

Stage and histological grade of gastrointestinal stromal tumors based on a new approach are strongly associated with clinical behaviors

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Tumor stage and grade for gastrointestinal stromal tumors are poorly defined. To develop a better evaluation system, we assessed 12 clinical and pathological parameters in 613 patients with follow-up information. These parameters were classified into two gross spread parameters including liver metastasis and peritoneal dissemination, five microscopic spread parameters including lymph node metastasis, vascular, fat, nerve and mucosal infiltration, and five histological parameters including mitotic count ≥ 10 per 50 high-power fields, muscularis propria infiltration, coagulative necrosis, perivascular pattern and severe nuclear atypia. The 5-year disease-free survival and overall survival of 293 patients without any of these predictive parameters of malignancy were 99% and 100%, respectively. They were regarded as nonmalignant and further evaluations on the stage and grade of these tumors were not performed. At least one and at most seven predictive parameters of malignancy were identified in 320 patients. For these patients, the 5-year disease-free survival and overall survival rates were 44% (mean 6.7 years) and 60% (mean 9.3 years), respectively. The disease-free survival showed significant difference between patients with and without gross spread ($P < 0.0001$), with and without microscopic spread ($P = 0.0009$). Disease-free survival and overall survival were associated with the number of predictive parameters of malignancy in patients without gross spread ($P < 0.0001$ for both disease-free survival and overall survival), but not in patients with gross spread ($P = 0.882$ and 0.441 , respectively). Malignant gastrointestinal stromal tumors could be divided into clinical stage I and II based on the absence and presence of gross spread, respectively. The degree of malignancy of patients in clinical stage I could be graded according to the number of predictive parameters of malignancy. Patients in clinical stage II were of the highest degree of malignancy regardless of the number of parameters. We found that the clinical stage and grade were strongly associated with prognosis.

Modern Pathology (2009) 22, 556–569; doi:10.1038/modpathol.2009.11; published online 27 February 2009

Keywords: gastrointestinal stromal tumor; grade; stage; disease-free survival; overall survival

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract. Recent progress in the understanding of the molecular mechanisms of their oncogenesis has contributed to the improvements in their diagnosis and treatment.^{1,2} The discovery that almost all GISTs express KIT/CD117 antigen has led to the development of imatinib mesylate

(imatinib, Glivec; Novartis, Basel, Switzerland) for the targeted therapy of GISTs.³ Several clinical trials have shown the effectiveness of imatinib in the treatment of advanced, metastatic and recurrent GISTs. The life expectancy of GIST patients has dramatically increased after treatment with imatinib. Although most patients with unresectable or metastatic GIST benefit from imatinib treatment, clinical resistance of this drug is a significant problem.⁴ The median time to disease progression is about 18 or 20 months when treated at 400 or 800 mg dose levels.^{5,6} Therefore, the best treatment regimen for patients with malignant GISTs, like doing imatinib preoperatively or postoperatively, is still under debate.^{7–9}

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Received 1 September 2008; revised 19 November 2008; accepted 2 December 2008; published online 27 February 2009

The guideline for the selection of patients for adjuvant therapy varies among experts, mainly due to the criteria predicting patients with a high risk of recurrence after the surgical removal of primary GISTs have yet to be established. Clinically, some patients with malignant GIST are highly aggressive, developing recurrence within short time after surgical removal of the primary tumor, whereas others can be treated effectively by surgical resection alone or had a long latency to develop recurrence. Many investigators made efforts to grade^{10–15} and/or stage GISTs.^{11,16–18} On the basis of these previous reports and our preliminary study, we selected 12 parameters that not only had predictive value for malignancy, but also had value to stage and grade GISTs effectively.¹⁹ We introduced a new and simple method based on these parameters for the staging and grading of GISTs. The objective of this study was to systematically correlate the clinical outcome of a large number of GIST patients with 12 predictive parameters of malignancy and obtain an effective grading and staging system for GISTs. Our new approach was also compared with the NIH consensus criteria and it could be valuable for the design of treatments for GIST patients.

Materials and methods

Tumor Specimens

Medical records and tissue specimens of 1155 primary mesenchymal tumors of GI tract were retrieved from 12 hospitals in Shanghai. The hospitals and the years patients were treated were Zhongshan Hospital, Fudan University, 1993–2006; Cancer Hospital, 1985–2002; Huashan Hospital, 1980–1999; Huadong Hospital, 1981–2000; Changning Centre Hospital, 1992–2002; Zhabei Centre Hospital, 1996–2003; Yangpu Center Hospital, 1990–2004; Chongming Centre Hospital, 1980–2001; Qipu Centre Hospital, 1971–2000; Putuo Centre Hospital, 1983–2000; The Tenth People's Hospital, 1998–2003 and Gongli Hospital, 1996–2003. The 1155 cases were primary mesenchymal tumors previously characterized as leiomyoma, leiomyosarcoma, leiomyoblastoma, schwannoma, stromal or smooth muscle tumors originated from GI tract, 771 of 1155 cases that underwent surgery were immunohistochemically or histologically identified as GISTs based on the positive immunohistochemical detection of KIT or the identical histopathological spectrum with KIT-positive tissues. All tumor slides were reviewed by two experienced pathologists. Another 69 GIST patients were collected from our own consultant file from January 2005 to March 2007. The institutional review boards approved tumor tissue collection and the following analyses.

Clinical Records

Patient demographics and clinical data were retrieved from the medical records. Parameters selected for analyses were the following: age (classified as <50 years or ≥50 years), sex, complaints and main symptoms, tumor size (stratified as <5, ≥5 to <10, and ≥10 cm), tumor site (stomach, duodenum, small intestine, rectum and others including esophagus, colon, extra-GI tract and unspecified), predominant growth pattern (chiefly submucosa, predominantly intramural, mainly outgrowth and others including extra-GI as well as unspecified), presence of ulceration, adhesion, tumor rupture, pedicle, liver metastases, peritoneal dissemination and surgical procedures (with curative or palliative intention).

Histological Evaluation

A total of 1–21 hematoxylin and eosin (H&E)-stained slides (with a median of 4 slides) for each patient were reviewed and the following features were recorded: predominant cell type, pleomorphism, nuclear atypia, necrosis, perivascular pattern, mitotic count and invasion.

