Research paper

Immune-infiltration based signature as a novel prognostic biomarker in gastrointestinal stromal tumour

Zhe-Wei Wei\textsuperscript{a,1}, Jing Wu\textsuperscript{a,b,1}, Wei-Bin Huang\textsuperscript{a,1}, Jin Li\textsuperscript{a,b,1}, Xiao-Fang Lu\textsuperscript{c}, Yu-Jie Yuan\textsuperscript{a}, Wen-Jun Xiong\textsuperscript{d}, Xin-Hua Zhang\textsuperscript{a}, Wei Wang\textsuperscript{d}, Yu-Long He\textsuperscript{a,h,1}, Chang-Hua Zhang\textsuperscript{b,1}

\textsuperscript{a}Department of Gastrointestinal Surgery, The First Affiliated Hospital of Sun Yat-sen University, 58 Zhongshan 2nd Road, Guangzhou, Guangdong 510080, China
\textsuperscript{b}Center of Digestive Diseases, The Seventh Affiliated Hospital of Sun Yat-sen University, 628 Zhenyuan Road, Shenzhen, Guangdong 518000, China
\textsuperscript{c}Department of Pathology, The Seventh Affiliated Hospital of Sun Yat-sen University, 628 Zhenyuan Road, Shenzhen, Guangdong 518000, China
\textsuperscript{d}Department of Gastrointestinal Surgery, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, 111 Dade Road, Guangzhou, Guangdong 510120, China

\textbf{Article History:}
Received 17 February 2020
Revised 3 June 2020
Accepted 4 June 2020
Available online xxx

\textbf{ABSTRACT}

\textbf{Background:} Accumulating evidence indicates that tumour-infiltrating lymphocytes (TILs) are the primary determinant of survival outcomes in various tumours. Thus, we sought to investigate the TIL distribution and density in gastrointestinal stromal tumours (GISTs) and to develop an immune infiltration (II)-based signature to predict prognosis.

\textbf{Methods:} The expression of 8 immune features in the tumour centre (TC) and tumour margin (TM) and PD-L1 in 435 GIST patients was investigated by immunohistochemistry. Then, a 4-feature-based II-GIST signature integrating the CD3\textsuperscript{+} TC, CD3\textsuperscript{+} TM, CD8\textsuperscript{+} TM and CD45RO\textsuperscript{+} TM parameters was developed using a LASSO Cox regression model in the training cohort and was validated in two separate validation cohorts.

\textbf{Findings:} High CD3\textsuperscript{+} TC, CD3\textsuperscript{+} TM, CD8\textsuperscript{+} TC, CD8\textsuperscript{+} TM and CD45RO\textsuperscript{+} TM correlated with improved survival. Patients with high II-GIST scores have better RFS and OS outcomes than those with low II-GIST scores. Multivariable analyses demonstrated that the II-GIST signature is an independent prognostic factor. The receiver operating characteristic (ROC) curve demonstrated that the prognostic accuracy of the II-GIST signature is superior to that of the NIH risk criteria. Further analysis showed that moderate- and high-risk GIST patients with high II-GIST scores could gain survival benefits from adjuvant imatinib therapy.

\textbf{Interpretation:} The novel II-GIST signature accurately predicted the survival outcomes of GIST patients. In addition, the II-GIST signature was a useful predictor of survival benefit from imatinib therapy amongst moderate- and high-risk patients with GIST.

\textbf{Funding:} This project was supported by National Natural Science Foundation of China (81702325), Natural Science Foundation of Guangdong Province (2017A030310565), and 3&3 Project of the First Affiliated Hospital of Sun Yat-sen University.

© 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license. (http://creativecommons.org/licenses/by-nc-nd/4.0/)

1. Introduction

Gastrointestinal stromal tumours (GISTs), first proposed by Mazur in 1983, have often been diagnosed as smooth muscle tumours or schwannomas due to the incomplete understanding of their origin and differentiation [1, 2]. With the development of molecular diagnostic approaches, GISTs have become the most frequently diagnosed mesenchymal malignancy of the gastrointestinal (GI) tract [3]. The US National Institutes of Health (NIH) risk criteria, the most commonly used staging system for GISTs, provide useful but insufficient information to predict prognosis. Therefore, interest in identifying new parameters to improve the predictive accuracy of the current GIST staging system is increasing.

