Factors Associated With Disease Progression in Patients With Gastrointestinal Stromal Tumors in the Pre-Imatinib Era

Igors Iesalnieks, MD,[1] Petra Rümmele, MD,[2] Wolfgang Dietmaier, MD,[2] Thomas Jantsch,[1] Carl Zülke, MD,[1] Hans J. Schlitt, MD,[1] Ferdinand Hofstädter, MD,[2] and Matthias Anthuber, MD[1]*

Key Words: Gastrointestinal stromal tumor; GIST; Prognostic factors; Surgery

DOI: 10.1309/AKK3VFF610CWM566

Abstract

The aim of this study was to determine the predictors of survival in 38 patients with curatively resected gastrointestinal stromal tumors (GISTs). The tumor was located in the stomach in 23 cases, the small bowel in 13, and the colon in 2. In 23 patients (61%), a mutation in exon 11 of the kit *gene was detected. In 7 cases, all small gastric tumors, a mutation in the platelet-derived growth factor receptor α (*PDGFRA*) gene was detected. The overall 5-year survival rate was 70%. In 9 patients, GISTs relapsed, leading to an actuarial 5-year disease-free survival of 78%. By multivariate analysis, the presence of distant metastases, the proliferative (MIB-1) index, and deletional mutation in codons 557 and/or 558 of* kit *exon 11 correlated significantly with poor outcome. None of the* PDGFRA *mutant GISTs relapsed. These findings suggest a strong relationship between various tyrosine kinase receptor mutations and survival outcome in patients with GISTs.*

Despite the definition of gastrointestinal stromal tumors (GISTs) as a separate entity among gastrointestinal tumors only 2 decades ago,[1] they already have undergone intense research. The rapid increase of knowledge has led to the introduction of an effective conservative therapy using the tyrosine kinase inhibitor imatinib, a unique development in the field of solid malignancy treatment.[2,3]

Determination of key survival predictors is of central importance for the development of further adjuvant treatment strategies. Tumor size and mitotic activity were recognized early as independent prognostic factors.[4] Further potential survival predictors remain controversial owing to a lack of clinical data and ongoing difficulties in standardization. The prognostic role of mutations in the *kit* gene remains a matter of serious debate.[5,6] As described by Hirota et al[7] in 1998, gain-of-function mutations in the exon 11 of this gene were first believed to be associated with a poor outcome. However, more recent studies have questioned this observation.[8,9]

Since then, further mutations of the *kit* gene have been described. It has been suggested that mutational activation of the kit receptor is, in fact, obligatory for the development of GISTs and, therefore, not related to prognosis.[5] Other authors have attempted to classify *kit* mutations by their individual predictive value.[10] The prognostic role of the recently described mutation in the tyrosine kinase receptor, platelet-derived growth factor receptor α (*PDGFRA*),[11] which may be found in many GISTs, remains unknown. In this retrospective analysis we sought to assess the prognostic value of various clinical, immunohistochemical, and molecular factors, with special focus on the impact of *kit* and *PDGFRA* mutations in patients undergoing surgery during the pre-imatinib era.

Material and Methods

Patients

Between November 1992 and July 2002, 53 patients with gastrointestinal mesenchymal tumors underwent surgery. GISTs were defined primarily as spindle or epithelioid cell gastrointestinal mesenchymal tumors that are kit+ (CD117+). In addition to the 38 patients with GISTs, 5 leiomyomas, 3 leiomyosarcomas, and 5 nerve sheath tumors were detected **Table 1**. In 2 cases, tumors could not be classified by structural or by immunohistochemical findings (ie, "null phenotype").

Sex distribution among the 38 patients with GISTs was 23:15 (male/female). The mean age was 59.9 years (range, 27-84 years). Three patients had synchronous peritoneal metastases. One patient underwent surgery for tumor relapse after duodenal wall resection for GIST 2 years earlier. In 6 cases, the GIST was an incidental finding during surgery for another malignant neoplasm (colorectal cancer, 4; gastric cancer, 1; esophageal cancer, 1). Of these 6 GISTs, 5 were smaller than 2 cm.

Twenty-four (69%) of 35 patients had symptomatic disease **Table 2**, in general unspecific abdominal pain followed by weight loss and gastrointestinal hemorrhage. All clinical information was derived from hospital records. Follow-up information was obtained by mail or phone communication with the primary care physician or the patient. Individual patients were seen at the outpatient office of the Department of Surgery at the University of Regensburg. Three patients received adjuvant chemotherapy with various agents. Imatinib was given to 1 patient following a recent diagnosis of tumor relapse.

Morphologic and Immunohistochemical Studies

All tissues were fixed in 4% neutral buffered formalin and were embedded routinely in paraffin. H&E-stained sections were reviewed for detailed histomorphologic findings, and all tumors were classified based on previously published criteria of the consensus approach[12] and according to the current World Health Organization classification. Variables routinely evaluated were presence of necrosis, mucosal invasion, *infiltration* of adjacent organs or structures (defined as an invasive growth), and mitotic activity. The *mitotic index* was defined as the number of mitoses per 10 high-power-fields (HPF), scored at representative tumor areas, using a microscope with a 40× objective and a 10× ocular.

Immunohistochemical analysis was performed on 1 representative block for each case. Antibodies to the following antigens were used: CD117 (c-kit proto-oncogene product, polyclonal antibody, dilution 1:200; DAKO, Hamburg, Germany), CD34 (QBend10, monoclonal antibody, dilution 1:20; DAKO), desmin (D33, monoclonal antibody, dilution 1:100; DAKO), smooth muscle actin (asm-1, monoclonal antibody, dilution 1:100; Roche Diagnostics, Mannheim, Germany), S-100 (polyclonal antibody, dilution 1:2,000; DAKO), Ki-67 (MIB-1, monoclonal antibody, dilution 1:10; DAKO), and vimentin (3B4, monoclonal antibody, dilution 1:50; Ventana Medical Systems, Tucson, AZ).