Predominant cell type in a tumor tissue refers to the cell type constituting greater than 70% of the total cells, including spindle cells, epithelioid cells or mixed type of cells.²⁰ The severity of pleomorphism of the tumor cells was defined by nuclear atypia and classified by the previously described criteria²¹ with modifications. No or mild pleomorphism indicates that the sizes of tumor cell nuclei show no or little difference from those of normal cells, or the average size of nuclei is roughly equal to one lymphocyte in diameter; moderate pleomorphism indicates some enlargement of the size of the nuclei equaling approximately to two lymphocytes in diameter; severe pleomorphism indicates obvious changes of nuclear sizes, or significant number of atypical nuclei are enlarged to a size transversely equal to three lymphocytes in diameter. The severity of nuclear atypia can be further classified as focal or diffused.^{22,23} A few cases of scattered focal and severe atypia were combined into the group of moderate atypia for statistical analysis, and diffused and severe nuclear atypia were classified as the severe group (Figure 1). Necrosis was classified as coagulate necrosis, when ghost of tumor cells was identified in the necrotic area²¹ (Figure 2). Perivascular pattern was identified when tumor cells packed and grew around vessels as perivascular collars^{23,24} (Figure 3). Mitotic count is the number of mitotically active cells in 50 consecutive high-power fields at a magnification of ×400 using an Olympus BX41 microscope with ×40 objective and ×10 ocular lens (0.159 mm²). All slides were first examined for the most proliferate areas before counting the mitotic cells. All mitotic

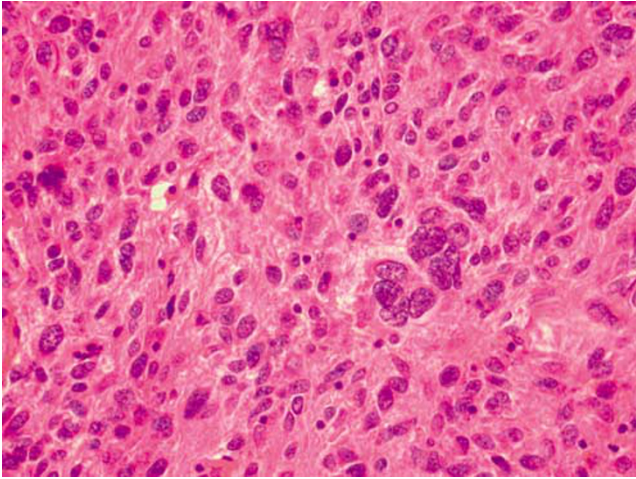


Figure 1 Image of an H&E-stained GIST specimen showing severe nuclear atypia under a light microscope, $\times 400$.

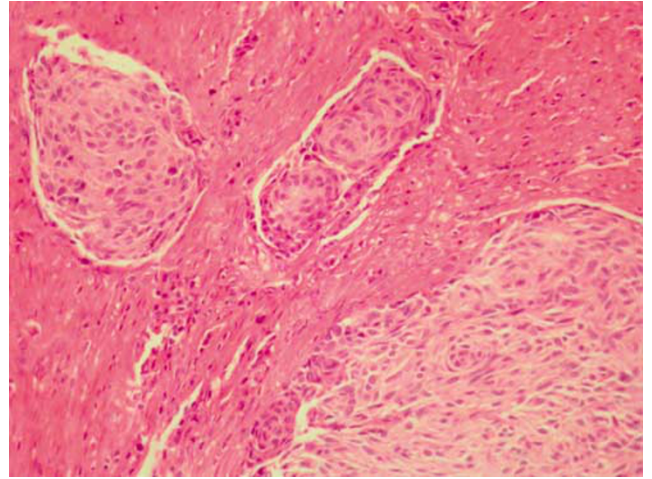


Figure 4 Image of H&E-stained GIST cells invaded into normal muscularis propria displaying a nest-like structure, $\times 200$.

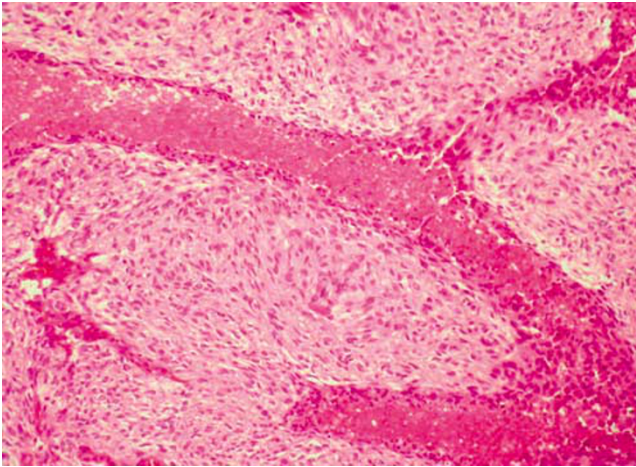


Figure 2 Image of an H&E-stained GIST specimen with coagulative necrosis showing map-like pattern, $\times 200$.

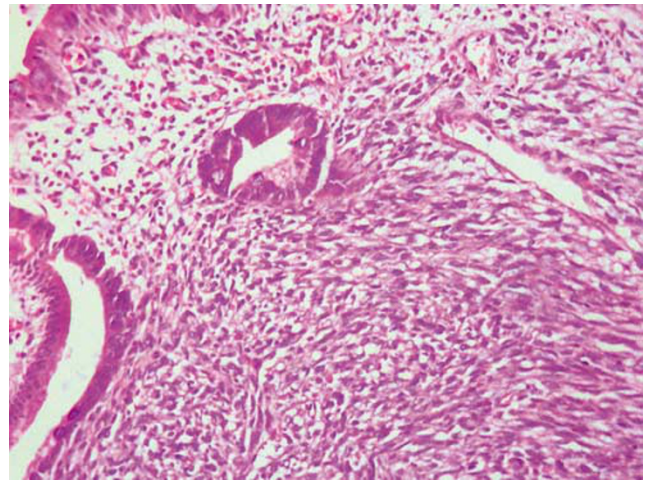


Figure 5 Image of H&E-stained GIST cells infiltrated into the native epithelial elements, $\times 200$.

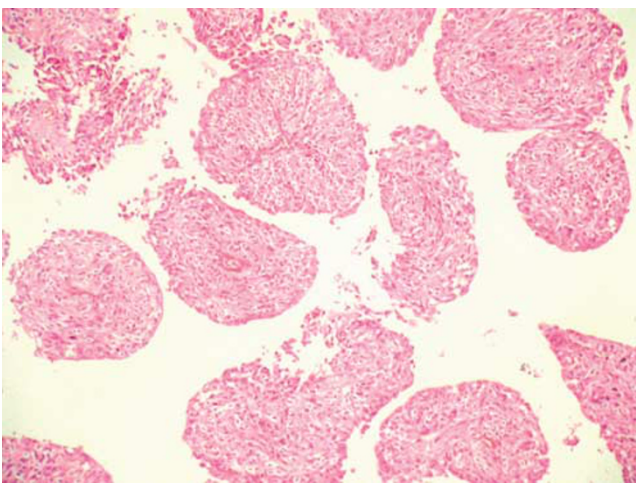


Figure 3 Image of H&E-stained GIST cells grown around vessels displaying a structure recorded as perivascular growth pattern, $\times 200$.

cells counted in 50 high-power fields were recorded as $<5/50$ high-power fields, or $5-10/50$ high-power fields and $\geq 10/50$ high-power fields.

The tumor-invaded tissues were identified under a light microscope by the previously reported methods^{11,20,21,25,26} with modifications. Muscular propria infiltration or 'muscle infiltration' indicates the presence of tumor cells between smooth muscle fibers as tongue-like, nest-like or sheet structures, and the fibers were splayed or dissected by tumor cells^{27,28} (Figure 4). Mucosal infiltration was registered when tumor cells infiltrated inside the normal epithelial layers^{23,27,28} (Figure 5). Invasion of fat^{29,30} (Figure 6) or nerve tissues (Figure 7), vascular infiltration or tumor emboli³¹ (Figure 8) and lymph node metastasis were all recorded. Pathological changes in the tumor stromal structures (cystic, hemorrhagic) were also recorded. Slides were reviewed by experienced pathologists who were blind to the patients' medical records or their disease outcomes.

