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Original contribution

# Prognostic and predictive values of the KIT11-mutated grading system in patients with gastrointestinal stromal tumors: a retrospective study☆,☆☆

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**Summary** The KIT11 mutation is the most frequent mutation pattern in gastrointestinal stromal tumors (GISTs). However, few studies have investigated the correlation between the KIT11-mutated grading system and imatinib mesylate (IM) sensitivity (the first choice for adjuvant treatment of GISTs). Here, we elucidated the clinical value of the KIT11-mutated grading system for prognostic prediction in patients with GISTs treated with IM. A total of 106 patients with GIST were treated with IM (8: intermediate-risk, 98: high-risk; 10: KIT9-mutated, 86: KIT11-mutated, 5: wild-type, and 5: other mutations). KIT11-mutated patients were divided into 3 grades based on the KIT11-mutated site and type. Clinical backgrounds and prognostic outcomes were retrospectively compared between the 3 groups. Of 86 KIT11-mutated patients treated with IM, 32 (37.21%) had grade 1 tumors, 37 (43.02%) had grade 2 tumors, and 17 (19.77%) had grade 3 tumors. The 5-year disease-free survival (DFS) was

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significantly worse in patients with grade 3 KIT11-mutated GISTs (41.96%, $p = 0.001$) than in those with grade 1 (93%) and grade 2 (70.64%) cases. The multivariable analysis suggested that the KIT11-mutated grading system was an independent risk factor for DFS in patients treated with IM (hazard risk, 2.512; 95% confidence interval, 1.370−4.607; p = 0.003). In conclusion, the KIT11-mutated grading system provides good prognostic stratification for DFS in patients treated with IM. Grade 1 tumors predict a favorable response to IM.
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## 1. Introduction

Gastrointestinal stromal tumors (GISTs) are rare mesenchymal neoplasms, most often occurring in the stomach and small intestine, and the incidence of GISTs is 12−14 per million [1]. GISTs are diagnosed by morphology and immunohistochemical staining for CD117 (KIT receptor) and DOG1 (discovered on GIST 1). GISTs arise from the interstitial cells of Cajal, frequently characterized by an oncogenic mutation in the KIT or platelet-derived growth factor receptor alpha (PDGFRA) genes [2−5]. It was reported that mutations most frequently occurred in KIT (75%) in patients with GISTs, followed by wild-type mutations (15%) and PDGFRA mutations (10%). Among those with KIT gene mutations, 87% occurred in exon 11 (KIT11), 11% occurred in exon 9 (KIT9), 1% occurred in exon 13 (KIT13), and 1% occurred in exon 17 (KIT17) [6]. There was a higher risk of progression in KIT9-mutated patients than in KIT11-mutated patients [7].

Imatinib mesylate (IM) is the first choice for adjuvant treatment of patients with GIST and high recurrence risk [8, 9], including intermediate and high-risk GISTs [10,11]. It was reported that patients with GIST with wild-type, KIT9, and PDGFRA18 mutations showed more significant IM resistance than those with KIT11 mutations [12−14]. Further analysis is needed of the factors related to prognosis and IM efficacy in patients with GIST and KIT11 mutations, which are the most common mutations in GISTs.

The aim of this study was to estimate the contribution of different types and sites of KIT11 mutations in the prognostic parameters and clinicopathological significance of GISTs with high recurrence risk.

## 2. Materials and methods

### 2.1. Subjects

A total of 490 cases of GISTs were collected from Shanghai General Hospital/Faculty of Basic Medicine, Shanghai Jiao Tong University School of Medicine, and Fudan University Shanghai Cancer Center from March 2008 to December 2016. Ethical, legal, and social implications were approved by the Shanghai Jiao Tong University Ethics Committee. Cases without prognosis data (n = 72), cases of recurrence (n = 13), and those with other cancers (n = 12) were excluded. There were 393 patients in the cohort study, consisting of 108 patients with very low-risk, 48 patients with low-risk, 42 patients with intermediate-risk, and 195 patients with high-risk GISTs as per the 2008 modified National Institutes of Health (NIH) classification system. Among the intermediate-risk patients, 16 underwent gene mutation detection. Of these 16 patients, 8 were treated with IM. Among the high-risk patients, 123 underwent gene mutation detection. Of these 123 patients, 98 patients were treated with IM (Fig. 1).

### 2.2. DNA extraction and mutation analysis

DNA was extracted from representative blocks of formalin-fixed/paraffin-embedded tissues with tumor cellularity >80%. Sections of 10-μm thickness were deparaffinized by serial xylene/ethanol washings. DNA was extracted using the EZ1 Biorobot (Qiagen GmbH, Hilden, Germany). KIT exons 9, 11, 13, and 17 and PDGFRA exons 12 and 18 were sequenced centrally during the study by Sanger sequencing using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California). The primers and mutational analysis are shown in Supplementary Fig. 1.