Emerging evidence suggests that immune systems interact with tumour cells and crucially influence multiple tumour processes, such as progression and metastasis [4]. Recent reports have demonstrated that infiltration of immune cells, especially lymphocytes, is critically important in tumours and is a primary determinant of survival outcomes in various types of cancers, including colon, ovarian, lung, gastric and breast cancers [5-8]. There has been considerable progress in
Evidence before this study

Mounting evidence demonstrated that immune systems interact with tumour cells and crucially influence multiple tumour processes. Tumour-infiltrating lymphocytes (TILs) have been demonstrated to reflect tumour inflammation and to correlate with prognostic outcomes in tumours. We searched PubMed for studies published in English reporting the prognostic effect of tumour-infiltrating lymphocytes in GISTs. The following terms were used as search terms: "gastrointestinal stromal tumors" or "GISTs", "tumour-infiltrating lymphocytes" or "lymphocytes". Twelve studies were identified. TILs were reported to be important in GISTs, but the prognostic role has not yet been clearly elucidated.

Added value of this study

The prognostic discrimination capability of the II-GIST signature, integrating the CD3+ TC, CD3+ TM, CD8+ TM and CD45RO+ TM, was significantly superior to that of the NIH risk criteria. Moderate- and high-risk patients with high II-GIST scores might obtain a greater survival benefit from postoperative imatinib therapy compared with those with low II-GIST scores.

Implications of all the available evidence

The current study indicated that the II-GIST signature based on immune infiltrations can effectively individualize prognostic predictions for GIST patients and serve as a useful predictor of the response to imatinib. Identifying and understanding additional immune abnormalities in GISTs may offer significant implications for future risk stratification and therapeutic opportunities.

The density and distribution of CD3+, CD8+, CD45RO+, Foxp3+ T cells and CD4+ TILs are diffusely infiltrated in GISTs [18]. However, the prognostic role of these TILs has not yet been clearly elucidated. Although seldom been reported in GIST, CD20 expressing on the surface of all B-cells, which are an important type of lymphocyte, has also been selected as a B-lymphocyte antigen. Hence, the current study sought to investigate the prognostic impact of CD3+, CD8+, CD45RO+, Foxp3+, CD4+, NKp46+ and CD20+ TILs in GISTs.

Considering the complexity of immune components, individual evaluation of each type of TILs might show only moderate predictive accuracy. Thus, an immune signature comprising multiple types of TILs might notably increase the precision of prognostic estimates. For instance, an immunoscore summarizing the distribution of CD3+ and CD8+TIL effectors in both the centre and invasive margin of the tumour has been demonstrated to be a reliable model for discriminating prognostic outcomes in colorectal cancer [7]. A recent study demonstrated that a 5-feature-based immunoscore signature was efficacious enough to estimate recurrence risk and predict chemosensitivity in gastric cancer [5]. Thus, we sought to investigate the distribution and density of TILs and to develop an immune infiltration (II)-based signature to predict prognosis and the efficacy of adjuvant imatinib therapy in patients with GIST.

2. Methods

2.1. Ethics statement

This study was approved by the Ethical Review Committee of the First Affiliated Hospital of Sun Yat-sen University and the Second Affiliated Hospital of Guangzhou University of Chinese Medicine. All patients in this study signed an informed consent form.

2.2. Patients and tissue samples

We retrospectively retrieved GIST patient information from the prospectively established database at the First Affiliated Hospital of Sun Yat-sen University and the Second Affiliated Hospital of Guangzhou University of Chinese Medicine. The inclusion criteria were as follows: (1) histologically proven GIST; (2) available archived samples with tumour components and margins; (3) lack of preoperative systemic treatment; (4) absence of synchronous or metachronous cancers; and (5) complete clinicopathological and follow-up information. Finally, 435 eligible formalin-fixed paraffin-embedded specimens were collected between 2005 and 2015 were used for the current study. We randomly assigned GIST patients from the First Affiliated Hospital of Sun Yat-sen University to the training (n = 130) and internal validation (n = 194) cohorts. Another 111 patients from the Second Affiliated Hospital of Guangzhou University of Chinese Medicine were enrolled into the external validation cohort. Moderate-risk and high-risk patients who received imatinib were previously analysed for mutations and were identified as having sensitive mutations according to the guidelines [19].