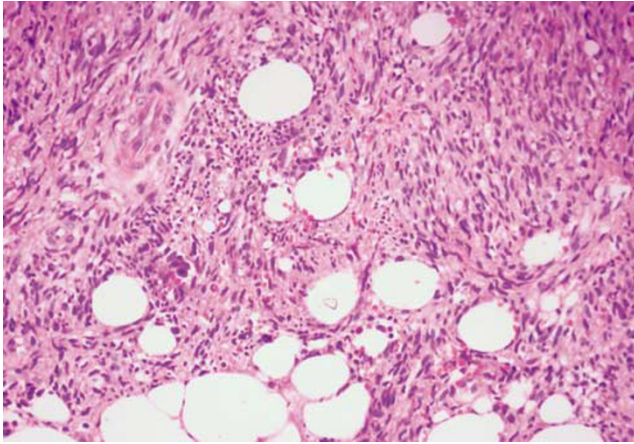


Figure 6 Image of H&E-stained GIST cells infiltrated into the fat tissue, $\times 200$.

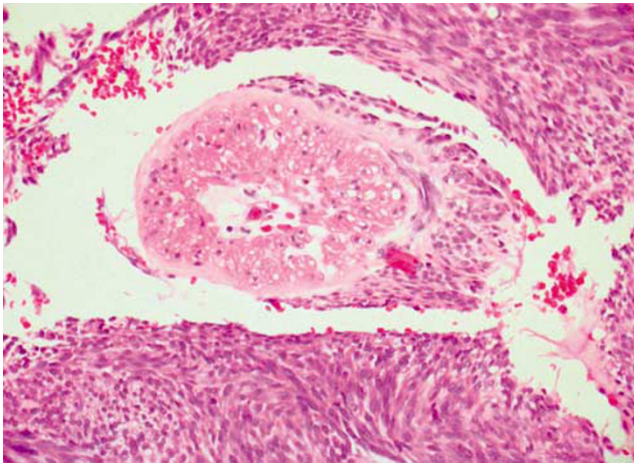


Figure 7 Image of H&E-stained GIST cells infiltrated into the nerve tissue, $\times 200$.

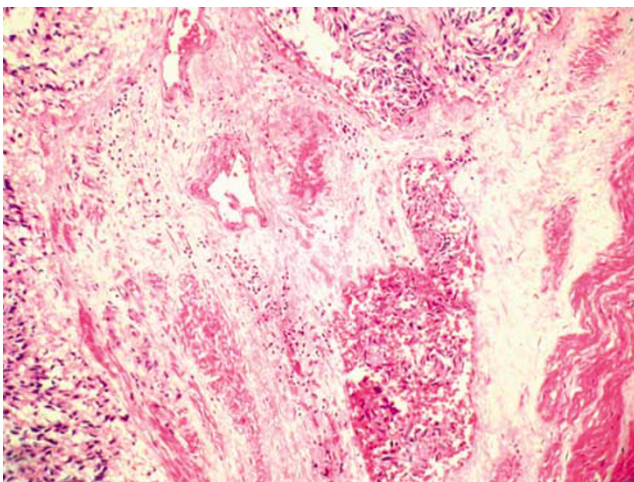


Figure 8 Image of H&E-stained GIST emboli found in the peripheral blood vessel, $\times 200$.

Immunochemical Evaluation

Immunohistochemical staining was performed based on previously reported method.²³ Formalin-fixed paraffin sections were prepared from one representative block and subjected to immunohistochemical staining with a panel of antibodies against CD117 (rabbit polyclonal anti-human c-KIT, diluted 1:150; Dako, Denmark), CD34 (mouse monoclonal antibody, clone QBEnd 10, diluted 1:200; Dako), α -smooth muscle actin (mouse monoclonal antibody, clone 1A4, diluted 1:200; Dako), desmin (mouse monoclonal antibody, clone D33, diluted 1:200; Dako) and S-100 protein (polyclonal, diluted 1:300; Dako). The slides were first treated with 0.01 M citrate buffer (pH 6.0) by microwave method for antigen retrieval, and incubated overnight at 4°C. Immunohistochemical detection was performed with EnVision- and avidin-biotin-based polymer system using a commercial kit (Dako). Diaminobenzidine was used as the chromogen, and all sides were counterstained with hematoxylin.

Patient Follow-up Information

The follow-up information for patients after surgeries and treatments was provided by the referring pathologists and clinicians, or obtained directly from patients and their family members.

Classification and Statistic Analysis

Among 840 patients, 181 patients with curative resection and 5 patients with palliative resection were lost during the follow-up because of relocation or telephone number change, 24 patients without recurrence or death with less than 1-year follow-up were not included in survival analysis, and 33 patients with palliative resection and 7 patients died of other reasons within 1-year were not included in disease-free survival analysis. Therefore, 250 patients were excluded from the disease-free survival analysis. In the end, 590 patients were evaluated for disease-free survival. Disease-free survival and overall survival were measured from the time of surgery to the time of first recurrence or most recent follow-up or death.

For the overall survival analysis, similar patient selection criteria were used, except patients with palliative resection were included and patients with imatinib therapy after recurrence were excluded. In the end, 562 patients were evaluated for overall survival. The total number of patients that underwent disease-free survival and overall survival was 613.

Statistic analysis was carried out using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Kaplan–Meier method was used to calculate the disease-free survival and overall survival functions. Log-rank test and Cox regression were used for univariate and multivariate

analyses. $P < 0.05$ was considered statistically significant.

Results

Clinical and Pathological Features

Of the patients, 373 were male and 240 were female. The patient age range was from 17 to 84 (median, 58 years). The patients most commonly presented with GI bleeding (206 patients), which was most often insidious bleeding with anemia and weakness. The other symptoms were abdominal pain or uncomfortable abdomen, and 86 patients had abdominal mass. In 127 patients, the tumor was incidentally detected during other medical procedure, for example, for physical examination ($n = 57$) and other abdominal surgery ($n = 70$). The median tumor size was 5 cm (range, 0.2–35), and 271 patients were with tumor < 5 cm, 187 with tumor ≥ 5 to < 10 cm and 155 with tumor ≥ 10 cm. There were 335 gastric tumors, 37 duodenal, 141 small intestinal, 60 rectal and 40 at another primary site (10 esophagus, 7 colon, 12 extra-GI tract, and 2 multiple sites and 9 unspecified). 421 were predominantly intramural, 19 were chiefly submucosal, 173 were mainly outgrowth or out of GI tract. 178 patients had adhesion and 25 patients had tumor rupture at operation. Thirteen patients had liver metastases, 22 had peritoneal dissemination and 4 had both liver metastases and peritoneal dissemination.

A majority of the GISTs were spindle-cell tumors (504, 82%). Epithelioid morphology was seen in 47 cases (8%). There were mixed spindle cell and epithelioid component in 62 patients (10%). Tumors exhibited mild to moderate cellularity or mild to moderate nuclear atypia. The degree of cellularity and atypia often varied at different areas of the same tumor. There was no or mild atypia in 136 cases, moderate atypia in 348 cases and severe atypia in 129 cases. Mitotic rates varied from 0 to > 100 per 50 high-power fields (mean, 17 mitoses per 50 high-power field). There were < 5 per 50 high-power fields in 364 patients (59%), ≥ 5 to < 10 per 50 high-power fields in 54 (9%) patients, ≥ 10 per 50 high-power field in 195 patients (32%).

