wall (50–60%), followed by the small bowel (20–30%), large bowel (10%), oesophagus (5%) and elsewhere in the abdominal cavity (5%).[5,7] In the present series, more tumours were located in the small bowel and outside the GI tract. This finding probably relates to the fact that this study only included patients with aggressive (metastatic and unresectable) tumours. In this respect, it is known that small intestinal GISTs behave more aggressively than stomach GISTs.[19,20] The immunohistochemical profile corresponds largely to data of the literature: CD117 expression in the vast majority, followed by CD34, alpha smooth muscle actin, S-100 protein and desmin. A major conclusion from the data is the complete absence of any correlation between the mitotic count, necrosis or immunohisto-

chemical profile and the mutation status, survival or any prognostic parameter. This finding strongly contrasts with the prognostic significance of the genotype.[12,17] It is of particular interest to note that CD34 did not correlate with survival, despite the fact that liver metastases were more frequently found in patients with a CD34 positive GISTs. Moreover, the CD34 negative GISTs were more frequently localised in the small intestine and were significantly less desmin positive. There is no clear-cut explanation for this finding, but it might relate to the fact that there are differences between interstitial cells of Cajal along the gut wall.[21]

In a small subset of GISTs, no KIT expression is found. This is reported to occur in less than 5% of GIST cases.[22] The majority of these KIT-immunonegative cases present as stomach wall tumours, and they often have an epithelioid phenotype. By genotype, two thirds have *PDGFRA* mutations (exon 18), about 23% are wild-type, and some 11% still have *KIT* mutations.[5,7] In our series, only 7 cases (1.4%) of KIT-immunonegative GISTs were present, five of which were localised in the stomach wall. Notably, amongst those that were genotyped only one had a *PDGFRA* mutation, whilst 3 of 5 others had an exon 11 *KIT* mutation. Of interest, four of seven CD117-immunonegative GISTs patients were still partially benefited from the imatinib therapy, although overall and progression-free survival was significantly worse in this group of patients in comparison with patients whose tumours showed expression of CD117 antigen. It is not clear whether the meager response to imatinib related to the level of target molecules for the drug in the tumour cells. Nevertheless, it is important to stress that those patients should not be deprived of the treatment.

In 8.8% of revised cases, the diagnosis of GIST was withdrawn. This finding underpinned again the need of vigorous pathology review in clinical trials. The final diagnosis was variable, including leiomyosarcoma, melanoma and carcinoma. Not surprisingly, the distribution of the location of these false positive GISTs differed from the *bona fide* GIST cases, with more colon and extra gastrointestinal locations. In addition, as expected from sarcomas different from GISTs, more lung metastases were found in this group. The very poor prognosis of this group confirms previous studies, thus the diagnosis of GIST should be double checked if there is any doubt, and particularly in clinical studies central pathology review remains of crucial importance in order to compare data. It is of interest that two of 47 tumours classified as non-GIST still had *KIT*/*PDGFRA* mutations. One of the tumours had a stop codon mutation, which is only sporadically reported in GISTs, and of which the functional role is not yet clear.[23,24] The second tumour, however, carried frequent *PDGFRA* D842V isoform, based on the presence of which the tumour should be classified as a regular GIST. Morphologically, these tumours were classified as leiomyosarcomas with the absence of KIT expression and with clear-cut desmin expression. Both cases highlight the important role of genetic screening in the differential diagnosis of CD117-immunonegative tumours originating from the gastrointestinal tract.

Several studies have emphasised the prognostic impact of GIST's genotype, reporting the association of tumours with *KIT* mutations with metastatic behaviour, and *PDGFRA* mutations with indolent course of the disease.[5,7,24–27] Particularly, *KIT* exon 11 deletions are indicated to confer a significantly higher risk of recurrence/metastasis in patients with GISTs.[24,27] Moreover, single-point mutations and insertions in the 3′ end of *KIT* exon 11 were associated with tumours of low malignant potential.[28,29] Consistent with these findings, the largest genotype subgroup amongst the analysed malignant GIST cases of the current clinical trial involved tumours harbouring *KIT* exon 11 deletions (55.2% of total tumours and 83.6% of *KIT* mutants), whilst *KIT* exon 11 single base substitutions and insertions were detected only in the small subsets of cases.[12] In addition, there was a low proportion of GISTs harbouring *PDGFRA* mutations in our study (3.2%), which reflects most likely the indolent biological features of these mutants.[22,30,19]

In summary, this analysis on 546 metastatic or unresectable GISTs illustrates that the response to imatinib is not defined by any histological or immunohistochemical parameter, including CD34 expression. In addition, in contrast to patients with non-GISTs, a high proportion of patients with CD117-immunonegative GISTs considerably benefited from the drug, reinforcing the need of a correct pathological diagnosis. Our study confirms indirectly the independent prognostic value of specific tumour genotypes that confer a significantly higher risk of recurrence in patients with GISTs, highlighting the value of *KIT*/*PDGFRA* mutational analysis in the proper diagnosis, prognosis and management of GISTs.

## Conflict of interest statement

Raf Sciot and Maria Debiec-Rychter have received from Novartis honoraria for participation in symposia and lectures. Jean-Yves Blay and Jaap Verweij have received honoraria from Novartis for consultancy. Pancras Hogendoorn has received honoraria from Novartis for lectures. Martine van Glabbeke has received a study grant for EORTC from Novartis.

Soren Daugaard, Cyril Fisher, Francoise Collin declare that they have no conflict of interest.

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