2.3. Assessment of TILs, PD-1 and PD-L1

IHC was performed as previously described [20]. Briefly, tissue sections (4–5 μm in thickness) were deparaffinized and rehydrated. Tissue slides were subjected to antigen retrieval by microwave heating in 10 mM citrate buffer (pH 6.0) and were quickly immersed in 3% H2O2 to quench endogenous peroxidase activity. Then, sections were incubated with primary antibodies against CD3 (Ventana, 790–4341; ready-to-use), CD8 (Dako, M7103; 1:100 dilution), CD45RO (Origene, TA807197; 1:100 dilution), CD4 (Abcam, ab213215; 1:300 dilution), Foxp3 (Abcam, ab214468; 1:500 dilution), CD20 (Abcam, ab27093; 1:200 dilution), programmed death-1 (PD-1) (Cell signaling Technology, 43,248; 1:50 dilution), and programmed death-ligand 1 (PD-L1) (Dako, M3653; 1:50 dilution) at 4°C overnights. The density and distribution of CD3+, CD8+, CD45RO+, Foxp3+, CD4+, NKp46+ and CD20+ TILs in the samples were assessed in two specific areas: the tumour centre (TC) and tumour margin (TM). The TM is defined as a region 800 μm wide centred on the border of the malignant cells with the fibrous trabeculae, and the TC is defined as the central tumour tissue surrounded by the TM (Fig. S1). Two
pathologists (Lu XF and Ding L.), who were blinded to the clinicopathological features, reviewed the stained slides to determine the TC and TM regions. Each pathologist independently selected 5 high-power fields (at 200 × magnification) in each region to assess the density of stained immune cells. ImageJ was used to calculate the absolute counts of TILs according to the operating parameters established by the two pathologists [21]. If scores were obviously different or if the inflation was heterogeneous, the two pathologists worked collaboratively to provide a result. If major discrepancies still existed, a third pathologist (Professor Xue L) assessed the samples and collaborated with the other two to provide a final result. The cases of major discrepancy between the two pathologists in review were 188 (2.4%).

Staining intensity of PD-L1 was graded as negative, weak, moderate, or strong. Samples with 10% of tumour cells exhibiting moderate to strong staining intensity for PD-L1 were deemed as positive.

2.4. Construction of the II-based signature

Considering the comparatively limited number of events in the training cohort relative to the number of variables and avoiding over-fitting due to the high dimensionality of the immune-infiltration-based signature, the least absolute shrinkage and selection operator (LASSO) was utilized to select significant features and obtain an optimal model. LASSO is a robust method that can be used with high-dimensional data for optimal selection of factors with strong diagnostic or prognostic value [23, 24]. LASSO shrinks the absolute size of coefficient estimates towards exactly zero depending on a penalized factor called λ, and thereby minimizes the prediction error. Briefly, as the value of λ increases, the coefficients of predictors in LASSO decrease (even to zero), which is pivotal for the establishment of a low-dimensional model with interpretability. In order to avoid over-fitting, 10-time cross-validation was used to determine the coefficients of LASSO modelling. Meanwhile, the mean-squared error should be as small as possible to improve the prediction accuracy. If λ with minimal partial likelihood deviance was used, 10 features would be chosen, which might lead to over-fitting. Hence, 1-SE criteria was used to select lambda (λ=0.0634) for further analysis. Here, a value λ=0.0634 with log(λ)=−2.759 was determined by 10-time cross-validation via 1-SE criteria in our study. Four parameters (CD3+ TC, CD3+ TM, CD8+ TM and CD45RO+ TM) were thereby minimizes the prediction error. Brieﬁly, 1-SE criteria was used to select lambda (λ=0.0634) with log(λ)=−2.759 was selected by 10-fold cross-validation via 1-SE criteria in our study. Four parameters (CD3+ TC, CD3+ TM, CD8+ TM and CD45RO+ TM) were finally included in the II-based signature in the training cohort (Fig. 1). The II-GIST score was calculated as follows: 0.0000019123 × CD3+ TC count + (0.0000559617 × CD3+ TM count) + (0.0028924318 × CD8+ TM count) + (0.0007187206 × CD45RO+ TM count). The R package “g illuminate” was used for the LASSO Cox regression analysis [25]. We used maximally selected rank statistics to define the threshold value for II-GIST as 0.58 in order to divide patients into the high II-GIST and low II-GIST groups. According to the above formula, high expression was defined as 1, and low expression was defined as 0.