Ulceration was common and present in 175 of 613 cases (29%). Coagulative necrosis was present in 150 (25%) cases, and muscle infiltration was present in 197 cases (32%). A perivascular growth pattern was seen in 120 cases (20%). Mucosal infiltration was seen in 57 cases (9%). Vascular, nerve and fat infiltration were uncommon and seen in 38 (6%), 18 (3%) and 13 (2%) cases, respectively. Lymph node metastasis was rare and seen in only four cases (1%). Hemorrhagic and cystic changes were common and seen in 284 (46%) and 296 (48%) cases, respectively.

Immunohistochemical Features

In the immunohistochemical assay for 383 patients of 613, expression of CD117 and CD34 was observed

in 95 and 80% of the 383 patients. α -Smooth muscle actin was expressed in 28% of the patients and S-100 was expressed in 16% of the patients. Positive immunostaining of desmin was observed in three patients (1%).

Outcome of Patients without Predictive Parameters of Malignancy

There were 293 patients without any predictive parameters of malignancy. These patients were followed up for at least 1 year (mean 4.8 years, ranging from 1 to 31.5 years). Among them, 117 patients were followed up over 5 years, and the longest follow-up time was 31.5 years. There were no death and only three patients developed local relapse. One patient with 2.5 cm duodenal GIST developed recurrence 3.84 years after first enucleation, which was located 2 cm away from the primary site according to surgical record. The patient was then treated by duodenopancreatectomy. The second and the third patient with rectal GIST developed local relapse 5.4 and 9.0 years after excision and subsequently treated with enucleations. All of the recurrent tumors did not present any of the predictive parameters of malignancy. The disease-free survival and overall survival for these patients are listed in Table 1.

Outcome of Patients with Predictive Parameters of Malignancy

There were 320 patients with predictive parameters of malignancy. For disease-free survival, 297 patients were selected for further analysis after excluding 23 patients with palliative resection. Of the 297 patients, 151 patients developed recurrences and the estimated 5-year disease-free survival rate was 44% (mean 6.7 years, median 4 years, ranging from 0.2 to 17.4 years). Of the 151 patients developed recurrences, 63 developed abdominal or pelvic recurrences, 50 had liver metastases without recurrence in primary site, 30 had abdominal or pelvic recurrences and liver metastases, 7 developed local recurrence and 1 had metastasis at unknown location. For overall survival, 269 patients were selected for further analysis after excluding 51 patients treated with imatinib therapy. A total of 93 patients died of GISTs. The estimated 5-year overall survival rate was 60% (mean 9.3 years, median 8.0 years, ranging from 0.2 to 17.4 years).

Evaluation of the Stage and Grade of Patients with 12 Predictive Parameters of Malignancy

A total of 12 predictive parameters of malignancy were used. For a single patient, at least one and at most six predictive parameters of malignancy were identified in 318 patients and 2 patients had seven parameters. Table 1 showed follow-up data by tumor groups defined by the number of parameters. The

Table 1 The 1- to 5-year disease-free survival and overall survival in patients with different number of the predictive parameters of malignancy

Number of parameters/number of patients*	Disease-free survival (%)					Overall survival (%)				
	1 year	2 year	3 year	4 year	5 year	1 year	2 year	3 year	4 year	5 year
0 (293)	100	100	100	99	99	100	100	100	100	100
<i>Without gross spread</i>										
1 (82)	87	69	60	51	46	98	90	82	76	68
2 (54)	96	95	90	78	78	100	99	97	94	90
3 (52)	93	77	74	74	60	98	98	88	88	79
4 (51)	92	69	62	49	44	100	88	78	78	65
5 (32)	80	53	36	27	22	95	78	72	68	51
6 (10)	75	31	17	13	8	91	71	46	26	20
	50	<15	—	—	—	100	75	—	—	—
<i>With gross spread</i>										
1 (3)	38	19	6	0	—	71	25	15	8	8
2 (9)	0	—	—	—	—	67	33	0	—	—
3 (6)	67	33	0	—	—	57	14	0	—	—
4 (7)	0	—	—	—	—	80	0	—	—	—
5 (5)	0	—	—	—	—	43	14	0	—	—
6 (7)	0	—	—	—	—	60	40	40	0	—
7 (2)	25	25	25	25	25	60	30	30	30	30
	50	50	0	—	—	50	50	50	0	—

Disease-free survival and overall survival indicated broad spectrum of GIST patients from indolent to highly aggressive.

Table 2 Nature tiers and combined tiers of patients with malignant GIST evaluated by the number of parameters presented in GIST patients based on log-rank test for disease-free survival and overall survival

Current classification	Disease-free survival	Overall survival
Presence of gross spread vs absence of gross spread	<0.0001	<0.0001
<i>Absence of gross spread</i>	<0.0001	<0.0001
GISTs with 1 parameter vs GISTs with 2 parameters	0.0390	0.1890
GISTs with 2 parameters vs GISTs with 3 parameters	0.0340	0.0367
GISTs with 3 parameters vs GISTs with 4 parameters	0.0120	0.0921
GISTs with 4 parameters vs GISTs with 5 parameters	0.0381	0.0502
GISTs with 5 parameters vs GISTs with 6 parameter	0.0225	0.7471
GISTs with 5 parameters vs GISTs with gross spread	0.0999	0.0032
GISTs with 6 parameters vs GISTs with gross spread	0.8957	0.0720
GISTs with 1+2 parameters vs GISTs with 3+4 parameters	<0.0001	<0.0001
GISTs with 3+4 parameters vs GISTs with 5+6 parameters	<0.0001	0.0033
Presence of microscopic spread vs absence of microscopic spread	0.0009	0.4611
<i>Presence of gross spread</i>	0.8820	0.4410
GISTs with 1 parameter vs GISTs with 2 parameters	0.9183	0.5254
GISTs with 2 parameters vs GISTs with 3 parameters	0.2212	0.7925
GISTs with 3 parameters vs GISTs with 4 parameters	0.3508	0.6129
GISTs with 4 parameters vs GISTs with 5 parameters	0.3173	0.1570
GISTs with 5 parameters vs GISTs with 6 parameters	0.7543	0.7339
GISTs with 1 parameter vs GISTs with 6 parameters	0.7543	0.5742

GIST, gastrointestinal stromal tumor.

Disease-free survival or overall survival can be stratified by the number of parameters for patients without gross spread, but not for patients with gross spread. In patients with 5 or 6 parameters and absence of gross spread exhibited consecutive biological behavior with patients presence of gross spread. Gray background indicate consecutive biological behavior.

disease-free survival and overall survival decreased with the increase of the number of parameters in patients without gross spread (or organ confined), but not in patients with gross spread.

Disease-free survival and overall survival were associated with the number of the predictive parameters of malignancy in patients without gross spread ($P < 0.0001$ for both disease-free survival and

overall survival), but not in patients with gross spread ($P = 0.882$ and 0.441) (Table 2).

Furthermore, in patients without gross spread (organ confined), disease-free survival exhibited significant difference between patients with consecutive number of parameters, like 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 ($P = 0.0390$, 0.0340 , 0.0120 , 0.0381 , 0.0225 , respectively). For overall

survival correlation, part of the pair of consecutive numbers exhibited significant difference. The correlation coefficients between 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 were 0.1890, 0.0367, 0.0921, 0.0502, 0.7471, respectively (Table 2).