Briefly, 4-μm sections were cut from tissue specimens, mounted on poly-L-lysine-coated slides, bar coded, and baked by placing the slides in a 37°C oven for 30 minutes. Specimens then were dewaxed by graded concentrations of ethanol (2× in xylene baths for 5-10 minutes each, 2× in 100% ethanol baths for 3 minutes each, 3 minutes in 95% ethanol, 3 minutes in 80% ethanol), followed by 10 dips in distilled water and incubation with the primary antibodies. Heat-induced epitope retrieval by microwave pretreatment was performed for antibodies directed against CD117, CD34, Ki-67, and desmin. Antibodies directed against S-100 were pretreated by Protease-1 digestion in a NEXES immunostainer (Ventana Medical Systems), in combination with the Basic DAB Detection Kit 250-001 (Ventana Medical Systems) using an avidin-biotin peroxidase method with diaminobenzidine (DAB) as the chromogen according to the manufacturer's instructions. The DAB-stained slides were counterstained with hematoxylin, dehydrated through graded alcohols, and mounted.

Blood vessels, small peripheral nerves, fibroblasts, myoblasts, and interstitial cells of Cajal served as internal control samples.

Table 1
Tumor Location*

	GISTs (n = 38)	Smooth Muscle Tumors (n = 8)	Nerve Sheath Tumors (n = 5)
Esophagus	0	1	0
Stomach	23	2	1
Small bowel	13	3	0
Colon/rectum	2	2	0
Mesentery	0	0	2
Greater omentum	0	0	1
Lesser omentum	0	0	1

GISTs, gastrointestinal stromal tumors.
* In 2 cases, tumors could not be classified (stomach, 1; small bowel, 1).

Table 2
Symptoms of Disease

Symptoms	GISTs (n = 38)	Smooth Muscle Tumors (n = 8)	Nerve Sheath Tumors (n = 5)
Asymptomatic	11	2	1
Pain	13	2	1
Bleeding	5	1	0
Weight loss	5	0	1
Perforation	1	0	0
Other	0	1	0
Unknown	3	2	2

GISTs, gastrointestinal stromal tumors.

DOI: 10.1309/AKK3VFF610CWM566

The proliferative (MIB-1) index was determined by counting 3 HPF or a minimum of 1,000 cells and expressed as the percentage of all positive nuclei.

kit and *PDGFRA* Mutation Analysis

DNA of all specimens was studied for the presence of an exon 11 mutation of *kit.* Specimens without exon 11 mutations that stained positive for CD117 were used for mutation analysis of *PDGFRA* exons 12 and 18.

DNA Isolation

Genomic tumor DNA was isolated from microscope-controlled, manually microdissected, formalin-fixed, paraffin-embedded tissue samples using the MagNA Pure System and LC DNA Isolation Kit II (Roche Diagnostics) according to the supplier's instructions.

Polymerase Chain Reaction Amplification

Polymerase chain reaction (PCR) amplification was carried out in a final volume of 20 µL containing 50 to 100 ng of purified genomic DNA, 1× PCR buffer (Roche Diagnostics), 200 µmol/L of each deoxynucleotide triphosphate (Roche Diagnostics), 0.3 µmol/L of each primer, 1.5 mmol/L of magnesium chloride, and 0.4 U of *Taq* polymerase (Roche Diagnostics). The *kit* exon 11 and *PDGFRA* exons 12 and 18 were amplified using the following primers:

kit exon 11, forward primer, 5'-GATCTATTTTTCCCTTTCTC-3'; reverse primer, 5'-AGCCCCTGTTTCATACTGAC-3'; *PDGFRA* exon 12, forward primer, 5'-TCCAGTCACTGTGCTGCTTC-3'; reverse primer, 5'-GCAAGGGAAAAGGGAGTCTT-3'; and *PDGFRA* exon 18, forward primer, 5'-ACCATGGATCAGCCAGTCTT-3'; reverse primer, 5'-TGAAGGAGGATGAGCCTGACC-3'.[11]

The annealing temperature was 51°C for *kit* exon 11, 59°C for *PDGFRA* exon 12, and 60°C for *PDGFRA* exon 18. After predenaturation at 95°C for 10 minutes, primer-specific annealing temperature for 1 minute, 72°C for 1 minute, 34 to 40 amplification cycles of 94°C for 1 minute, and a final step of 72°C for 8 minutes were performed using an MJ Research Thermocycler (PTC100, MJ Research, Watertown, MA).

DNA Sequencing

PCR products were purified by polyethylene glycol precipitation and subsequently used for cycle sequencing using the Dye v1.1 sequencing kit (Applied Biosystems, Darmstadt, Germany) according to the manufacturer's recommendations using an ABI310 automatic sequencer (Applied Biosystems) and sequencing analysis v3.7 software (Applied Biosystems). All sequencing reactions were performed in both directions. Sequencing was performed without knowledge of the clinical course by one of us (T.J.).

Statistics

Only deaths due to GIST or relapse of GIST were included in the analysis of long-term results. Patients dying of other causes were stated as free of disease at the time of death. One postoperative death due to surgical complications was stated as "death due to disease" and included in the calculation of disease-free survival. Comparisons between patient groups were made by using the Fisher exact or Mann-Whitney tests for nonnormal distribution for continuous variables and by χ^2 analysis for categorical variables. The Kaplan-Meier method was used for survival analysis. The log-rank test was applied to calculate the prognostic value of various variables by univariate analysis. Multivariate analysis of factors related to outcome was performed using the Cox proportional hazards model.