2.5. Statistical analysis

For categorical variables, the associations between the TIL densities and the clinicopathological features were analysed via the χ² test or Fisher’s exact test. For continuous variables, parametric or non-parametric tests were used to compare the differences between groups.

Survival curves were calculated by the Kaplan-Meier method and were compared by log-rank tests. We conducted univariate analysis to investigate the associations between clinicopathological features and survival outcomes. Variables with P<0.25 in the univariate analysis were incorporated into a multivariable analysis that employed a Cox proportional hazards model using the backward method. The Cox model was also used to investigate the interactions between the II-GIST signature and adjuvant imatinib therapy. The time-dependent area under the receiver operating characteristic (ROC) curve (AUC) was calculated to compare the prognostic discrimination capability of each immune infiltration and the prediction models for GISTs. In addition, Harrell’s concordance index (C-index) with 95% confidence interval (CI) was calculated to evaluate discrimination by the II-GIST signature and the NIH risk criteria. The relative importance of each parameter to survival risk was assessed using the χ² statistics by R package “rms” [26]. All statistical analyses were performed with SPSS (Inc., Chicago, IL, USA) and R software.

3. Results

3.1. Density and distribution of TILs in GISTs

Initially, CD3+, CD8+, CD45RO+, Foxp3+, CD4+, NKp46+ and CD20+ cells were evaluated separately in the TC and TM (Fig. S2). More CD3+, CD8+, CD4+ and NKp46+ cells were detected in the TM than in the TC (Fig. S3a, b, e and f). The densities of CD45RO+, Foxp3+ and CD20+ cells were similar between the TC and TM (Fig. S3c, d and g).

Then, we analysed the associations between the clinicopathological variables and the absolute counts of CD3+, CD8+, CD45RO+, Foxp3+, CD4+, NKp46+ and CD20+ cells in the TC and TM. Localized GISTs exhibited higher CD8+ (Wilcoxon Rank Sum test, P=0.008) and Foxp3+ (Wilcoxon Rank Sum test, P=0.001) cell densities in the TM compared with metastatic GISTs (Fig. S4b and d). The CD45RO+ cell counts in the TC were higher in tumours ≤5 cm in size than in tumours >5 cm in size (Wilcoxon Rank Sum test, P=0.023) (Fig. S5c). Stomach GISTs exhibited a lower prevalence of CD8+ cells in both the TC (Wilcoxon Rank Sum test, P=0.001) and TM (Wilcoxon Rank Sum test, P=0.001) than non-gastric GISTs (Fig. S6b).

Next, we investigated associations between the density of TILs in the TC or TM and the prognosis of GIST patients. The Kaplan-Meier analysis results suggested that high levels of CD3+, CD8+, CD45RO+, NKp46+ and CD20+ cell infiltration in both the TC and TM correlated with improved recurrence-free survival (RFS) and overall survival (OS) outcomes (Fig. 2 and Fig. S7–9). The time-dependent ROC curve analysis revealed the prognostic discrimination capability of individual TILs (Fig. S10 and Table S1–2). Multivariable Cox proportional regression analyses after adjustment for other clinicopathological variables demonstrated that high levels of CD3+ TC, CD3+ TM, CD8+ TC, CD8+ TM, CD45RO+ TM, NKp46+ TM and CD20+ TM infiltrations were independent prognostic factors for GIST outcomes (Table S3). In addition, moderate correlations were detected in pairs (TC and TM)

Fig. 1. Construction of the II-GIST signature comprising 4 immune features. (a) Tenfold cross validation for tuning parameter selection in the LASSO model. The solid vertical lines represent partial likelihood deviance ± standard error (SE) values. The dotted vertical lines are drawn at the optimal values according to the minimum criteria and 1-SE criteria. Here, a value of λ=0.0634 with log(λ)=−2.759 was selected by 10-fold cross validation with the 1-SE criteria. (b) LASSO coefficient profiles of the Gise associated immune features.
of CD3+, CD8+, CD45RO+, CD20+ and NKp46+ TILs, as shown in Figure S11.