### 2.3. Construction of the KIT11-mutated grading system

Patients with KIT11 mutations who were in the NIH high-risk category were divided into six groups based on mutation sites, including single-site mutations, mutations in codon 550−555, mutations in codon 555−561, mutations in codon 561−579, mutations in codon 579−587, and others (across different mutation site groups, eg, codon 550−558). We divided the patients with KIT11 mutations who were in the NIH high-risk category into five groups based on the mutation types (insertion, deletion, duplication, point mutation, and mixed mutation). Mutation sites in KIT11-mutated patients were significantly associated with

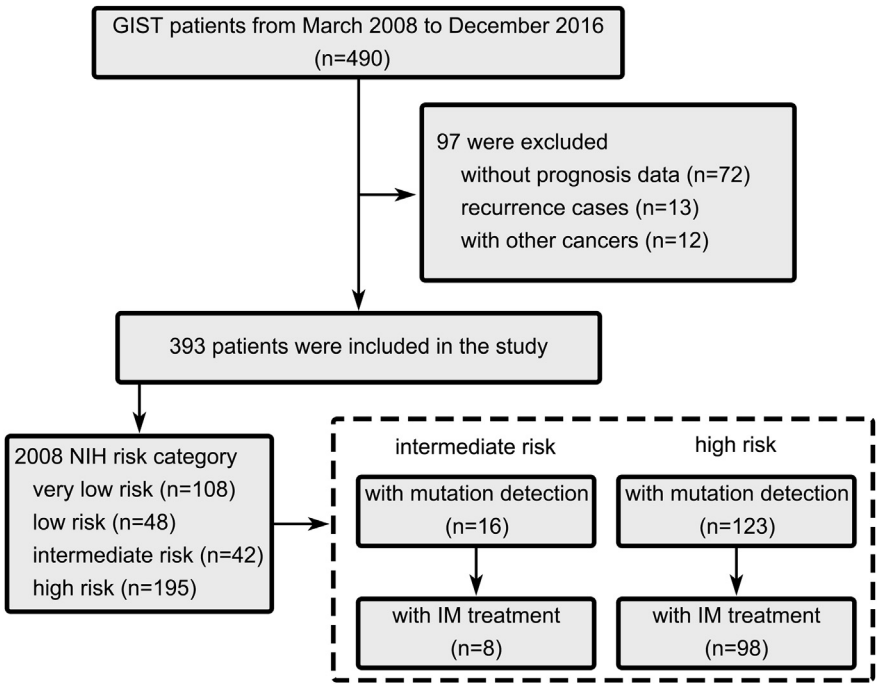


**Fig. 1** Flow chart of the analyzed patients. GIST, gastrointestinal stromal tumor; NIH, National Institutes of Health.

disease-free survival (DFS; $p = 0.020$, Supplementary Fig. 2A) but not with overall survival (OS; $p = 0.956$, Supplementary Fig. 2B). The DFS for single-site mutations, mutations in codon 550−555, mutations in codon 555−561, mutations in codon 561−579, mutations in codon 579−587, and others was 94.12% (32/34), 50% (2/4), 73.08% (19/26), 58.33% (7/12), 100% (3/3), and 75% (12/16), respectively. The results indicated that the DFS for insertion, deletion, duplication, point mutation, and mixed mutation was 100% (4/4), 71.70% (38/53), 100% (1/1), 95.83% (23/24), and 69.23% (9/13), respectively ($p = 0.059$, Supplementary Fig. 2C). However, there was no difference in OS between the mutation type groups ($p$ was not comparable, Supplementary Fig. 2D). Mutation sites and types of KIT11 mutations were chosen as the criteria for the grading system. The scoring scheme is summarized in Table 1. Each tumor was assigned as grade 1 (score = 0−1), grade 2 (score = 2), or grade 3 (score = 3).

## 2.4. Statistical analysis

Statistical analysis was performed using SPSS version 23.0 software (IBM Corporation, Armonk, NY, USA). Categorical variables were compared using the exact chi-square test, and continuous variables were compared using the independent samples $t$-test. The factors related to prognosis in GISTs were analyzed using univariate and multivariate analyses. All variables that were statistically significant on univariate analysis ($p < 0.05$) were included in a multivariable Cox proportional hazards regression model (Cox regression, Parameter, Forward: LR). DFS and OS were analyzed using standard Kaplan-Meier analysis with a log-rank test. A two-tailed $p$ value $<0.05$ was considered statistically significant.

# 3. Results

## 3.1. Clinicopathological data

A total of 393 patients with primary GIST who underwent surgical resection from 2008 to 2016 were investigated. A female predominance was observed (male/female = 160:233). The age of the patients ranged from 14 to 91 years (median age, 60 years). The most common primary tumor location was the stomach (59.29%, n = 233), followed by the small intestine (28.24%, n = 111), large intestine (4.07%, n = 16), and other locations (8.40%, n = 33). DFS and OS analyses were performed, and significant differences were observed in tumor location between the stomach and other sites (including the small intestine, large intestine, and other locations), but there were no differences between the small intestine, large intestine, and other locations ($p > 0.05$, Supplementary Fig. 3A and B). In this study, tumors were assigned as either stomach (n = 233) or others (including the small intestine, large intestine, and other locations; n = 160). Patients were divided into very low-risk (n = 108), low-risk (n = 48), intermediate-risk (n = 42), and high-risk (n = 195) groups as per the NIH risk category. Gene mutation detection was performed for 139 patients (intermediate-risk, n = 16; high-risk, n = 123; male/

female = 65:74; median age, 58 years, range from 29 to 87 years). Among the 16 intermediate-risk patients who underwent mutation detection, 8 patients received IM therapy after surgical resection. Among 123 high-risk patients who underwent mutation detection, 98 patients were treated with IM after surgical resection.

### 3.2. Survival analyses between the different NIH risk categories

The median follow-up time for very low-risk, low-risk, intermediate-risk, and high-risk cases was 26.5, 40, 39.5, and 51 months, respectively. The 5-year DFS for very low-risk, low-risk, intermediate-risk, and high-risk patients was 100%, 100%, 100%, and 79.04%, respectively (Fig. 2A), while the 5-year OS was 100%, 100%, 100%, and 96.39%, respectively (Fig. 2B). The NIH risk category was significantly associated with DFS ($p = 0.000$) but not OS ($p = 0.310$).