Results

Surgery

In 20 cases, GISTs could be removed by local full-thickness transmural excisions. In the remaining 18 cases, 6 patients underwent limited small bowel resection, 4 total gastrectomy, 3 partial pancreatoduodenectomy, 3 partial gastrectomy, 1 left colon resection, and 1 debulking surgery. An extended lymphadenectomy was performed in 8 cases with no subsequent evidence of nodal metastases in any case. Complete removal of the disease (R0) was achieved in 35 cases. One patient with a large perforated gastric GIST had microscopically positive resection margins (R1). Another 2 patients with synchronous peritoneal spread had macroscopic residual disease at the end of surgery (R2). All 3 of these patients experienced relapse.

Postoperative complications occurred in 7 patients (18%). Anastomotic leakage developed in 2 cases (in one following partial pancreatoduodenectomy; in the other following local excision of a rectal GIST), anastomotic stenosis following antrectomy in 1, pancreatic fistula in 1 (partial pancreatoduodenectomy), pneumonia in 1, and seizures in 1. One patient died of small bowel ischemia following total gastrectomy leading to a postoperative mortality rate of 3%.

Histologic Features

Of the GISTs, 29 (76%) had spindle cell–type morphologic features, and 9 (24%) had an epithelioid appearance. Of the 9 epithelioid-type GISTs, 7 were located in the stomach. There was no statistical difference between spindle and epithelioid cell tumors in size, mitotic activity, invasive growth, presence of *PDGFRA* or *kit* exon 11 mutations, and outcome. The mean tumor size for all GISTs was 6.3 cm (median, 5.2 cm; range 0.3-20 cm). Of the tumors, 19 (50%)

DOI: 10.1309/AKK3VFF610CWM566

were larger than 5 cm. The mean mitotic index was 5 per 10 HPF (median, 1/10 HPF; range, 0-28/10 HPF). Ten tumors had more than 2 mitoses per 10 HPF, and 8 had more than 5. Histologic invasion of the serosa, adjacent structures, or both was found in 7 GISTs (18%). Tumor necrosis was observed in 17 cases. Tumor size correlated significantly with necrosis (Pearson coefficient, 0.61; $P < .001$).

Marked differences in clinical and histopathologic features were observed between gastric and small bowel GISTs. The latter tended to be larger and more often symptomatic and had higher rates of mitoses, invasive growth, tumor necrosis, and proliferative index **Table 3**.

Immunohistochemical Analysis

All GISTs were positive for kit (CD117) staining. Whereas most cases showed diffuse, strong cytoplasmic positivity **Image 1**, focal staining was found in 2 tumors. Nevertheless, tumor cells positive for kit could be differentiated clearly from interstitial cells of Cajal, which served as internal positive control samples. Of the GISTs, 21 (55%) were positive for CD34, 11 (29%) for smooth muscle actin, 5 (13%) for desmin, 1 (3%) for S-100, and 35 (92%) for vimentin. The mean MIB-1 index was 3% (median, 1%; range, <1%-30%). Only 8 patients had a proliferative index of more than 3%.

kit Mutations

Exon 11 mutations of the *kit* gene were observed in 23 cases (61%). All were in-frame mutations. There were 9 simple deletions, 8 deletions with preceding or following amino acid change **Table 4**, 3 point mutations, and 3 internal tandem duplications (ITDs) at the 3' end of exon 11 **Table 5**. The ITDs were observed in 3 gastric GISTs (in 2 men and 1 woman). All 3 were spindle cell tumors, 1.5, 10, and 17 cm in size, with mitotic rates of 0, 1, and 5 per 10 HPF, respectively. More than half of the mutations of exon 11 began or were between codons 550 and 560 (n = 14). Deletional mutations in codons 557 and/or 558 were associated with inferior survival; 5 of the 10 patients with these mutations experienced relapse of or died of GIST.

PDGFRA Mutations

Mutations in the *PDGFRA* gene were identified in 7 cases (in 6 men and 1 woman), accounting for 18% of all GISTs **Table 6**. The *PDGFRA* and *kit* mutations were exclusive. Four cases had mutations in the juxtamembrane domain (exon 12), including 1 case with the point mutation V561D and another with the deletion SPDGHE566-571R. Both mutations have been described previously.[11] Two cases had novel duplications at the 3' end of the juxtamembrane domain of *PDGFRA*. Three cases had mutations in the tyrosine kinase domain (exon 18) of the *PDGFRA* gene; a point mutation, D842V, in 2; and 1 deletion, HDSN845-848P. Both mutations also have been described previously.[11] These tumors all were small (range, 1-3.5 cm) gastric tumors with low mitotic activity (mitotic rate, 0/10 HPF in all but 1 case). Of the tumors, 3 were epithelioid type and 4 were spindle cell type. Three tumors were incidental. None of the patients in this group experienced relapse by 11 to 82 months (median, 33 months) after surgery. Comparisons of GISTs with mutations in *kit* exon 11, *PDGFRA* mutations, and no mutations are shown in **Table 7**.