3.2. II-GIST signature and survival of GIST patients

The baseline characteristics of the training, internal validation and external validation cohorts are listed in Table 1. First, we developed a prediction model using a LASSO Cox regression model based on the training cohort, which ultimately included 4 immune features (Fig. 1).

As shown in Table 1, the baseline clinicopathological features were similar between the high II-GIST and low II-GIST groups in both the training and internal validation cohorts, while in the external validation cohort, the low II-GIST group seemed to have more advanced disease characteristics. In the training cohort, 77 patients were classified into the high II-GIST group, and 53 were assigned to the low II-GIST group. In the training cohort, the 5-year DFS and OS rates were 94.1 and 94.8%, respectively, in the high II-GIST group and 52.6 and 57.2%, respectively, in the low II-GIST group (Fig. 3a). In the internal validation cohort, the DFS (HR=0.110; 95% CI, 0.044−0.274; P<0.001) and OS (HR=0.162; 95% CI, 0.061−0.426; P<0.001) rates in the high II-GIST group were significantly superior to those in the low II-GIST group (Fig. 3b). Similarly, the high II-GIST group exhibited higher DFS and OS rates than the low II-GIST group in the external validation cohort (Fig. 3c). Multivariable Cox regression analyses demonstrated that II-GIST was an independent prognostic factor for both DFS and OS (Table S4−7).

Table 1
Clinical characteristics of patients according to the II-GIST Signature in the training and validation cohorts.

| Characteristic | Training Cohort (n = 130) | Internal Validation Cohort (n = 194) | External Validation Cohort (n = 111) |
|---------------|---------------------------|-------------------------------------|-------------------------------------|
|               | High-II-GIST | Low-II-GIST | P | High-II-GIST | Low-II-GIST | P | High-II-GIST | Low-II-GIST | P |
| Median age (range) | 56 (23−77) | 56 (27−80) | 0.542 | 0.229 | 56 (26−94) | 59 (28−76) | 0.934 | 0.707 | 60 (27−83) | 61 (44−87) | 0.070 | 0.803 |
| Sex | Male | 72 | 46 | 26 | 120 | 73 | 47 | 0.607 | 0.154 | 66 | 41 | 25 | 0.176 |
| | Female | 58 | 31 | 27 | 74 | 43 | 31 | 109 | 70 | 39 | 66 | 45 | 21 | 0.901 |
| Tumour size | ≤5cm | 75 | 43 | 32 | 109 | 70 | 39 | 0.369 | 0.152 | 45 | 25 | 20 | 0.091 |
| | >5cm | 45 | 34 | 21 | 85 | 46 | 39 | 36 | 23 | 13 | 0.041 |
| Primary tumour site | Stomach | 82 | 51 | 31 | 120 | 67 | 53 | 0.202 | 0.251 | 75 | 47 | 28 | 0.036 |
| | Non-Stomach | 48 | 26 | 22 | 74 | 49 | 25 | 34 | 17 | 17 | 26 | 12 | 14 |
| Mitotic index | ≤5 | 60 | 96 | 36 | 131 | 82 | 49 | 0.055 | 0.165 | 85 | 58 | 27 | 0.036 |
| | >5 | 34 | 17 | 17 | 63 | 34 | 29 | 33 | 15 | 18 | 21 | 16 | 5 |
| NIH criteria | High risk | 54 | 26 | 28 | 90 | 48 | 42 | 33 | 15 | 18 | 33 | 15 | 18 |
| | Intermediate risk | 31 | 23 | 8 | 32 | 19 | 13 | 21 | 16 | 5 | 26 | 12 | 14 |
| | Very Low/Low risk | 45 | 28 | 17 | 72 | 49 | 23 | 57 | 39 | 18 | 57 | 39 | 18 |
| | Imatinib therapy | Yes | 18 | 12 | 6 | 20 | 10 | 10 | 0.478 | 0.362 | 31 | 17 | 14 | 0.264 |
| | No | 112 | 65 | 47 | 174 | 106 | 69 | 80 | 53 | 27 |