However, when some of the consecutive number groups were combined together, both disease-free survival and overall survival showed significance difference. For example, when group with 1+2 parameters vs 3+4 parameters and 3+4 vs 5+6 were compared, $P < 0.0001$ was obtained for disease-free survival. $P < 0.0001$ and 0.0033 were obtained for 1+2 vs 3+4 and 3+4 vs 5+6, respectively, for overall survival (Table 2). In addition, the disease-free survival showed significant difference between patients with and without microscopic spread ($P = 0.0009$) (Table 2).

On the basis of the above results, we therefore divided GISTs with predictive parameters of malignancy into clinical stage I (or organ confined) and II based on the absence or presence of gross spread at the time of diagnosis, and clinical stage I was divided into pathological stage I and II based on the absence or presence of microscopic spread.

The degree of malignancy of patients in clinical stage I could be graded according to the number of malignant parameters and patients were classified into low (patients with one and two parameters), moderate (patients with three and four parameters) and high (patients with five and six parameters) degree of malignancy. Patients in clinical stage II were of the highest degree of malignancy regardless the number of parameters, and there was no need to grade patients with gross spread further.

Correlation of Prognosis with Clinical and Pathological Parameters, and Tumor Nature, Grade and Stage

First, we showed that most of the clinical and pathological parameters affected the prognosis (disease-free survival) of patients with GIST by univariate analysis when all of the patients with GIST were regarded as one group. A total of 11 clinical and 12 pathological parameters associated with adverse prognosis are listed in Table 3.

Second, the correlation of clinical and pathological parameters with prognosis was changed or lost when tumor nature (malignant or nonmalignant) was classified. For example, in 293 patients with nonmalignant GIST, only rectal GIST associated with adverse prognosis since 2 patients developed local recurrence in 28 patients with rectal GIST and 1 patient developed local recurrence in 265 patients with non-rectal GIST; in malignant group, there were seven clinical and seven pathological parameters associated with adverse prognosis (Table 3).

Third, when these 14 parameters were further analyzed by multivariate analyses, it revealed that 4 clinical and 5 pathological parameters were inde-

pendent prognostic factors. The clinical parameters were adhesion ($P = 0.001$), tumor rupture ($P = 0.006$), gross spread ($P = 0.008$) and anorectal GISTs ($P = 0.003$); the pathological parameters were severe nuclear atypia ($P = 0.043$), mitoses ≥ 10 per 50 high-power field ($P < 0.0001$), coagulative necrosis ($P < 0.0001$), vascular infiltration ($P < 0.0001$) and fat infiltration ($P = 0.014$). The tumor size had no prognostic function in these multivariate analyses system (Table 4).

However, when two stages and three-tier grade system were classified (Table 3), only a small number of parameters were associated with prognosis in different grades and stages, and the parameters were not consistent. Furthermore, tumor grade and stage as classified by our new system were strongly associated with disease-free survival independent of the clinical (Table 5) and pathological parameters (Table 6). The Kaplan-Meier plots of disease-free survival and overall survival are shown in Figures 9 and 10 for patients with nonmalignant GIST and malignant GIST including low, moderate, high grade in clinical stage I and patients in clinical stage II ($P < 0.0001$ for both). The mean and median time of disease-free survival for these groups was 18.1 years, not reached; 10.7 years, 14.4 years; 4.8 years, 3.0 years; 1.7 years, 1.2 years and 1.3 years, 0.8 years, respectively. Patients with nonmalignant GIST could be cured by surgical resection alone. Therefore, there is no mean and median time for overall survival in these patients. The mean and median time of overall survival for the malignant patients including low, moderate, high grade in clinical stage I and patients in clinical stage II were 14.1 years, 15.6 years; 7.2 years, 6.8 years; 3.8 years, 3.0 years; 1.9 years, 1.2 years, respectively.

Relationship of Tumor Nature, Grade and Stage with the NIH Consensus Criteria

When the 613 patients with GIST were classified into different risk levels according to the NIH consensus criteria, 81 cases were classified into the very-low-risk level, 179 into the low-risk level, 98 into the intermediate-risk level, and 255 into the high-risk level. There were 5, 10, 14 and 125 patients who developed recurrence in the four risk levels. Among the lower three levels, only marginal difference was presented ($P = 0.06$) for disease-free survival (Figure 11); for the high-risk level, patients could be classified further into three subgroups based on the NIH criteria: tumor size > 5 cm and mitoses $> 5/50$ per high-power field, tumor size > 10 cm with any mitoses and mitoses > 10 per 50 high-power field with any size. Among these subgroups, significant difference ($P < 0.0001$) was presented for disease-free survival (Figure 12).

The distributions of all patients with GIST in various groups classified by the NIH system and by our current tumor nature, grade and stage

Table 3 Univariate analysis of factors that influence disease-free survival in all of the patients stratified with the new staging and grading system by log-rank test

	<i>All patients</i>	<i>Nonmalignant</i>	<i>Malignant</i>	<i>Low grade</i>	<i>Moderate grade</i>	<i>High grade</i>	<i>Stage cII</i>
Sex	<0.0001	0.5969	0.025	0.3007	0.9852	0.5113	0.1234
Age (year)	0.001	0.1983	0.070	0.0922	0.2972	0.3361	0.7433
Gastrointestinal bleeding	0.548	0.9864	<i>0.042</i>	0.1788	0.1210	0.6779	0.7867
Abdominal pain	0.257	0.4484	0.716	0.8150	0.5704	0.8204	0.7864
Abdominal discomfort	0.114	0.3984	0.120	0.0562	0.8506	0.5873	0.2095
Ulceration	0.579	0.3055	0.155	0.4354	<i>0.0007</i>	0.3497	0.0649
Abdominal mass	<0.0001	0.5227	0.045	0.5067	0.1101	0.9825	0.8611
Adhesion	<0.0001	0.7267	<0.0001	0.0014	0.0007	0.0236	0.9215
Tumor rupture	<0.0001	No value	0.001	0.2510	0.0044	0.6974	0.0111
Gross spread	<0.0001	No value	<0.0001	No value	No value	No value	No value
Pedicle	<i>0.009</i>	0.6303	<i>0.007</i>	0.0511	0.4974	No value	0.9397
<i>Tumor size (cm)</i>							
<5: ≥5 to <10: ≥10	<0.0001	0.4888	<0.0001	0.7411	0.0078	0.1425	0.9274
<i>Tumor site</i>							
Stomach	<0.0001	<i>0.0325</i>	<i>0.006</i>	<i>0.0039</i>	0.0567	0.9717	0.3664
Duodenum	0.962	0.0814	0.747	0.3006	0.7753	0.5099	No value
Small bowel	<0.0001	0.5269	0.229	0.8118	0.2744	0.8818	0.3112
Anorectum	0.001	0.0201	0.001	<0.0001	0.0962	0.8629	No value
Other sites	0.687	0.7137	0.571	0.3526	0.7918	0.3672	0.8020
<i>Growth pattern</i>							
Submucosa	<i>0.009</i>	0.6615	<i>0.025</i>	0.2231	—	No value	No value
Transmural	0.157	0.2990	0.542	0.3347	0.4334	0.4579	0.0491
Subserosa	0.045	0.4187	0.837	0.6946	0.4039	0.5808	<i>0.0162</i>
Other patterns	0.042	0.7798	0.308	0.8018	0.1278	0.4686	0.8615
<i>Cell type</i>							
Spindle	<i>0.006</i>	0.5529	0.653	0.0894	0.4597	0.3985	0.4601
Epithelioid	0.618	0.6456	0.208	0.1138	0.8357	0.7279	0.6649
Mixed	<0.0001	0.7267	0.089	0.4962	0.2025	0.4871	0.3507
Nuclear atypia	<0.0001	0.6731	0.001	0.0198	0.1147	0.2578	0.4665
<i>Mitotic count (50 HPFs)</i>							
<5: ≥5 to <10: ≥10	<0.0001	0.6623	<0.0001	0.0025	0.2624	0.7344	0.7803
Muscle infiltration	<0.0001	—	0.025	0.9952	<i>0.0104</i>	<i>0.0324</i>	0.9234
Coagulative necrosis	<0.0001	—	<0.0001	0.0692	0.0707	0.1113	0.4034
Coin-like pattern	<0.0001	—	<0.0001	0.9596	0.8550	0.5167	0.6235
Hemorrhagic change	<0.0001	0.2145	0.376	0.6286	0.2603	0.4389	0.2011
Cystic change	0.005	0.2087	0.963	0.4933	0.9688	0.4195	0.1238
Mucosal infiltration	<0.0001	—	0.076	0.8195	0.5207	0.7657	0.2227
Vascular infiltration	<0.0001	—	0.004	0.6606	0.4771	0.1891	0.8879
Fatty infiltration	<0.0001	—	0.034	—	0.8798	0.6419	0.0186
Nerve infiltration	<0.0001	—	0.128	—	0.5329	0.4143	0.7867

