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ORIGINAL ARTICLE

Immune Cell Infiltration and the Expression of PD-1 and PD-L1 in Primary PDGFRA-Mutant Gastrointestinal Stromal Tumors

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Abstract

Purpose To characterize the immune cell profile and expression of PD-1, PD-L1, and IDO in PDGFRA-mutant gastrointestinal stromal tumors (GISTs).

Methods The clinicopathological data of PDGFRA-mutant GIST patients who received surgical resection in Zhongshan Hospital between January 2013 and August 2019 were reviewed retrospectively. The specimens of tissue chips were detected for immune cell infiltration and the expression of PD-1, PD-L1, and IDO by immunohistochemical staining.

Results $CD3^+$, $CD8^+$, and $CD68^+$ cells were the main infiltrating immune cells in the 42 patients included in this study. In addition, $CD4^+$, $CD56^+$, $Foxp3^+$, and $CD20^+$ cells were also observed. A higher $CD8^+$ T cell count was associated with smaller tumor size and PDGFRA D842V mutation ($P = 0.047$, $P = 0.005$). A higher $CD3^+$ and $CD68^+$ cell count was associated with a higher mitotic index ($P = 0.022$, $P = 0.006$). $CD4^+$ and $CD20^+$ cell count was associated with tumor morphology ($P = 0.002$, $P = 0.045$). PD-1 expression was present in 37 (88%) samples. Eighteen samples were positive for PD-L1 expression, and it was higher in small vs. large tumors ($P = 0.012$) and epithelioid and mixed cell type vs. spindle cell type GISTs ($P = 0.046$). IDO expression was positive in all 42 patients. The number of $CD4^+$ cells was significantly greater in the specimens with high IDO expression ($P = 0.012$).

Conclusion There were abundant infiltrating immune cells in PDGFRA-mutant GISTs. PD-L1 expression was negatively associated with tumor size. The immunotherapy targeting PD-1/PD-L1 checkpoint and IDO may be valuable.

Keywords Gastrointestinal stromal tumors · Immunotherapy · Immune infiltrate · Programmed cell death-ligand 1

Abbreviations

IDO	Indoleamine-2,3-dioxygenase
PD-1	Programmed cell death protein-1
PD-L1	Programmed cell death protein ligand-1
GISTs	Gastrointestinal stromal tumors
PDGFRA	Platelet-derived growth factor receptor alpha
TKIs	Tyrosine kinase inhibitors
NIH	National Institutes of Health
TIL	Tumor-infiltrating lymphocyte
Treg	Regulatory T cell
TMA	Tissue microarray
TPS	Tumor proportion score
NCCN	National comprehensive cancer network
NSCLC	Non-small cell lung cancer
HPF	High-power field

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract, with an

annual incidence of 10–20 per million.[1] About 80% of GISTs contain mutations of the c-Kit (KIT), and 5–10% of GISTs harbor activating mutations of the platelet-derived growth factor receptor alpha (PDGFRA).[2] The advent of tyrosine kinase inhibitors (TKIs) has significantly improved the prognosis of GIST patients.[3–5] However, sensitivity to TKIs depends on the type of mutation and the prognosis of TKI-resistant tumors remains poor. The exon 18 D842V is the most common PDGFRA mutation, which leads to resistance to imatinib and sunitinib, and there is no effective treatment for these patients.[6]

The role of immunotherapy in cancer treatment has aroused increasing attention. At present, the tumor-infiltrating inflammatory cells and relevant immune checkpoint inhibitors have become the focuses of research. Studies have demonstrated that these tumor-infiltrating inflammatory cells are associated with clinicopathological features and have an additional predictive value in GIST patients.[7, 8] Immune checkpoint is a way to maintain self-tolerance and limit damage during immune responses, but immune escape occurs when malignant tumors express immune checkpoint-related ligands. The most common immune checkpoint is the programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) pathway.[9] The PD-1/PD-L1 immune checkpoint inhibitors showed a lasting anti-tumor response and improved the survival rate in several malignant tumors (kidney cancer, bladder cancer, melanoma, and lung cancer).[10–13] Moreover, PD-1 and PD-L1 are abundantly expressed in GISTs, and the expression of PD-L1 in GISTs is heterogeneous and could be used as an independent prognostic factor.[14] PD-1 and PD-L1 blockade in vivo was found to enhance the anti-tumor efficacy of imatinib in the presence of indoleamine-2,3-dioxygenase (IDO) inhibition.[15] To provide preliminary clinical evidence for the immunotherapy of PDGFRA-mutant GISTs, the current study mainly explored the infiltration of inflammatory cells and the expression of PD-1, PD-L1, and IDO in PDGFRA-mutant GISTs.