Abbreviation: II, Immune-infiltration (II) based Signature; NIH, National Institutes of Health.
Mitotic index, mitoses/50 high-power field.
To explore the prognostic discrimination capability of the II-based signature in GIST patients, we performed a time-dependent ROC curve analysis. The AUC values for RFS at 1, 3 and 5 years were 0.81 (95% CI: 0.66–0.96), 0.91 (95% CI: 0.84–0.97) and 0.92 (95% CI: 0.86–0.99), respectively, in the training cohort and 0.87 (95% CI: 0.80–0.95), 0.87 (95% CI: 0.80–0.95) and 0.91 (95% CI: 0.83–0.98), respectively, in the internal validation cohort. The AUC values for RFS at 1, 3 and 5 years were 0.82 (95% CI: 0.71–0.92), 0.81 (95% CI: 0.72–0.90) and 0.88 (95% CI: 0.81–0.95), respectively, in the external validation cohort. The ROC curve analysis suggested that the II-GIST signature is potentially more accurate than the NIH risk criteria in terms of prognostic prediction in the internal and external validation cohorts (Fig. S12). Furthermore, subgroup analyses stratified by clinicopathological features were performed to explore the ability of the II-GIST signature to predict the prognosis of GIST patients in the internal and external validation cohorts. High II-GIST patients showed better OS and RFS outcomes than both low II-GIST patients with moderate/high risk and GIST patients with very low/low risk (Fig. 5). When the cohorts were stratified by tumour size, mitotic index and primary tumour location, the II-GIST signature remained an efficient prognostic model (Fig. S13–15).

3.3. II-GIST signature and the benefit of adjuvant imatinib therapy

Current clinical practice recommends adjuvant imatinib therapy after surgery for moderate- and high-risk patients with GIST. Thus, the association between the II-GIST signature and the benefit of imatinib treatment was explored amongst moderate- and high-risk groups of patients with GISTs. The benefit of adjuvant imatinib therapy in the high II-GIST group was obvious for both RFS (HR=0.134;
95% CI, 0.025–0.724; \( P = 0.020 \); \( P = 0.001 \) for the interaction) and OS (HR=0.050; 95% CI, 0.006–0.440; \( P = 0.007 \); \( P = 0.006 \) for the interaction) (Table S9). The Kaplan-Meier survival curves revealed that adjuvant imatinib therapy significantly improved RFS (log-rank \( P = 0.020 \)) and OS (log-rank \( P = 0.007 \)) outcomes in the high II-GIST group, although no obvious differences were detected in the low II-GIST group (Fig. 6). Taken together, our data suggest that moderate- and high-risk patients with high II-GIST scores may gain survival benefits from adjuvant imatinib therapy.

3.4. PD-1 and PD-L1 in GISTs

Moreover, we analysed the expression of PD-1 and PD-L1 in GIST patients in the whole cohort (Fig. S16–17). The densities of PD-
1\(^+\) cells were similar between the TC and TM (Fig. S3h). The Kaplan-Meier analysis results indicated that high PD-1 expression in TC or TM are not associated with RFS or OS (Fig. S18a-b). PD-L1 expression was found to be positive in only 75 cases (16.3%). No significant difference of survival outcomes was detected between the PD-L1-positive and -negative group, either (Fig. S18c). Seifert et al. suggested that PD-1/PD-L1 blockade enhance T-cell activity and antitumor efficacy of imatinib in murine GISTs [27]. Then, the correlation between PD-L1 expression and response to imatinib was also evaluated amongst moderate- and high-risk patients. However, the benefit from adjuvant imatinib therapy were not discernable in neither PD-L1 positive nor negative group (Fig. S19).