HPF, high-power field.
Italics indicate parameters associate with favorable prognosis.
Bold indicate parameters associate with adverse prognosis.

classification system are shown in Table 7. There were 3 (1.0%) patients with recurrences in the non-malignant group, 34 (25%) in the low-grade group, 65 in the moderate-grade group (63%), 36 (86%) in the high-grade group, and all of the 16 patients in clinical stage II treated with surgical resection with curative intention developed recurrence (100%). For each risk level stratified by the NIH criteria, there is a significant correlation of disease-free survival with our current classification system (all *P*'s <0.0001). However, for each group stratified by our grading and staging system, there is no significant correlation of disease-free survival with the different risk

levels classified by the NIH criteria, except for low-grade group (*P* = 0.001).

Discussion

Tremendous progress has been made in the diagnosis and treatment of GIST since Hirota *et al* reported the molecular basis of GISTs. Most GISTs have a mutation in the c-kit proto-oncogene, leading to the constitutive expression of KIT protein. In addition, an activating mutation in the *PDGFRA* gene was identified in about 5% of GISTs.³² GISTs

Table 4 Multivariate analyses of parameters that influence disease-free survival for 297 patients with malignant GIST evaluated by Cox regression

Parameters	Classification	Multivariate analyses 95% CI for Exp			P-value
		Wald	Lower	Upper	
Sex	Male/female	0.596	0.598	1.251	0.440
Palpable mass	Yes:no	0.007	0.663	1.564	0.934
Adhesion	Yes:no	10.095	0.329	0.769	0.001
Tumor rupture	Yes:no	7.513	0.265	0.802	0.006
Gross spread	Yes:no	7.120	1.240	4.082	0.008
Tumor size (cm)	<5				
	≥5 to <10	0.383	0.478	1.468	0.536
	≥10	0.049	0.585	1.958	0.825
Tumor site	Anorectum (Yes:no)	8.794	0.302	0.783	0.003
Nuclear atypia	Mild:Moderate:Severe	4.094	1.012	2.056	0.043
Mitotic count (50 HPFs)	<5				
	≥5 to <10	0.799	0.652	3.141	0.371
	≥10	24.118	2.003	5.039	< 0.0001
Muscle infiltration	Yes:no	0.012	0.676	1.420	0.913
Coagulative necrosis	Yes:no	14.520	0.316	0.691	< 0.0001
Perivascular pattern	Yes:no	3.369	0.483	1.024	0.066
Vascular infiltration	Yes:no	19.465	0.212	0.550	< 0.0001
Fatty infiltration	Yes:no	6.016	1.226	6.217	0.014

HPF, high-power field.

Bold indicate parameters associate with adverse prognosis.

Table 5 Effect of malignant grade on disease-free survival and overall survival with respect to clinical parameters evaluated by log-rank test

Parameters	Classification	5-year disease-free survival low:moderate:high	Disease-free survival	Overall survival
Sex	Male	64:36:5	< 0.0001	< 0.0001
	Female	79:35:14	< 0.0001	< 0.0001
Age (year)	<50	60:35:9	0.0001	< 0.0001
	≥50	76:32:7	< 0.0001	< 0.0001
GI bleeding	Yes	77:39:7	< 0.0001	< 0.0001
	No	65:30:8	< 0.0001	< 0.0001
Abdominal pain	Yes	70:34:29	0.0020	0.003
	No	70:34:4	< 0.0001	< 0.0001
Abdominal discomfort	Yes	79:32:17	0.0001	0.001
	No	68:34:7	< 0.0001	< 0.0001
Ulceration	Yes	79:54:0	< 0.0001	< 0.0001
	No	67:21:13	< 0.0001	< 0.0001
Abdominal mass	Yes	69:18:11	< 0.0001	0.009
	No	71:37:5	< 0.0001	< 0.0001
Adhesion	Yes	54:17:4	< 0.0001	< 0.0001
	No	82:50:10	< 0.0001	< 0.0001
Tumor rupture	Yes	60:17:0	0.0203	0.041
	No	71:36:7	< 0.0001	< 0.0001
Pedicle	Yes	100:67:no	0.0555	0.046
	No	67:33:7	< 0.0001	< 0.0001
Tumor size (cm)	<5	76:41:30	0.0015	< 0.0001
	≥5 to <10	71:44:0	< 0.0001	< 0.0001
	≥10	66:20:7	< 0.0001	< 0.0001
Tumor site	Stomach	86:50:9	< 0.0001	< 0.0001
	Duodenum	58:33:0	0.2327	0.049
	Small bowel	68:26:12	< 0.0001	0.001
	Anorectum	31:20:0	0.1358	0.013
	Others	86:50:25	0.0004	0.008
Growth pattern	Submucosa	100:100:no	—	—
	Transmural	66:38:10	< 0.0001	< 0.0001
	Subserosa	74:23:0	< 0.0001	< 0.0001
	Others	80:0:0	0.0005	0.083

GI, gastrointestinal.

Bold indicate significant difference.