### 3.3. Association between gene mutation patterns with clinicopathological features

Among 139 patients who underwent mutation detection, 9.35% (n = 13) occurred in KIT9, 79.14% (n = 110) occurred in KIT11, 3.60% (n = 5) occurred in PDGFRA18, 5.76% (n = 8) were wild-type mutations, 0.72% (n = 1) occurred in KIT13, and 1.44% (n = 2) occurred in KIT11 and KIT17 (Fig. 2C). The association between clinicopathological features and gene mutation patterns is summarized in Supplementary Table 1. Tumors with PDGFRA18 mutations were more likely to show a low mitotic count (KIT9 versus PDGFRA18, $p = 0.029$; KIT11 versus PDGFRA18, $p = 0.002$). Tumors with KIT9 mutations were more likely to be located in the other sites (not stomach, KIT9 versus KIT11, $p = 0.001$). The results showed significant differences in mutation types between the groups (KIT9 versus KIT11, $p = 0.000$; KIT9 versus PDGFRA18, $p = 0.000$; KIT11 versus PDGFRA18, $p = 0.022$). Of the 13 tumors with KIT9 mutations, insertion (53.85%, n = 7) and duplication (46.15%, n = 6) were identified. Tumors with KIT11 mutations included insertion (4.55%, n = 5), deletion (53.64%, n = 59), duplication (0.91%, n = 1), point mutation (28.18%, n = 31), and mixed mutation (12.73%, n = 14). The point mutation of D842V (100%, n = 5) was identified in patients with PDGFRA18 mutations.

### 3.4. Survival analyses between different gene mutation patterns of GISTs in high-risk patients

The results showed that DFS of high-risk patients with GIST and KIT9, KIT11, PDGFRA18, wild-type, KIT13, and multiple mutations (KIT11 and KIT17) was 61.54% (8/13), 78.95% (75/95), 100% (4/4), 87.5% (7/8), 100% (1/1), and 0% (0/2), respectively. The OS of high-risk patients with GIST and KIT9, KIT11, PDGFRA18, wild-type, KIT13, and multiple mutations (KIT11 and KIT17) was 76.92% (10/13), 97.90% (93/95), 100% (4/4), 87.5% (7/8), 100% (1/1), and 50% (1/2), respectively. The mutation pattern was significantly associated with DFS (pooled over strata, $p = 0.022$, Fig. 2D) but not with OS (pooled over strata, $p = 0.053$, Fig. 2E). But through pairwise over strata analysis, KIT9-mutated patients had poorer OS than KIT11-mutated patients (pairwise over strata, $p = 0.003$). However, no significant difference was observed in OS between the other groups (PDGFRA18-mutated, wild-type, KIT11-mutated, and multiple mutations) as per pairwise over strata analysis ($p > 0.05$). Patients with multiple mutations had poorer DFS than KIT11-mutated patients ($p = 0.001$) and PDGFRA18-mutated patients ($p = 0.046$). However, no significant difference was observed in DFS between KIT9-mutated patients, PDGFRA18-mutated patients, patients with wild-type mutations, and KIT11-mutated patients as per pairwise over strata analysis (KIT11 versus KIT9, $p = 0.096$; KIT11 versus PDGFRA18, $p = 0.371$; KIT11 versus wild-type, $p = 0.902$).

### 3.5. Association between KIT11-mutated grade and clinicopathological features

Using the KIT11-mutated grading system described in Table 1, 95 KIT11-mutated patients in the NIH high-risk

**Table 1** Criteria of the KIT11-mutated grading system.

| | Score | | |
|---|---|---|---|
| | 0 | 1 | 2 |
| Mutation site | Single-site mutation and codon 579–587 | Codon 555–561 and others[a] | Codon 561–579 and codon 550–555 |
| Mutation type | Insertion, duplication, and point mutation | Deletion and mixed mutation | |
| Grade | 1 | 2 | 3 |
| Total score | 0–1 | 2 | 3 |

[a] Across different mutation site groups, eg, codon 550–558.