Long-Term Results and Predictors of Survival

No patient was lost to follow-up. The mean follow-up time was 49 months (range, 12-113 months). Of the 38 patients, 9 died, 1 during the early postoperative course, 4 owing to GIST relapse, 3 of concurrent malignancy, and 1 following femur fracture. The actuarial 5-year survival rate was 70%. Of 38 patients, 9 (24%) had disease relapse; the 5-year

Table 3
Comparison of Gastric and Small Bowel GISTs*

Characteristic†	Gastric GISTs (n = 23)	Small Bowel GISTs (n = 13)
Symptomatic	15 (65)	11 (85)
Invasive growth	3 (13)	3 (23)
Tumor necrosis	8 (35)	9 (69)
Mean tumor size (cm)	5.9	7.6
Mean mitotic index (/10 high-power fields)	2.3	6.3
Mean proliferative (MIB-1) index (%)	2.3	4.4
Deletion of codon 557 and/or 558 of *kit*	6 (26)	3 (23)

GISTs, gastrointestinal stromal tumors.
* Data are given as number (percentage) unless otherwise indicated.
† No difference was statistically significant.

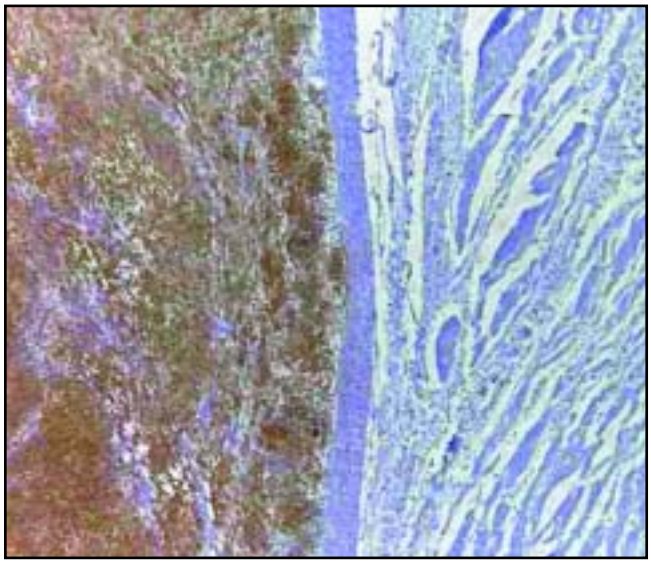

Image 1 Staining for kit in a patient with rectal gastrointestinal stromal tumor. Diffuse, strong, cytoplasmic positivity in the majority of tumor cells (×40).

DOI: 10.1309/AKK3VFF610CWM566

Table 4
Deletional Mutations in Exon 11 of the *kit* Gene[*]

Case	550		560		570		580
WT	S P Q K	P M Y E V	Q W K V V	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
23[†]	S **Q** **K** —	— — — — V	Q W K V V	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
22[†]	S P Q —	— — — — —	— — — V V	E E I	N G N N Y V Y	I D P T Q L Y Y D	H K
37	S P Q **Q**	**R** — — — **I**	Q W K V V	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
9	S P Q K	P — — — —	— — K V V	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
8[†]	S P Q K	P M **S** **E** —	— — — V V	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
26	S P Q K	P M Y E —	— — — — —	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
50	S P Q K	P M Y E V	**H** — — — —	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
42	S P Q K	P M Y E V	— — — — —	— — —	— — — — — — —	— — P T Q L P Y D	H K
47[†]	S P Q K	P M Y E V	**H** — — **F** V	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
55	S P Q K	P M Y E V	Q — — V V	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
52[†]	S P Q K	P M Y E V	Q **C** — — —	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
13[†]	S P Q K	P M Y E V	Q W — — V	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
11	S P Q K	P M Y E V	Q W K V —	E E I	N G N N Y V Y	I D P T Q L P Y D	H K
7, 30	S P Q K	P M Y E V	Q W K V V	E E I	— — — — — — —	— — — — — — P Y D	H K
29, 35[†]	S P Q K	P M Y E V	Q W K V V	E E I	N G N N Y V Y	I D P T Q L P Y —	H K

WT, wild type; —, deletion.
[*] Bold type indicates an amino acid substitution.
[†] Patient died of gastrointestinal stromal tumor or experienced relapse.

Table 5
Point and Insertion Mutations of *kit* Exon 11[*]

Case	550 558 560 572 … 580 … 590 … 600
WT	Q K · K V V · D P T Q L P Y D H K W E F P R N R L S F G Q
21	Q K · K V V · D P *D P* T Q L P Y D H K W E F P R N R L S F G Q
2	Q K · K V V · D P T Q L P Y D H K W E **S** *T Q L P Y D H K W E* F P R N R L S F G Q Y
12	Q K · K V V · D P T Q L P Y D H K W E F P R N R L S F **A** *Y D H K W E F P R N R L S* F G Q
17	Q K · K **D** V · D P T Q L P Y D H K W E F P R N R L S F G Q Y
54, 57	Q K · K V V · D P T Q **P** P Y D H K W E F P R N R L S F G Q Y

WT, wild type.
[*] Bold type indicates an amino acid substitution; italic type, duplication; ·, codons 551-557 and 561-571, respectively.

Table 6
Summary of *PDGFRA* Mutations in *kit*-WT GISTs

PDGFRA Region/Case No.	Mutation
Juxtamembrane (exon 12)	
38	V561D
27	Del SPDGHE566-571R
36	Dup WEFPRDGLV586-595R
44	Dup EIRWRVIESISPDGHEYIYVDPMQ LPYDSRWEFPRDGLVL556-595
Activation loop (exon 18)	
48, 49	D842V
4	Del HDSN845-848P

PDGFRA, platelet-derived growth factor receptor α.