Table 6 Effect of malignant grade on disease-free survival and overall survival with respect to pathological parameters evaluated by log-rank test

Parameters	Classification	5-year disease-free survival low:moderate:high	Disease-free survival	Overall survival
Cell type	Spindle	67:35:5	<0.0001	<0.0001
	Epithelioid	100:32:0	0.0022	0.045
	Mixed	85:22:21	0.0034	0.004
Nuclear atypia	Mild	—		
	Moderate	62:38:0	<0.0001	0.003
	Severe	90:22:8	<0.0001	<0.0001
Mitotic count (50 HPFs)	<5	80:57:0	<0.0001	<0.0001
	5 to 10	80:29:0	0.0678	—
	≥10	48:29:7	<0.0001	0.003
M-proprria infiltration	Yes	70:40:7	<0.0001	<0.0001
	No	70:12:0	<0.0001	<0.0001
Coagulative necrosis	Yes	53:29:6	<0.0001	0.001
	No	74:43:33	<0.0001	0.002
Coin-like pattern	Yes	70:37:8	<0.0001	0.010
	No	70:31:13	<0.0001	<0.0001
Hemorrhagic change	Yes	72:32:5	<0.0001	<0.0001
	No	67:35:9	<0.0001	0.002
Cystic change	Yes	75:39:5	<0.0001	<0.0001
	No	64:29:9	<0.0001	0.001
Mucosal infiltration	Yes	89:42:0	0.0001	0.007
	No	69:31:9	<0.0001	<0.0001
Vascular infiltration	Yes	67:14:11	<0.0001	0.216
	No	71:37:8	<0.0001	<0.0001
Fatty infiltration	Yes	100:50:0	0.0716	0.368
	No	70:34:8	<0.0001	<0.0001
Nerve infiltration	Yes	100:40:28	0.2377	—
	No	71:33:6	<0.0001	<0.0001

HPF, high-power field.
Bold indicate significant difference.

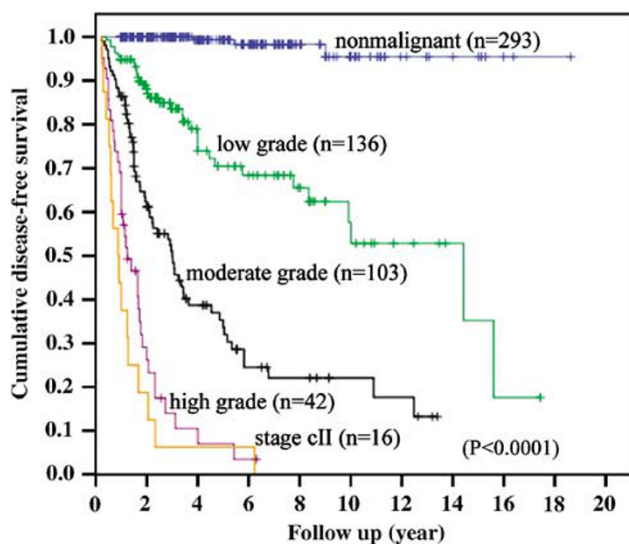


Figure 9 Kaplan–Meier representation of cumulative disease-free survival of the nonmalignant, different grades and clinical stage II ($P<0.0001$) according to the current classification.

have a wide spectrum of clinical behavior that spans from indolent and curable lesions by surgical resection alone to highly malignant diseases that metastasize and become lethal.³³ The treatment of GIST is complete excision when possible, and treatment with KIT/PDGFR tyrosine kinase inhibitors,

such as imatinib, when the tumor is unresectable or in metastatic stage. Unfortunately, tumor resistance to imatinib treatment is a significant problem, and the long-term success is limited. The imatinib resistance is usually generated by the development of the secondary mutations in the KIT or PDGFRA tyrosine kinase domains.^{4,34–36} Although the tyrosine kinase inhibitor SU11248 and PKC412 can be used as the second-line treatments and some patients with imatinib-resistant GIST showed response to these drugs,³⁷ the median time to disease re-progression was only about 6 months. Because imatinib adjuvant as well as neoadjuvant treatment can dramatically improve the prognosis for high-grade malignant GISTs,⁹ there are potential benefits to apply imatinib therapy preoperatively or post-operatively in patients with malignant GIST. Up to now, there is still no consensus on the selection of candidates for adjuvant therapy, mainly because the criteria predicting patients with a high risk of recurrence after the surgical removal of primary GISTs have yet to be established.

Initially it is proposed that the presence of genetic mutations could be used as a marker to predict malignancy or poor prognosis.^{38–43} Since approximately 90% of the GISTs contain either *KIT* or *PDGFRA* gene mutations, it is apparently not a reliable criterion.⁴⁴ Studies indicated that the type of mutation may predict prognosis.^{40,41,45–47} On the

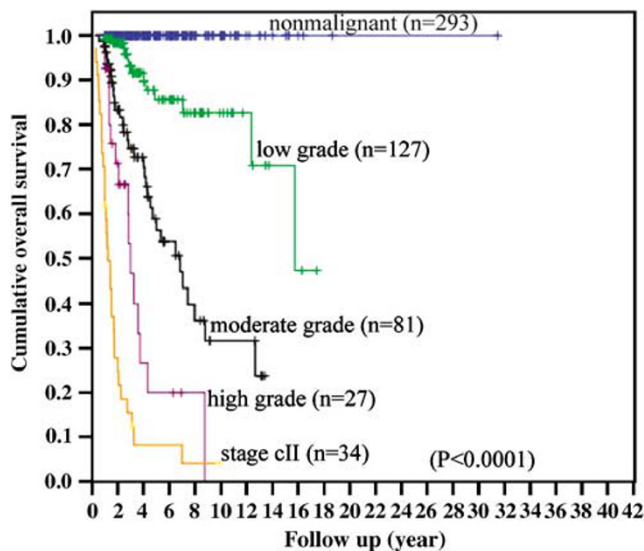


Figure 10 Kaplan–Meier representation of cumulative overall survival of the nonmalignant, different grades and clinical stage II ($P < 0.0001$) according to the current classification.

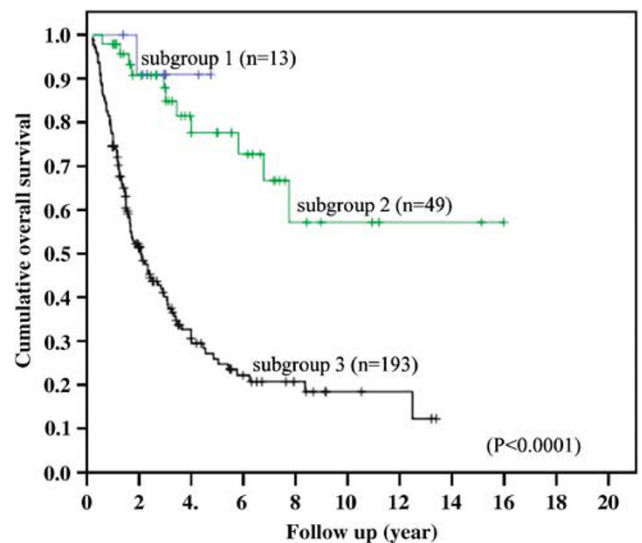


Figure 12 Kaplan–Meier representation of cumulative disease-free survival of the subgroups in high-risk level based on the NIH criteria ($P < 0.0001$).