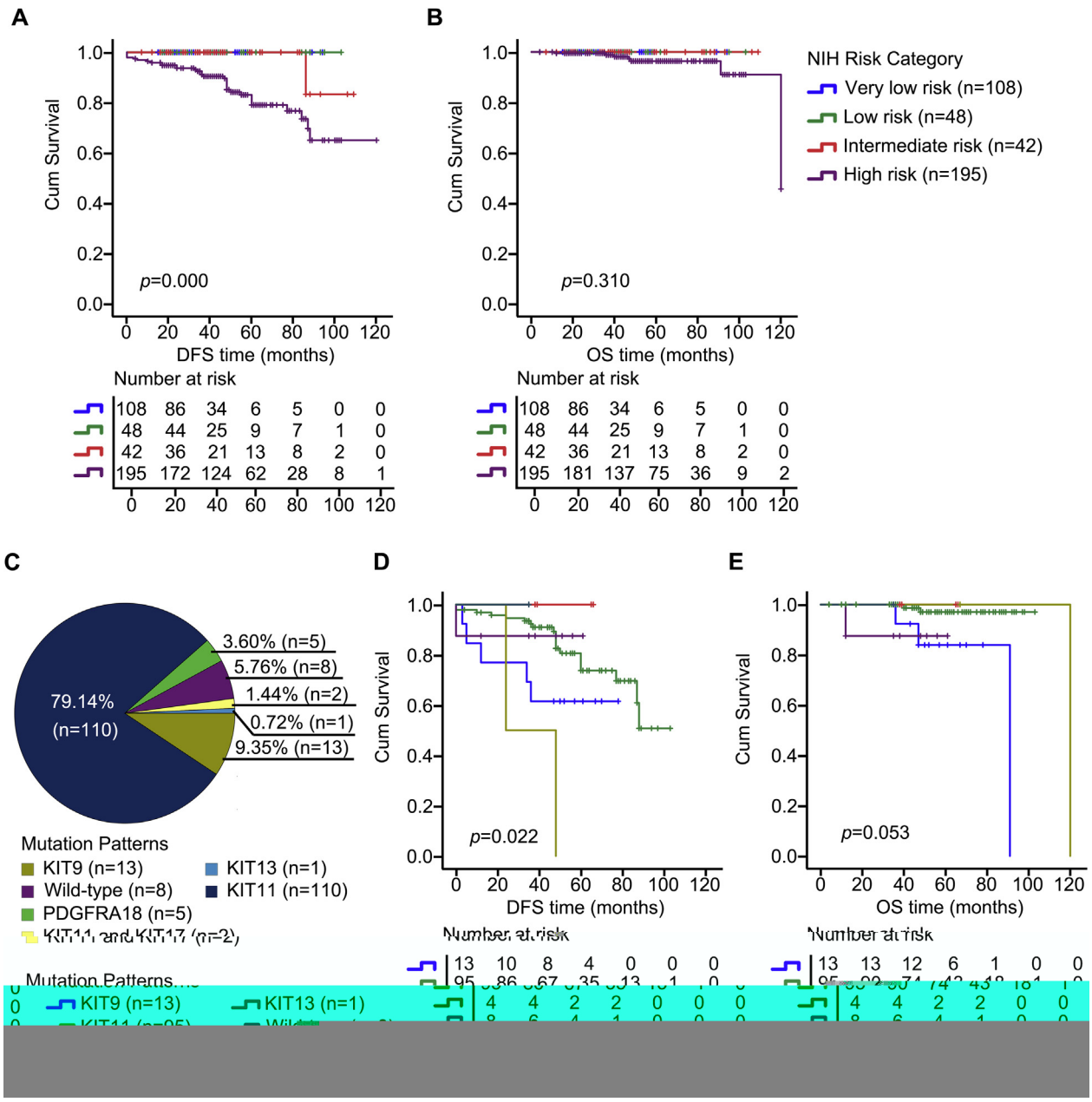


**Fig. 2** Kaplan-Meier curves for DFS (A) and OS (B): patients in the NIH very low- (n = 108), low- (n = 48), intermediate- (n = 42), and high-risk (n = 195; panel A, $p = 0.000$; panel B, $p = 0.310$) category. The pie graph depicts that the highest percentage is KIT11-mutated mutations (79.14%, n = 110), followed by KIT9-mutated (9.35%, n = 13), wild-type (5.76%, n = 8), PDGFRA18-mutated (3.60%, n = 5), multiple (KIT11-and KIT17-mutated, 1.44%, n = 2), and KIT13-mutated (0.72%, n = 1) mutations (C). Kaplan-Meier curves for DFS (D) and OS (E): patients with KIT9-, KIT11-, PDGFRA18-, KIT13-mutated, multiple, and wild-type mutations (panel D, $p = 0.022$; panel E, $p = 0.053$). DFS, disease-free survival; OS, overall survival; NIH, National Institutes of Health.

**Table 2** Association between KIT11-mutated grade and clinicopathological features[a] in NIH high-risk GISTs.

| Risk category | Grade 1 (n = 40) | Grade 2 (n = 39) | Grade 3 (n = 16) | *P* value[b] | *P* value[b] | | |
|---|---|---|---|---|---|---|---|
| | | | | | Grade 1 vs 2 | Grade 1 vs 3 | Grade 2 vs 3 |
| Sex | | | | 0.411 | 0.216 | 0.351 | 0.991 |
| Male | 17 (42.5) | 22 (56.41) | 9 (56.25) | | | | |
| Female | 23 (57.5) | 17 (43.59) | 7 (43.75) | | | | |
| Age (years), mean (SD) | 60.03 (10.04) | 57.56 (11.77) | 52.81 (10.53) | 0.085 | 0.320 | **0.020** | 0.167 |
| Tumor size, cm | | | | 0.829 | 0.525 | 0.710 | 0.901 |
| ≤2.0 | 0 (0) | 1 (2.56) | 0 (0) | | | | |
| 2.1−5.0 | 1 (2.5) | 3 (7.69) | 1 (6.25) | | | | |
| 5.1−10.0 | 26 (65) | 24 (61.54) | 11 (68.75) | | | | |
| >10.0 | 13 (32.5) | 11 (28.21) | 4 (25) | | | | |
| Mitotic count/50 HPF | | | | 0.274 | 0.151 | 0.860 | 0.189 |
| ≤5 | 14 (35) | 8 (20.51) | 6 (37.5) | | | | |
| >5 | 26 (65) | 31 (79.49) | 10 (62.5) | | | | |
| Location | | | | **0.010** | 0.741 | **0.014** | **0.008** |
| Stomach | 18 (45) | 19 (48.72) | 1 (6.25) | | | | |
| Other sites | 22 (55) | 20 (51.28) | 15 (93.75) | | | | |
| Mutation sites of KIT11 | | | | **0.000** | **0.000** | **0.000** | **0.000** |
| Single-site | 34 (85) | 0 (0) | 0 (0) | | | | |
| Codon 550−555 | 0 (0) | 0 (0) | 4 (25) | | | | |
| Codon 555−561 | 2 (5) | 24 (61.54) | 0 (0) | | | | |
| Codon 561−579 | 0 (0) | 0 (0) | 12 (75) | | | | |
| Codon 579−587 | 3 (7.5) | 0 (0) | 0 (0) | | | | |
| Others[c] | 1 (2.5) | 15 (38.46) | 0 (0) | | | | |
| Mutation type | | | | **0.000** | **0.000** | **0.000** | 0.879 |
| Insertion | 4 (10) | 0 (0) | 0 (0) | | | | |
| Deletion | 11 (27.5) | 30 (76.92) | 12 (75) | | | | |
| Duplication | 1 (2.5) | 0 (0) | 0 (0) | | | | |
| Point mutation | 24 (60) | 0 (0) | 0 (0) | | | | |
| Mixed mutation | 0 (0) | 9 (23.08) | 4 (25) | | | | |