Table 7
Tumor Characteristics in Patients With Mutations in Exon 11 of *kit* and With Mutations in the *PDGFRA* Gene Compared With Those Without Mutations[*]

	No Mutations (n = 8)	*kit* Exon 11 Mutation (n= 23)	*PDGFRA* Mutation (n = 7)
Mean tumor size (cm)	5.2	8.2	1.4[†‡]
Mean mitotic index (/10 high-power fields)	6.7	4.2	0.6[†]
Mean MIB-1 index (%)	4.9	3.2	1.2[†‡]
Invasive growth	2 (25)	5 (22)	0 (0)
Relapse or died of GIST	3 (38)	7 (30)	0 (0)

GIST, gastrointestinal stromal tumor; *PDGFRA*, platelet-derived growth factor receptor α.
[*] Data are given as number (percentage) unless otherwise indicated.
[†] Statistically significant difference ($P < .05$) between GISTs with *PDGFRA* mutations and *kit* exon 11 mutations.
[‡] Statistically significant difference ($P < .05$) between GISTs with *PDGFRA* mutations and no mutations.

disease-free survival was 78% (median, 103 months). Disease recurrence was observed at a median of 10 months postoperatively, whereas death from GIST occurred at a median of 38 months following surgery. The majority of patients with disease recurrence developed liver metastases (n = 8); other metastatic localizations were peritoneal (3), pulmonary (2), and bone (1).

DOI: 10.1309/AKK3VFF610CWM566

Univariate analysis revealed that the following factors were related to inferior disease-free survival **Table 8**: location of GIST in the small bowel ($P = .03$), presence of distant metastases ($P < .0001$), positive resection margins ($P < .0001$), invasive growth pattern ($P < .0001$), presence of tumor necrosis ($P = .02$), mitotic index ($P = .002$), proliferative (MIB-1) index ($P = .002$), tumor size ($P = .04$), and deletional mutation in codons 557 and/or 558 of *kit* exon 11 ($P = .022$).

Multivariate analysis revealed that the presence of distant metastases ($P = .0002$), proliferative (MIB-1) index ($P = .01$), and deletional mutation in codons 557 and/or 558 of *kit* exon 11 ($P = .02$) correlated significantly with a poorer outcome **Figure 1** and **Figure 2**.

Table 8
Outcome According to Various Variables

Factor	No. of Events/ No. of Patients (%)*	P†
Age (y)		
<60	3/16 (19)	
≥60	7/22 (32)	.6
Male	6/23 (26)	
Female	4/15 (27)	.63
Tumor location		
Stomach	3/23 (13)	
Small bowel	7/13 (54)	.03
Symptoms		
Yes	9/27 (33)	
No	1/11 (9)	.19
Invasive growth		
Yes	6/7 (86)	
No	4/31 (13)	<.0001
Tumor necrosis		
Yes	8/17 (47)	
No	2/21 (10)	.02
Mitotic index (/10 high-power fields)		
≤2	3/28 (11)	
>2	7/10 (70)	.002
CD34+		
Yes	3/21 (14)	
No	7/17 (41)	.14
Proliferative (MIB-1) index (%)		
<4	5/30 (17)	
≥4	5/8 (63)	.002
Distant metastases		
Yes	3/3 (100)	
No	7/35 (20)	<.0001
Positive resection margins		
Yes	3/3 (100)	
No	7/35 (20)	<.0001
kit exon 11 mutation		
Yes	7/23 (30)	
No	3/15 (20)	.67
Deletions in *kit* exon 11		
Yes	7/17 (41)	
No	3/21 (14)	.13
Deletion in *kit* codons 557/558		
Yes	5/10 (50)	
No	5/28 (18)	.022
PDGFRA mutation		
Yes	10/31 (32)	
No	0/7 (0)	.13
Size of tumor (cm)‡		
≤2	0/11 (0)	
>2	10/27 (37)	.04
≤10	6/32 (19)	
>10	4/6 (67)	.003

PDGFRA, platelet-derived growth factor receptor α.
* An event indicates the patient died of gastrointestinal stromal tumor or experienced relapse.
† By log-rank test.
‡ $P = .057$ when a cutoff value of 5 cm was used.

Discussion

Favorable long-term results were observed in this study, with disease relapse developing in only 9 of 38 patients. This corresponds to a disease-free survival at 5 years of 78%. The majority of patients could be cured by local excision alone. Therefore, our results are in contrast with poorer overall outcome findings observed by many others.[9,13,14] In the analysis by DeMatteo et al,[13] only 45% of all patients were free of disease at 5 years despite curative resection of their primary tumor. However, in their study a higher proportion of patients had tumors of 5 cm or larger. Furthermore, some referral bias was suggested by the authors. A higher proportion of intestinal GISTs, together with a higher degree of metastatic disease and larger primary tumors were reported by Antonescu et al,[9] leading to relapse in two thirds of the patients. In our study, only local patients were referred. Therefore, our series might more adequately reflect the average population with resectable primary GISTs than many of the previous series. In fact, our results are in accordance with the recent large population-based study from western Sweden,[15] in which a median overall survival of 10.9 years and a mere 17% tumor recurrence rate after complete surgical removal were observed.

During the last decade, prognostic determinants of GISTs have been studied extensively.[16] The predictive value of tumor size and mitotic activity were soon recognized and have been confirmed by most authors,[4,13,17-20] as well as by our study. A substantial fraction of GISTs must still be defined as possessing "uncertain malignant potential"[4] when applying only these 2 factors as prognostic determinants. Numerous reports, therefore, have addressed the question of additional survival predictors, suggesting cellularity, pleomorphism, proliferation markers proliferating cell nuclear antigen and Ki-67, p53, chromosomal aberrations, ploidy, the presence of tumor necrosis, and atypical mitoses as further possible predictive factors.[19-24] Unfortunately, many of these factors are highly investigator-dependent, difficult to standardize, or very expensive to study, making their use in large clinical studies impractical.