4. Discussion

The immune microenvironment has attracted intense attention in the past decade because of the astounding progress in immunotherapy [28]. TILs, critical components of the immune microenvironment, participate in a complex interplay with their surroundings through which they influence tumour progression, development and metastasis [29]. During the past several decades, revolutionary progress has occurred in the diagnosis, management and understanding of the molecular mechanisms of GIST, a disease that has been historically misdiagnosed as leiomyoma. However, the role of TILs in GISTs has rarely been addressed. The present study investigated the distribution of imatinib in murine GISTs [27]. Then, the correlation between PD-L1 expression and response to imatinib was also evaluated amongst moderate- and high-risk patients. However, the benefit from adjuvant imatinib therapy were not discernable in neither PD-L1 positive nor negative group (Fig. S19).

Accumulating literature indicates that TILs in tumours are efficient predictive indicators of prognosis [33, 41]. Higher infiltration levels of CD8\(^+\) CTLs, important components of tumour-specific cellular adaptive immunity, were associated with better prognosis and pathological complete response to chemotherapy [29, 42]. In addition, CD3\(^+\) T lymphocytes have been associated with prolonged RFS in hepatocellular carcinoma [12]. NKp46\(^+\) NK cells, a population of lymphocytes that are part of the innate immune system, were found to correlate significantly with more favourable survival in gastrointestinal malignancies [43, 44]. Tumour-infiltrating CD20\(^+\) B cells (CD20\(^+\)TILs) play a vital role and are correlated with increased survival in ovarian cancer [45]. In GISTs, accumulating evidence indicates that several haematologic biomarkers, including the platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR), which are precise predictors of prognosis, should include lymphocytes to reflect immunity and inflammation [46-50]. Regarding TILs, Rusakiewicz et al. reported that NKp46\(^+\) and CD3\(^+\) cell infiltrates were associated with lower rates of recurrence and smaller tumour sizes amongst 53 localized GISTs [16]. In the present study, we investigated the prognostic impact of each TIL population and suggested that high infiltration levels of CD3\(^+\) TC, CD3\(^+\) TM, CD8\(^+\) TC, CD8\(^+\) TM, CD45RO\(^+\) TM, NKp46\(^+\) TM and CD20\(^+\) TM infiltrations are associated with favourable prognosis. Infiltrating immune cells contribute to immunosurveillance, which eliminates tumour cells and slows immune evasion [49, 50]. Convincing evidence indicates that the presence of tumour immune infiltrates is associated with a good response to treatment in different malignancies [35, 42, 51]. The abovementioned mechanisms might
clarify the association between high infiltration levels of TILs and favourable prognoses of patients with GISTS.

Relapse remains a common clinical occurrence even after complete resection of GISTs [52], and precise estimation of the recurrence risk is crucial for the management of GISTs [53]. Currently, the survival outcomes vary amongst GIST patients with identical NIH risk criteria. Due to the inadequate accuracy of the NIH risk criteria, a novel scoring system to evaluate the prognosis of patients with GISTs is urgently needed. The innate immune system performs critical functions in protecting the host from tumours; thus, the focus on immune profiling has increased. TILs have been shown to be positively correlated with the prognosis of patients with various kinds of solid tumours, including GISTs [5, 6, 16, 54]. Historically, the role of TILs has been largely investigated in individual TIL populations despite the complexity of immune features, which has led to the discovery of moderate predictive ability of TIL-related markers. Integrating numerous immune infiltrates into a single model has resulted in improved prognostic performance for individualized recurrence risk in several cancer types [5, 32, 35]. Hence, we developed a novel II-based signature with 4 immune features to independently predict the prognosis of GIST patients. The II-GIST signature is an indicator of bio-immunological characteristics, while the NIH risk criteria are determined mainly according to the following pathologic parameters: tumour size, mitotic index, nuclear pleomorphism, and tumour necrosis [55]. Our data indicated that the prognostic discrimination capability of the II-GIST signature was significantly superior to that of the NIH risk criteria and exhibited the greatest ability to predict survival when it was combined with other clinicopathological features into one model. The II-GIST signature is a comprehensive reflection of the immune environment and is correlated with tumour surveillance and progression and exhibited excellent accuracy for prognostic predictions. When patient cohorts were stratified by other clinicopathological parameters, the II-GIST signature still improved the accuracy of prognostic predictions.