Abbreviations: GIST, gastrointestinal stromal tumor; SD, standard deviation; HPF, High Power Field.

[a] Values are presented as number (%), unless otherwise indicated.

[b] Bold values indicate significance, $p < 0.05$.

[c] Across different mutation site groups, eg, codon 550−558.

category were subdivided into grade 1 (n = 40), grade 2 (n = 39), and grade 3 (n = 16). The association between grading and clinicopathological features is summarized in Table 2. Grade 3 tumors were significantly associated with young age (grade 1 versus 3, $p = 0.020$) and were more likely to be located in the other sites (not stomach, grade 1 versus 3, $p = 0.014$; grade 2 versus 3, $p = 0.008$). The results showed significant differences in mutation sites ($p = 0.000$) and types ($p = 0.000$) of KIT11 mutations between the groups. Of 40 grade 1 tumors, single-site mutations (85%, n = 34), mutations in codon 555−561 (5%, n = 2), mutations in codon 579−587 (7.5%, n = 3), and other mutations (2.5%, n = 1) were identified. Grade 2 tumors included mutations in codon 555−561 (61.54%, n = 24) and others (38.46%, n = 15), whereas grade 3 tumors included mutations in codons 550−555 (25%, n = 4) and 561−579 (75%, n = 12). Grade 1 tumors with KIT11 mutations included insertion (10%, n = 4), deletion (27.5%, n = 11), duplication (2.5%, n = 1), and point mutation (60%, n = 24). Grade 2 KIT11-mutated tumors included deletions (76.92%, n = 30) and mixed mutations (23.08%, n = 9; grade 1 versus 2, $p = 0.000$). Grade 3 KIT11-mutated tumors included deletions (75%, n = 12) and mixed mutations (25%, n = 4; grade 1 versus 3, $p = 0.000$).

### 3.6. Survival analyses between the different KIT11-mutated grade groups

The 5-year DFS for KIT11-mutated patients with grade 1, 2, and 3 tumors who were in the NIH high-risk category was 93%, 70.64%, and 41.96%, respectively. KIT11-mutated grade was significantly associated with DFS ($p = 0.001$, Fig. 3A) but not with OS ($p = 0.780$, Fig. 3B).

### 3.7. Association between IM sensitivity and clinicopathological features

In this study, 106 patients were treated with IM, including 8 cases in the NIH intermediate-risk category and 98 cases in the high-risk category. To investigate the association between IM sensitivity and clinicopathological