The prognostic value of the proliferation marker Ki-67 (MIB-1 index) was proposed by studies of Hillemanns et al,[22] Rudolph et al,[19] and Carrillo et al.[25] In these older analyses, however, kit staining was not performed. Therefore, cases of other gastrointestinal mesenchymal tumors might have been included. In a more recent study, Toquet et al[26] described 35 patients with gastric GISTs. The authors concluded that MIB-1 immunostaining, when used in combination with tumor size

DOI: 10.1309/AKK3VFF610CWM566

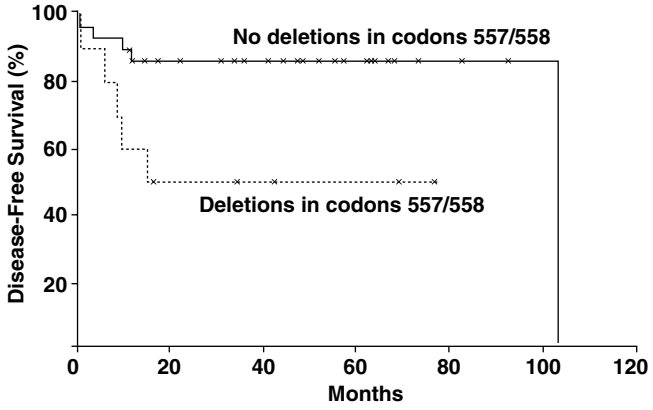

Figure 1 Kaplan-Meier estimates of disease-free survival according to presence of deletional mutations in codons 557 and/or 558 of *kit* exon11.

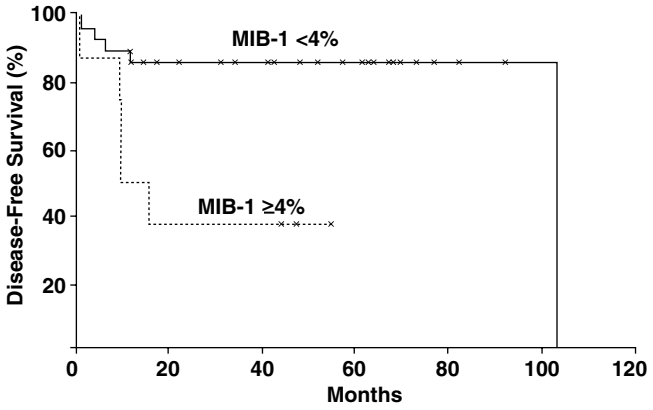

Figure 2 Kaplan-Meier estimates of disease-free survival according to proliferative (MIB-1) index.

and mitotic count, represented an additional marker to identify patients at high risk of recurrence in the case of gastric GISTs. Unfortunately, the authors did not report multivariate analysis of their data. Another recent retrospective analysis by Wong et al[27] did not show a significant advantage of Ki-67 immunohistochemical analysis over simple mitotic count as an outcome predictor in 108 cases of gastric GISTs. Two further retrospective analyses[18,28] also failed to demonstrate a correlation between MIB-1 index and survival, yet again, kit staining had not been performed in either study. In our own study, we found a significant correlation between MIB-1 index and mitotic rate; however, only the MIB-1 index and not the mitotic rate per se independently correlated with reduced disease-free survival in multivariate analysis.

Another problem in the implementation of proliferation markers as prognostic variables is the adequate choice of a cutoff value. Values ranging from 4% to 22% were suggested in some studies,[19,22,25,26,29] and in others, definite cutoff values were not calculated.[15,30] In our study population, merely 1 patient had an MIB-1 index higher than 10%; a cutoff value of 4% was able to identify patients likely to have a poorer outcome. Our results, as well as the aforementioned articles, underline the potential prognostic value of the proliferative activity in GISTs. The ongoing difficulties in standardizing the calculation of the MIB-1 index allow the mitotic rate to remain the most widely accepted determinant of outcome.

Activating mutations of the tyrosine kinase receptor kit are found in 60% to 90% of GISTs and now are widely accepted as an important step in tumorigenesis.[5,7] The most frequent mutation in the *kit* gene is located in exon 11, which encodes the juxtamembrane domain of the kit receptor. This mutation can be detected in 50% to 70% of all cases.[8,9,31] Mutations in exons 9, 13, and 17 together occur in 10% to 20% of GISTs.[8,9,31,32]

Although the prognostic value of the exon 11 mutation has been studied extensively, controversy remains. Early studies documented a significantly higher proportion of exon 11 mutations in patients with malignant (ie, disseminated or recurrent) GISTs,[6,14,24,33] with an overall incidence of exon 11 mutations ranging between 30% and 57%. Taniguchi et al[14] also found a significant correlation between tumor size and occurrence of *kit* exon 11 mutations. In our study, we were able to show that GISTs with exon 11 mutations were, on average, larger than those without. In contrast, Corless et al[8] detected exon 11 mutations in 77% of small (<1 cm), incidental, and clinically benign GISTs, a finding supported by Antonescu et al.[9] More recent studies also have revealed a higher overall incidence of *kit* mutations without any true correlation with prognosis.[5,8,9,31,32,34] *kit* mutations were proposed to be a ubiquitous feature of GISTs.[5] Refinements of DNA analysis methods, using fresh frozen specimens,[5,9,31,34] enable the detection of *kit* mutations in smaller, benign GISTs, thus severely altering the calculated prognostic value.