Adjuvant imatinib therapy has been recommended as a first-line postoperative treatment for moderate- and high-risk patients with GISTs harboring sensitive mutations [19]. However, the population of moderate- and high-risk patients with GISTs exhibits considerable heterogeneity, which has led to great difficulties in distinguishing patients who could benefit from imatinib treatment. Emerging evidence suggests that TILs are independent predictors of the chemotherapeutic response of solid tumours. Asano et al. demonstrated that a high CD8⁺/Foxp3⁺ TIL ratio correlates with pathological complete response to neoadjuvant chemotherapy in aggressive breast cancer [51]. Balachandran et al. demonstrated that in GISTs, intratumoural CD3⁺ and CD8⁺ cell infiltration is associated with a favourable response to imatinib [18]. Rusakiewicz et al. reported that the NKP46/Foxp3 ratio in the TC increased significantly after imatinib treatment [16]. Thus, we investigated correlations between the novel II-GIST signature and the benefit of adjuvant imatinib treatment in moderate- and high-risk patients with GISTs. Our data suggested that moderate- and high-risk patients with high II-GIST scores might obtain a greater survival benefit from postoperative imatinib therapy compared with those with low II-GIST scores. Balachandran et al. showed that imatinib amplifies a pre-existing immune response in mouse GIST and that CD8⁺ T cells are required for its maximal effects [18]. Borg indicated that imatinib promotes NK cell activation and NK cell-dependent antitumour effects in GISTs [56]. Taken together, lymphocytes contribute substantially to the antitumour effects of imatinib. The II-GIST signature based on TILs is a comprehensive reflection of the immune status of GIST patients, which may correspondingly predict the effect of imatinib in GISTs. Hence, the II-GIST signature may have the potential to serve as a useful predictor of the response to imatinib and offer better guidance than existing indices for clinical selection of patients who can benefit from imatinib therapy. Considering the limited number of patients received imatinib, the predictive accuracy of the II-GIST signature to imatinib therapy needs to be further validated in larger datasets. Furthermore, Seifert et al. reported that PD-L1 inhibition might augment both the therapeutic effect of imatinib and T cell activity in GISTs [27]. But in the present study the benefit from adjuvant imatinib therapy are not associated with PD-L1 status (Fig. S19). Consequently, application of the II-GIST signature may contribute to treatment optimization, including that of future immunotherapies.

Some limitations of our study need to be addressed. First, this was a retrospective study, and all enrolled patients were of Chinese ethnicity. Second, the sample size seemed to be limited. However, given the comparatively low incidence of GISTs, the current study is one of the largest clinical studies conducted to date. Therefore, the prognostic impact of the II-GIST signature must be validated in further prospective studies to guide clinicians in the identification of patients who require more frequent monitoring and intensive therapy.

5. Conclusion
In conclusion, the novel II-GIST signature can effectively individualize prognostic predictions for GIST patients and improve the predictive accuracy of common clinicopathological indices. Moreover, the II-GIST signature may have the potential to predict the therapeutic response to imatinib in moderate- and high-risk patients with GISTs. Therefore, the II-GIST signature might guide patient counselling, decision-making regarding individualized adjuvant treatment, and follow-up scheduling.

Funding
This study was supported by National Natural Science Foundation of China, No. 81702325; Natural Science Foundation of Guangdong Province, No. 2017A030310565; 3&D Project of the First Affiliated Hospital of Sun Yat-sen University. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethics approval and consent to participate
This study was reviewed and approved by the Ethics Committee of the first affiliated hospital of Sun Yat-sen University and the second affiliated hospital of Guangzhou University of Chinese Medicine.

Author contributions
CH Zhang, YL He and ZW Wei contributed to the study design. J Wu, J Li, WJ Xiong, ZW Wei and XF Lu performed immunohistochemistry. WB Huang, W Wang, J Wu and XF Lu performed statistical analysis and interpretation. XH Zhang, J Wu and WB Huang drafted the manuscript. All authors contributed to critical revision of the final manuscript and approved the final version of the manuscript.

Declaration of Competing Interest
The authors declare that they have no conflict of interest.

Acknowledgment
The authors would like to acknowledge the assistance of Donglian Chen in maintaining our database.

Supplementary materials
Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2020.102850.