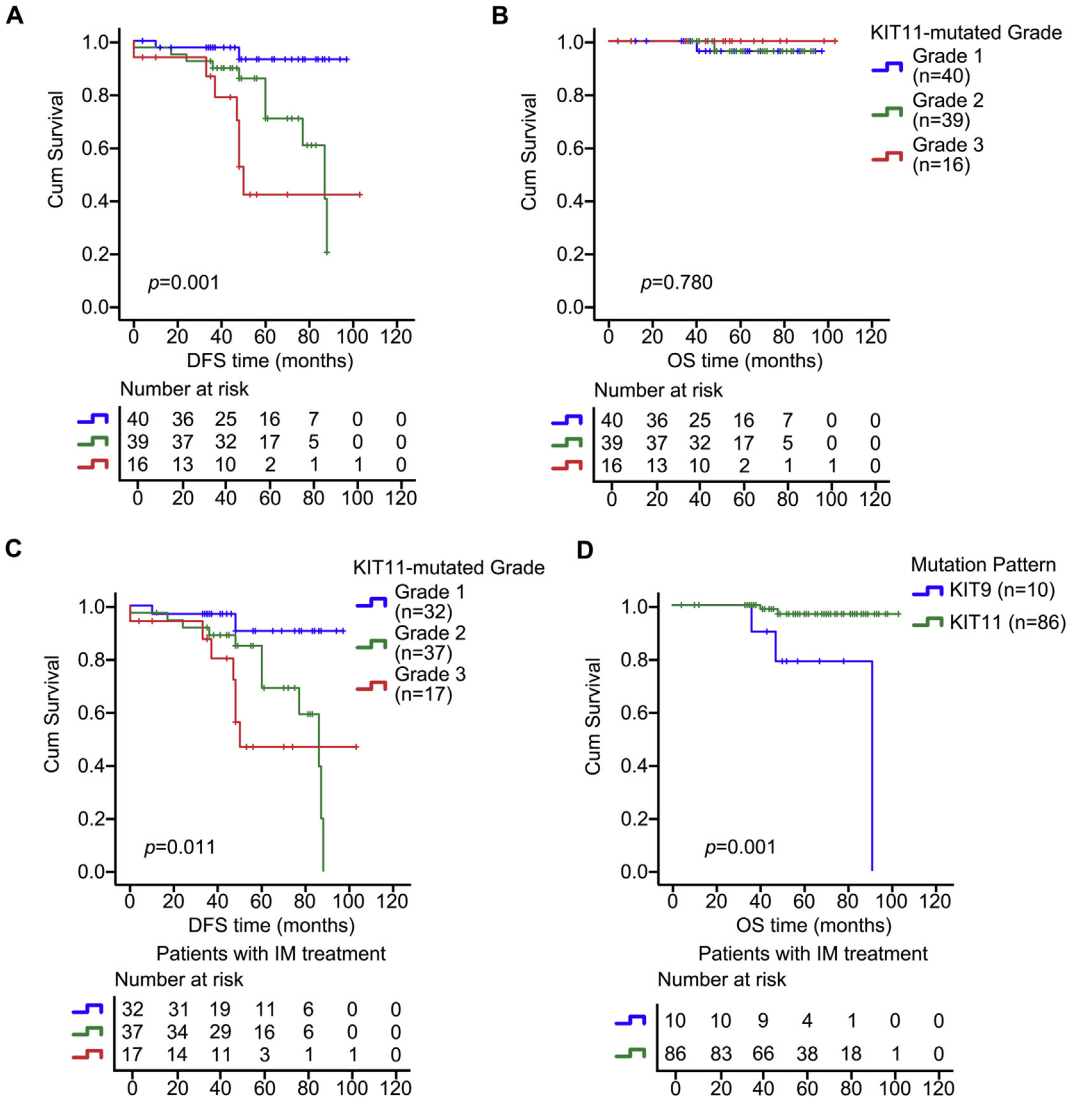


**Fig. 3** Kaplan-Meier curves of DFS (A) and OS (B) in patients in the NIH high-risk category in accordance with the KIT11-mutated grading system (grade 1, n = 40; grade 2, n = 39; grade 3, n = 16; panel A, $p = 0.001$; panel B, $p = 0.780$). Kaplan-Meier curves for DFS in patients treated with IM: KIT11-mutated with grade 1 (n = 32), 2 (n = 37), and 3 (n = 17; $p = 0.011$) (C). Kaplan-Meier curves for OS in patients treated with IM: KIT9-mutated (n = 10), KIT11-mutated (n = 86; $p = 0.001$) (D). DFS, disease-free survival; OS, overall survival; IM, imatinib mesylate; NIH, National Institutes of Health.

features, we analyzed data through univariate and multivariate analyses (Cox regression, Parameter, Forward: LR). Univariate analysis results showed that tumor size ($p = 0.020$), mutation pattern ($p = 0.010$), and KIT11-mutated grade ($p = 0.011$) significantly influenced DFS (Table 3). Multivariate analysis revealed that KIT11-mutated grade (hazard risk, 2.512; 95% confidence interval, 1.370−4.607; $p = 0.003$) remained significantly associated with DFS in the forward stepwise (likelihood ratio) multivariate analysis model. KIT11-mutated grade was significantly associated with DFS in patients undergoing IM treatment ($p = 0.011$, Fig. 3C). The 5-year DFS for patients with grade 1 (n = 32), grade 2 (n = 37), or grade 3 (n = 17) tumors undergoing IM treatment was 90.42%, 68.93%, and 46.73%, respectively. KIT9 mutations (5-year OS with IM, 78.75%) were significantly associated with poor OS, compared with KIT11 mutations (5-year OS with IM, 96.63%, $p = 0.001$, Fig. 3D).

## 4. Discussion

GISTs are often located in the gastrointestinal tract, and mutations in KIT or PDGFRA were found in approximately 80−85% of cases [15−17]. The recurrence risk assessment system for the primary GIST includes the American Joint Committee on Cancer staging system (8th edition), American Forces Institute of Pathology criteria, National Comprehensive Cancer Network Guideline biological