Recently Singer et al[31] studied the prognostic value of various types of *kit* mutations. Deletion and insertion mutations were found to be associated with reduced disease-free survival compared with exon 11 point mutations. In another study, mutations in exon 9 of *kit* were associated with poor outcome.[9] Finally, Wardelmann et al[10] observed an impact on the biologic behavior of GISTs by deletional mutations of exon 11, especially when codons 557 and/or 558 were lost. We confirmed the results of this study because significantly poorer long-term outcome resulted if codons 557 and/or 558 of exon 11 were deleted (mean disease-free survival, 42 vs 89 months; $P = .02$).

In contrast, so-called ITDs at the 3' end of exon 11, as described by Antonescu et al,[9] were suggested to be associated with a favorable prognosis. The ITDs in this study were located exclusively in gastric GISTs and revealed a benign clinical course, although the follow-up period in this study was short.[9]

DOI: 10.1309/AKK3VFF610CWM566

Our own results confirm the positive prognostic value of GISTs with this type of mutation. Three patients with ITDs in our study had gastric tumors of 1.5, 10, and 17 cm, and none of the tumors recurred by 4.5 to 5.5 years after surgery.

Recently, Heinrich et al[11] described mutations in *PDGFRA*, another receptor tyrosine kinase, in 35% of patients with GISTs lacking *kit* mutations. Ten patients in their study had mutations in the exon 18 encoding kinase region and 4 in exon 12 encoding the juxtamembrane region of *PDGFRA*. No patient with a *PDGFRA* mutation had a simultaneous mutation in *kit*. Therefore, the authors stated that *kit* and *PDGFRA* mutations are mutually exclusive in GISTs. Hirota et al[35] later found that *PDGFRA* mutations have gain-of-function properties. They seem to have a crucial role in the pathogenesis of GISTs without *kit* mutations. However, the morphologic and clinical characteristics of patients with *PDGFRA* mutations are still not well understood. In our series, we were able to study 7 patients with *PDGFRA* mutations, 6 of whom were men. All tumors were gastric GISTs smaller than 3.5 cm, and none relapsed by 11 to 82 months after surgery. However, owing to small numbers, the positive effect did not reach statistical significance ($P = .13$). Heinrich et al[36] recently reported on response to imatinib in a group of patients with advanced (metastatic or unresectable) GISTs. Of 127 patients in their study, 6 (4.7%) had *PDGFRA* mutations. This number seems to be lower than the overall incidence of *PDGFRA* mutations in our population (18%). Therefore, it may be assumed that a certain number of GISTs with *PDGFRA* mutations might manifest with malignant behavior.

We were able to demonstrate that the *kit* mutational status in patients with GISTs might predict the biologic behavior of many tumors. Half of our patients with deletional mutations in codons 557 and/or 558 of *kit* exon 11 experienced disease recurrence despite complete resection. It could be speculated whether such patients might benefit from adjuvant treatment with a tyrosine kinase inhibitor, particularly because imatinib was shown to have a high response rate in patients with *kit* exon 11 mutations.[36] In contrast, patients with ITDs of *kit* exon 11 seem to have a favorable prognosis, even with large gastric tumors. Many questions about the natural history of GISTs with *PDGFRA* mutations remain unanswered. Although 7 patients with small gastric tumors and long-term recurrence-free survival were found in our study population, advanced disease has been reported in the presence of *PDGFRA* mutation by others. The future will show whether the treatment strategy for patients with GISTs might be guided by the specific genetic changes detected in each case. With development of novel therapeutic agents aimed at the tumorigenesis of GISTs at various steps, this could become a reality.

From the Departments of [1]Surgery and [2]Pathology, University of Regensburg, Regensburg, Germany.

[*] Dr Anthuber is currently with the Augsburg Clinic, Augsburg, Germany.

Address reprint requests to Dr Iesalnieks: Dept of Surgery, University of Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany.

References

1. Schaldenbrand JD, Appelman HD. Solitary solid stromal gastrointestinal tumors in von Recklinghausen's disease with minimal smooth muscle differentiation. *Hum Pathol.* 1984;15:229-232.
2. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med.* 2002;347:472-480.
3. van Oosterom AT, Judson I, Verweij J, et al. European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet.* 2001;358:1421-1423.
4. Miettinen M, El-Rifai W, Sobin L, et al. Evaluation of malignancy and prognosis of gastrointestinal stromal tumors: a review. *Hum Pathol.* 2002;33:478-483.
5. Rubin BP, Singer S, Tsao C, et al. kit activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res.* 2001;61:8118-8121.
6. Lasota J, Jasinski M, Sarlomo-Rikala M, et al. Mutations in exon 11 of c-*kit* occur preferentially in malignant versus benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. *Am J Pathol.* 1999;154:53-60.
7. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-*kit* in human gastrointestinal stromal tumors. *Science.* 1998;279:577-580.
8. Corless CL, McGreevey L, Haley A, et al. *kit* mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. *Am J Pathol.* 2002;160:1567-1572.
9. Antonescu CR, Sommer G, Sarran L, et al. Association of *kit* exon 9 mutations with nongastric primary site and aggressive behavior: *kit* mutation analysis and clinical correlates of 120 gastrointestinal stromal tumors. *Clin Cancer Res.* 2003;9:3329-3337.
10. Wardelmann E, Losen I, Hans V, et al. Deletion of Trp-557 and Lys-558 in the juxtamembrane domain of the c-*kit* protooncogene is associated with metastatic behavior of gastrointestinal stromal tumors. *Int J Cancer.* 2003;106:887-895.
11. Heinrich MC, Corless CL, Duensing A, et al. *PDGFRA* activating mutations in gastrointestinal stromal tumors. *Science.* 2003;299:708-710.
12. Fletcher CDM, Berman JJ, Corless C, et al. Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Hum Pathol.* 2002;33:459-465.
13. DeMatteo RP, Lewis JJ, Leung D, et al. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. *Ann Surg.* 2000;231:51-58.
14. Taniguchi M, Nishida T, Hirota S, et al. Effect of c-*kit* mutation on prognosis of gastrointestinal stromal tumors. *Cancer Res.* 1999;59:4297-4300.
15. Nilsson B, Bumming P, Meis-Kindblom JM, et al. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era: a population-based study in western Sweden. *Cancer.* 2005;103:821-829.