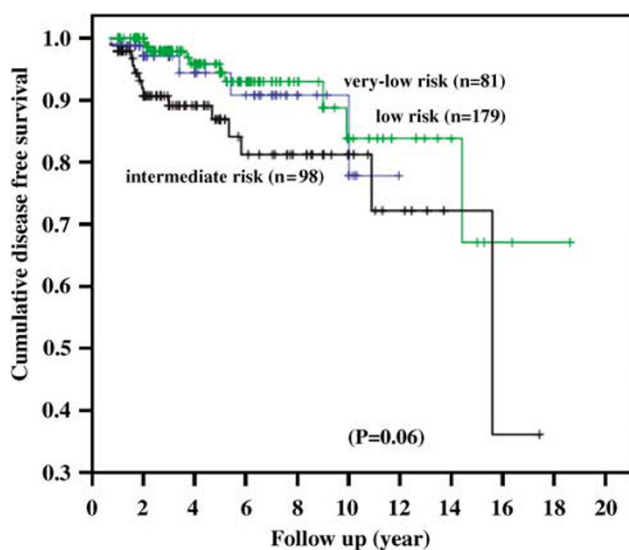


Figure 11 Kaplan–Meier representation of cumulative disease-free survival of the very low-risk, low-risk and intermediate-risk levels based on the NIH criteria ($P = 0.06$).

basis of our unpublished data and recent reports, the most reliable mutation type with independent prognostic role was homogenous deletion in exon 11 of *KIT* gene.⁴⁸ However, the frequency of this homogenous mutation is rare. DNA ploidy, telomerase activity, immunohistochemical staining for cell proliferation antigens and other factors involved in the regulation of the cell cycle (p53, p16, p21, bcl-2, CD44, cox-2) have been investigated as prognostic factors, but no consistent results were obtained.⁴⁹

It is known that GISTs exhibited a broad range of biologic behaviors. There is a strong need for a practical and reliable classification system that can

be used to predict the clinical course of GIST patients and facilitate the design of treatment regimen. On the basis of previous investigations and our preliminary work,^{10–18} we collected the pathological and clinical data of a large number of GIST patients and assessed a simple and new grading and staging system. Patients from multiple hospitals were analyzed because it is expected to be more likely free of selection bias, which could occur in different hospitals or treatment trials.

We used 12 clinical and pathological predictive parameters of malignancy, and these predictive parameters of malignancy were divided into three types according to the distance of spread from the primary sites. Gross spread (clinical stage II) parameters included liver metastasis and peritoneal dissemination; microscopic spread (pathological stage II) parameters included lymph node metastasis, vascular, fat, nerve and mucosal infiltration; and localized (pathological stage I) parameters included mitotic count ≥ 10 per 50 high-power fields, muscularis propria infiltration, coagulative necrosis, perivascular pattern and severe nuclear atypia.

The malignant and nonmalignant tumors were separated first by the presence and absence of any of these parameters; second, the clinical stage II and stage I were classified based on presence and absence any of gross spread parameters; and finally the natural tiers or combined tiers of malignant tumors were represented by the number of parameters that were identified for the tumor. When the tumors were staged and graded by the new system, strong correlations of disease-free survival and overall survival with the tumor stage and grade were obtained. This is the first time that the degree of malignancy was demonstrated by the number of the predictive parameters of malignancy.

Table 7 Distribution of patients based on the new system and NIH criteria and their correlations with respective grade and risk evaluated by log-rank test

NIH criteria	Tumor nature, grade and stage					Total/recurrent event	P-value
	Nonmalignant	Malignant			Stage cII		
		Stage cI					
		Low	Moderate	High			
Very low risk	77	4	0	0	0	81/5	<0.0001
Low risk	146	28	5	0	0	179/10	<0.0001
Intermediate risk	48	36	10	3	1	98/14	<0.0001
High risk	22	68	88	39	38	255/125	<0.0001
Total	293	136	103	42	39	613/154	—
Recurrent event	3	34	65	36	16 ^a	—	—
P-value	0.759	0.001	0.540	0.867	—	—	—

^aOnly patients with curative excision included in disease-free survival.

Previous studies have demonstrated that the adverse outcome of GISTs correlates with the increase in the tumor size,^{12,16,25,50–53} mitotic rate,^{20,25,51,52,54} cellularity^{51,52,55} and nuclear atypia.¹⁶ In addition, tumor site^{23,56,57} from the upper to lower GI tract seemingly had an effect on the outcome too. The similar observations were made in our study. When all patients were treated as one group, each pathological parameter and most of the clinical parameters showed effect on the disease-free survival in a way as previously reported. However, when patients were grouped into more homogenous groups by nonmalignancy, malignant degrees and stages, less and less clinical and pathological factors had significant correlation with disease-free survival by univariate analysis. For example, only one parameter correlated with disease-free survival in nonmalignant GISTs, four correlated with disease-free survival in low-grade tumors, three in moderate-grade tumors, one in high-grade tumors and three in clinical stage II tumors. Moreover, these parameters are not consistent in different stage and grade of tumors. In addition, tumor size no longer showed any prognostic function in multivariate analyses.

On the contrary, when tumors were graded and staged by our system with the identified parameters, the disease-free survival nearly always strongly correlated with the malignant grade, indicating that the clinical stage and grade were closely associated with the prognosis of GISTs. On the basis of our analytic method and results, it can be concluded that the predictive role of a single predictive parameter of prognosis varied with the tumor nature, grade and stage. Therefore, the role of a single parameter is limited in prognosis prediction.

The NIH consensus criteria based on the tumor size and mitotic count have gained general acceptance to predict the prognosis of GISTs. By comparing the NIH consensus criteria with our staging and grading system, interesting differences were observed. We found our predictive parameters of

malignancy showed malignant course for some patients with small and mitotically inactive GISTs, for whom the NIH consensus criteria only predict low risk. Moreover, some patients with GISTs of bigger size did not show any of the predictive parameters of malignancy experienced indolent outcome, for whom the NIH consensus criteria should predict high risk. These observations indicated that tumor size/mitoses alone are not adequate for the accurate prediction of prognosis. In addition, on the one hand, we found only marginal significant difference among the very low level, low level and intermediate level, indicating that these levels could be combined together; on the other hand, we found significant difference existed in the subgroups of the high-risk level, indicating that these subgroup should be stratified further, especially for mitoses >10 per 50 high-power field with any size. These problems of the grading system in the NIH criteria were also pointed out by Huang *et al*,⁵⁸ and sometimes existed in other soft sarcomas.⁵⁹

In conclusion, we showed that the stage and grade of tumors classified by our 12 clinical and pathological parameters strongly correlated with the prognosis of GISTs. Nonmalignant and malignant tumors were first identified, the stage was second classified and the number of predictive parameters of malignancy was used to represent the degree of malignancy in organ-confined sarcoma. The prognosis of malignant GISTs was strongly associated with tumor stage and grade. Initial comparison showed that our system can predict the prognosis better than the NIH consensus criteria. For non-malignant patients, it is unnecessary for further therapy, for patients with malignant GIST especially higher degree of malignancy, adjuvant therapy should be recommended postoperatively. The usefulness of our new staging and grading system in predicting the outcome of imatinib therapy will be studied in future randomized trials with imatinib as a neoadjuvant or adjuvant treatment for GISTs.

Acknowledgement

This study was performed under the auspices of National Natural Science Foundation of China (no. 30300152) and Shanghai Science Technique Planning Foundation (no. 064119622).

Disclosure/conflict of interest

All authors read and approved the final paper. The opinions contained herein are the expressed views of the authors. We declare that we have no conflict of interest.

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