**Table 3** Association between IM sensitivity and clinicopathological features.

| Characteristic | No. (%) | DFS (univariate) | | DFS (multivariate) | | OS (univariate) | |
|---|---|---|---|---|---|---|---|
| | | 5-year DFS | *P* value[b] | HR (95% CI) | *P* value[b] | 5-year OS | *P* value[b] |
| Sex | | | 0.451 | | | | 0.208 |
| Male | 49 (46.23) | 70.57% | | | | 96.97% | |
| Female | 57 (53.77) | 67.67% | | | | 92.91% | |
| Age (years) | | | 0.934 | | | | 0.650 |
| ≤57 | 53 (50) | 70.33% | | | | 97.56% | |
| >57 | 53 (50) | 67.27% | | | | 92.03% | |
| Tumor size (cm) | | | **0.020** | NA | 0.060 | | 0.817 |
| ≤2.0 | 2 (1.89) | 50% | | | | 100% | |
| 2.1−5.0 | 10 (9.43) | 100% | | | | 100% | |
| 5.1−10.0 | 66 (62.26) | 71.76% | | | | 95.45% | |
| >10.0 | 28 (26.42) | 53.48% | | | | 91.48% | |
| Mitotic count (/50 HPF) | | | 0.434 | | | | 0.802 |
| ≤5 | 30 (28.57) | 80.17% | | | | 96.30% | |
| >5 | 75 (71.43) | 64.63% | | | | 93.97% | |
| Location | | | 0.173 | | | | 0.115 |
| Stomach | 38 (35.85) | 78.02% | | | | 100% | |
| Other sites | 68 (64.15) | 64.80% | | | | 92.44% | |
| NIH risk category | | | 0.400 | | | | 0.609 |
| Intermediate risk | 8 (7.55) | 100% | | | | 100% | |
| High risk | 98 (92.45) | 66.30% | | | | 94.48% | |
| Mutation pattern | | | **0.010** | NA | 0.653 | | 0.067 |
| KIT9 | 10 (9.43) | 50% | **0.025** (KIT9 vs KIT11) | | | 78.75% | **0.001** (KIT9 vs KIT11) |
| KIT11 | 86 (81.13) | 70.49% | | | | 96.63% | |
| PDGFRA | 2 (1.89) | 100% | | | | 100% | |
| Wild-type | 5 (4.72) | 100% | | | | 100% | |
| KIT11 and KIT17 | 2 (1.89) | 0% | | | | 100% | |
| KIT13 | 1 (0.94) | 100% | | | | 100% | |
| Mutation sites of KIT11 | | | 0.103 | | | | NA |
| Single-site | 29 (33.72) | 89.66% | | | | 94.74% | |
| Codon 550−555 | 4 (4.65) | 37.50% | | | | 100% | |
| Codon 555−561 | 23 (26.74) | 79.71% | | | | 94.44% | |
| Codon 561−579 | 13 (15.12) | 49.85% | | | | 100% | |
| Codon 579−587 | 2 (2.33) | 100% | | | | 100% | |
| Others[a] | 15 (17.44) | 48.75% | | | | 100% | |
| Mutation type | | | 0.062 | | | | NA |
| Insertion | 2 (2.33) | 100% | | | | 100% | |
| Deletion | 50 (58.14) | 67.40% | | | | 97.06% | |

**Table 3** (*continued*)

| Characteristic | No. (%) | DFS (univariate) | | DFS (multivariate) | | OS (univariate) | |
|---|---|---|---|---|---|---|---|
| | | 5-year DFS | *P* value[b] | HR (95% CI) | *P* value[b] | 5-year OS | *P* value[b] |
| Duplication | 0 (0) | NA | | | | NA | |
| Point mutation | 21 (24.42) | 95.24% | | | | 93.33% | |
| Mixed mutation | 13 (15.12) | 26.25% | | | | 100% | |
| KIT11-mutated grade | | | **0.011** | 2.512 (1.370−4.607) | **0.003** | | 0.686 |
| Grade 1 | 32 (37.21) | 90.42% | | 1 (reference) | NA | 95.00% | |
| Grade 2 | 37 (43.02) | 68.93% | | 4.683 (1.006−21.807) | **0.049** | 96.00% | |
| Grade 3 | 17 (19.77) | 46.73% | | 13.211 (2.556 −68.293) | **0.002** | 100.00% | |

Abbreviations: IM, imatinib mesylate; DFS, disease-free survival; OS, overall survival; NIH, National Institutes of Health; HR, hazard ratio; 95% CI, 95% confidence interval; NA, not applicable; HPF, High Power Field.

[a] Across different mutation site groups, eg, codon 550−558.

[b] Bold values indicate significance, $P < 0.05$.

behavior predictor system (2018), and the 2008 NIH classification system. The Chinese Society of Clinical Oncology Expert Committee recommends the modified NIH classification (2017-revised edition), which may be more suitable for Asian populations [18]. The NIH risk category was assigned based on tumor location, tumor size, mitotic count, and tumor rupture. In our study, the 5-year DFS for patients in the NIH very low-, low-, intermediate-, or high-risk category was 100%, 100%, 100%, and 79.05% ($p = 0.000$), respectively, and the 5-year OS was 100%, 100%, 100%, and 96.39% ($p = 0.310$, Fig. 2A and B), respectively. It is noteworthy that our cohort had a much higher DFS and OS in the high-risk group (79.05%; 96.39%) than in previous studies (45−47%; 38−83%) [19,20]. One possible explanation is that 59.29% of our cohort had tumors located in the stomach and showed a better DFS and OS than those with tumors in other locations (including the small intestine, large intestine, and other locations). Colombo et al. [19] only analyzed the clinical, pathological, and surgical characteristics of GISTs located in the duodenum (5-year DFS, 47%; 5-year OS, 83%). Another possibility is the usage of IM. It was reported that IM resulted in prolonged 5-year OS in patients with advanced GISTs from 16 to 19 months [21−23] to 5 years [24−29]. In a study by Hassan et al. [20], the most frequent primary site of the tumors was the stomach (54%), which was consistent with our data (stomach, 59.29%), but none of the patients in this cohort received IM (5-year DFS, 45%; 5-year OS, 38%). In our cohort, 98 patients (50.26%, 98/195) were treated with IM.