DOI: 10.1309/AKK3VFF610CWM566

16. Franquemont DW. Differentiation and risk assessment of gastrointestinal stromal tumors. *Am J Clin Pathol.* 1995;103:41-47.
17. Seidal T, Edvardsson H. Expression of c-kit (CD117) and Ki67 provides information about the possible cell of origin and clinical course of gastrointestinal stromal tumours. *Histopathology.* 1999;34:416-424.
18. Goldblum JR, Appelman HD. Stromal tumors of the duodenum: a histologic and immunohistochemical study of 20 cases. *Am J Surg Pathol.* 1995;19:71-80.
19. Rudolph P, Gloeckner K, Parwaresch R, et al. Immunophenotype, proliferation, DNA ploidy, and biological behavior of gastrointestinal stromal tumors: a multivariate clinicopathologic study. *Hum Pathol.* 1998;29:791-800.
20. Tworek JA, Appelman HD, Singleton TP, et al. Stromal tumors of the jejunum and ileum. *Mod Pathol.* 1997;10:200-209.
21. Franquemont DW, Frierson HF Jr. Proliferating cell nuclear antigen immunoreactivity and prognosis of gastrointestinal stromal tumors. *Mod Pathol.* 1995;8:473-477.
22. Hillemanns M, Pasold S, Bottcher K, et al. Prognostic factors of gastrointestinal stromal tumors of the stomach [in German]. *Verh Dtsch Ges Pathol.* 1998;82:261-266.
23. Cunningham RE, Federspiel BH, McCarthy WF, et al. Predicting prognosis of gastrointestinal smooth muscle tumors: role of clinical and histologic evaluation, flow cytometry, and image cytometry. *Am J Surg Pathol.* 1993;17:588-594.
24. Debiec-Rychter M, Lasota J, Sarlomo-Rikala M, et al. Chromosomal aberrations in malignant gastrointestinal stromal tumors: correlation with c-*kit* gene mutation. *Cancer Genet Cytogenet.* 2001;128:24-30.
25. Carrillo R, Candia A, Rodriguez-Peralto JL, et al. Prognostic significance of DNA ploidy and proliferative index (MIB-1 index) in gastrointestinal stromal tumors. *Hum Pathol.* 1997;28:160-165.
26. Toquet C, Le Neel JC, Goillou L, et al. Elevated (≥10%) MIB-1 proliferative index correlates with poor outcome in gastric stromal tumor patients: a study of 35 cases. *Dig Dis Sci.* 2002;10:2247-2253.
27. Wong NACS, Young R, Malcomson RDG, et al. Prognostic indicators for gastrointestinal tumors: a clinicopathological and immunohistochemical study of 108 resected cases of the stomach. *Histopathology.* 2003;43:118-126.
28. Emory TS, Derringer GA, Sobin LH, et al. Ki-67 (MIB-1) immunohistochemistry as a prognostic factor in gastrointestinal smooth-muscle tumors. *J Surg Pathol.* 1997;2:239-242.
29. Bucher P, Taylor S, Villiger P, et al. Are there any prognostic factors for small intestinal stromal tumors? *Am J Surg.* 2004;187:761-766.
30. Yan H, Marchettini P, Acherman YI, et al. Prognostic assessment of gastrointestinal stromal tumor. *Am J Clin Oncol.* 2003;26:221-228.
31. Singer S, Rubin BP, Lux ML, et al. Prognostic value of *kit* mutation type, mitotic activity, and histologic subtype in gastrointestinal stromal tumors. *J Clin Oncol.* 2002;20:3898-3905.
32. Miettinen M, Furlong M, Sarlomo-Rikala M, et al. Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the rectum and anus: a clinicopathologic, immunohistochemical, and molecular genetic study of 144 cases. *Am J Surg Pathol.* 2001;25:1121-1133.
33. Ernst SI, Hubbs AE, Przygodzki RM, et al. *kit* mutation portends poor prognosis in gastrointestinal stromal/smooth muscle tumors. *Lab Invest.* 1998;78:1633-1636.
34. Andersson J, Sjogren H, Meis-Kindblom JM, et al. The complexity of *kit* gene mutations and chromosome rearrangements and their clinical correlation in gastrointestinal stromal (pacemaker cell) tumors. *Am J Pathol.* 2002;160:15-22.
35. Hirota S, Ohashi A, Nishida T, et al. Gain-of-function mutations of platelet-derived growth factor α gene in gastrointestinal stromal tumors. *Gastroenterology.* 2003;125:660-667.
36. Heinrich MC, Corless CL, Demetri GD, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumors. *J Clin Oncol.* 2003;21:4342-4349.

DOI: 10.1309/AKK3VFF610CWM566

HOLOGIC®

First and Only FDA Cleared
Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager

Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer
with the Advanced Technology of the
Genius™ Digital Diagnostics System

Click or Scan
to discover more

genius™
DIGITAL DIAGNOSTICS