IM is the first-line therapy for advanced GISTs, including intermediate- and high-risk GISTs [10, 11]. In our cohort, there was no significant difference between NIH intermediate-risk and high-risk in DFS ($p = 0.400$) and OS ($p = 0.609$) in patients undergoing IM treatment (intermediate-risk, n = 8; high-risk, n = 98). It was previously reported that a high Ki67 index was related to a worse prognosis in GISTs [1, 30, 31], and Ki67 index >8% may act as an unfavorable factor for IM treatment [32]. Besides, there was much research on the guiding significance of molecules in IM therapy. Patients with KIT9 mutations were resistant to IM, compared with those with KIT11 mutations (OS, $p = 0.001$; DFS, $p = 0.025$, Table 3), which was in concordance with previous studies [33−35]. It was reported that patients with GIST and PDGFRA18-mutated and wild-type mutations showed more significant IM resistance than those with KIT11-mutated [24, 36]. However, in our data, no significant difference was observed in DFS or OS between PDGFRA18, wild-type, and KIT11 mutations, which may be related to insufficient data of PDGFRA18 and wild-type mutations. Of 106 IM-treated patients, KIT9-mutated (9.43%, n = 10), KIT11-mutated (81.13%, n = 86), PDGFRA18-mutated (1.89%, n = 2), wild-type (4.72%, n = 5), and other (KIT13, 0.94%, n = 1; KIT11 and KIT17, 1.89%, n = 2) tumors were identified. Further analysis is needed of the factors related to prognosis in patients with GIST and KIT11 mutations because this was the most frequent mutation pattern of GISTs. However, to our knowledge, there are currently few studies with regard to the KIT11-mutated grading system. Several studies have focused on KIT11 mutations at codon 557−558. It was reported that KIT exon 11 mutations at codon 557−558 showed a favorable response to IM [37] but poor progression-free survival [38,39]. Ramaswamy et al. [40] divided patients into mutations upstream to 557, mutations involving codon 557−558, and mutations downstream to codon 558. This study showed that there was a trend toward an inferior

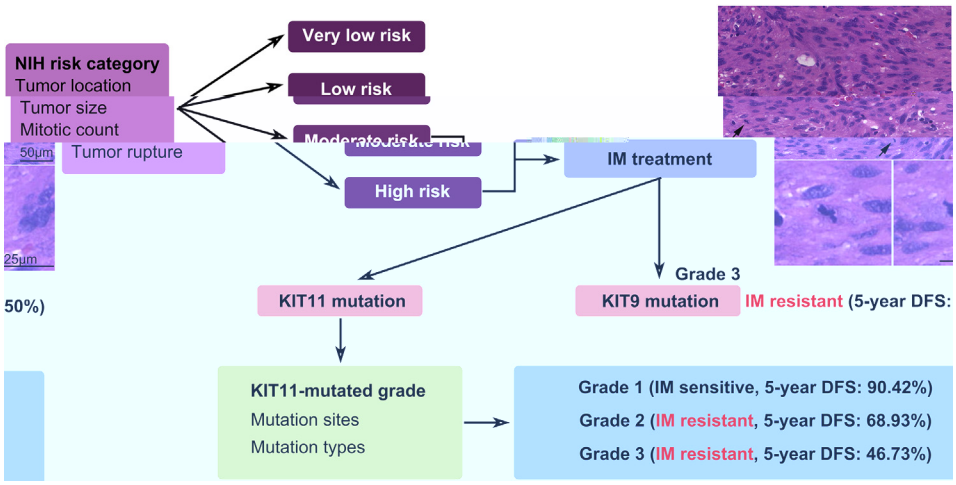


**Fig. 4** Prognostic stratification for DFS in patients with GISTs treated with IM. The image shows the H&E staining section of GIST. Most GISTs show spindle cell morphology, and the mitotic count is one of the main prognostic indicators of the NIH risk category. (black arrow, mitosis; enlarged below). NIH, National Institutes of Health; H&E, hematoxylin and eosin; GIST, gastrointestinal stromal tumor; DFS, disease-free survival; IM, imatinib mesylate.

survival in patients with the codon 557−558 mutation subset even when treated with IM, but this was not statistically significant.

In our study, we have shown that a three-tier KIT11-mutated grading system, using the mutation site and type of KIT11 mutations, stratifies KIT11-mutated GISTs into three prognostic groups. In IM-treated patients, we found that although no significant difference was observed in OS with regard to the KIT11-mutated grading system, the KIT11-mutated grading system was an independent prognostic factor in DFS in multivariate analysis ($p = 0.003$). The 5-year DFS rates for patients with grade 1 (n = 32), grade 2 (n = 37), or grade 3 (n = 17) GISTs undergoing IM treatment were 90.42%, 68.93%, and 46.73%, respectively ($p = 0.011$). Using this system, we observed younger patients with grade 3 tumors (mean age, 52.81 ± 10.53) compared with grade 1 tumors (mean age, 60.03 ± 10.04, $p = 0.020$). In comparison, the grade 1 and 2 KIT11-mutated GISTs in our study had a significantly higher rate of location in the stomach (grade 1, 45%; grade 2, 48.72%) than grade 3 tumors (6.25%, $p = 0.010$). A significantly higher rate of single-site mutation (85%) and point mutation (60%) was observed in grade 1 KIT11-mutated GISTs. The rate of mutation of codon 555−561 (61.54%) was higher in the grade 2 KIT11-mutated GISTs. In addition, the rate of mutation of codon 561−579 (75%) was higher in the grade 3 KIT11-mutated GISTs. Deletion mutation was higher in grade 2 (76.92%) and 3 (75%) than in grade 1 (27.5%) GISTs.

There are several limitations to our study that merit further discussion. In our study, the KIT11-mutated grading system was associated with DFS in patients undergoing IM treatment, but not with OS. And we did not have more details on dosage and duration of IM treatment. Furthermore, some of the subsets of mutation sites (codon 550−555, n = 4; codon 579−587, n = 3) and types (duplication, n = 1; insertion, n = 4) have low numbers. It is necessary to further amplify the cases of mutations of codon 550−555, codon 579−587, duplication, and insertion to study the response to IM treatment.

In summary, we proposed a KIT11-mutated grading system that was a good predictor of DFS in patients with high recurrence risk. Grade 3 tumors identified using this system showed more aggressive clinical behavior with poor DFS. In addition, grade 1 tumors predicted a favorable response to IM (Fig. 4).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2021.01.001.

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