Materials and Methods

Patient Selection

The study was approved by the ethics review committee of Fudan University Zhongshan Hospital (Shanghai, China). Written informed consent was obtained from all participating patients. All patients were pathologically diagnosed with primary PDGFRA-mutant GISTs who underwent surgical resection in our hospital between January 2013 and August 2019. None of the patients had been treated with anti-PD-1/PD-L1 medicine before the surgery. The clinicopathological features were investigated based on medical records. According to the modified National Institutes of Health (NIH) consensus criteria,[16] all the patients were classified as a very low-low risk or a moderate-high-risk group.

Follow-up

Patients were followed up every 3–6 months, including medical history, physical examination, laboratory, and imaging examinations (tumor markers, chest radiographs, abdominal B-ultrasound, abdominal pelvic enhanced CT, and gastroscopy). The above information was obtained by telephone in four patients who were not regularly followed up at our hospital. Recurrence-free survival (RFS) was calculated from the date of primary tumor resection to the date of disease recurrence (including local relapse and metastasis) or the last follow-up date. Overall survival (OS) was defined as the time from the date of surgery to patient death or the last follow-up date. The survival and recurrence status was last updated in December 2019.

Tissue Microarray and Immunohistochemistry

IHC assay was carried out on a total of 42 GIST specimens with PDGFRA mutations. Tissue microarray (TMA) was created by paraffin-embedded tissue blocks from the pathology department of our hospital. Two pathologists without knowledge of the clinical information reviewed the sections and selected a representative tumor region from each case. The tissue from the donor wax block (2 mm × 6 mm) was inoculated vertically into the recipient wax block to make TMA. IHC was performed on 5-micron slices TMA by the fully automated immunohistochemistry machine (Leica Bond-Max) using the following antibodies: the monoclonal antibody (McAb) of mouse anti-human CD3 (LN10, Leica), CD4 (4B12, Dako), Foxp3 (236A1E7, Abcam), CD20 (L26, Dako), CD56 (1B6, Leica), CD68 (PGM1, Dako), and rabbit polyclonal antibodies against human CD8 (poly, Abcam), aimed at identifying T lymphocytes, helper T cells, regulatory T cells (Treg), B cells, natural killer (NK) cells, macrophages, and cytotoxic T cells,[17–20] and rabbit monoclonal antibodies against human PD-1 (EPR4877, Abcam) and PD-L1 (EIL3N, CST) for staining of PD1 and PD-L1, and rabbit monoclonal antibodies against human IDO (D5J4E, Cell Signaling) for staining of IDO. A fully automatic digital slice scanning system (Leica Aperio AT2) was used to scan the IHC staining images of each tissue chip and count the IHC-stained cells. Firstly, the whole section was observed under a low-power field to look for higher lymphocyte density, among which five high-power fields were selected to count the infiltrating immune cells. Two pathologists counted them independently and re-counted them when the difference was greater than 5%. PD-L1 expression was evaluated by estimating the proportion of tumor cells (tumor proportion score, TPS) with membranous staining of any intensity after counting at least

100 tumor cells, excluding normal parenchymal and tumor-associated immune cells. PD-1 expression was defined as negative when TPS was < 1%, and as positive when TPS was ≥ 1%. PD-L1 expression was defined as negative (TPS < 1%), low (1% ≤ TPS < 50%), and high (TPS ≥ 50%). The expression level of IDO was defined as negative (TPS < 1%), low (1 ≤ TPS < 10%), and high (TPS ≥ 10%).[21]

Statistical Analysis

Data are expressed as means ± standard deviation (SD) or median. Significant differences in the number of immune cells were evaluated using Student's *t* test or one-way analysis of variance (ANOVA). Associations between categorical variables were assessed using Pearson's chi-square test, continuity correction, or Fisher's exact test, as appropriate. The binomial logistic regression model was used for multivariate analysis. All statistical tests were two-tailed at the 5% level of significance. All statistical analyses were performed using SPSS statistical software version 20.0 (SPSS Inc, Chicago, IL, USA).

Results

Patient Clinicopathological Characteristics

All clinicopathological characteristics available are presented in Table 1. Altogether, 42 patients were included in this study, with a median age of 60 (32–76) years and a male predominance (73.8%, 31/42). All tumors were located in the stomach, including the epithelioid type in 11 (26.2%), spindle type in 15 (35.7%), and the mixed type in 16 (38.1%). Of them, 28 (66.7%) patients were classified as the very low-low-risk group and 14 (33.3%) as the moderate-high-risk group. The median follow-up period was 18 (13–27) months. There was only one case of relapse and no patient died of the disease until the last follow-up date. There was no significant difference in tumor size, mitotic index, and risk grade between the D842V and non-D842V groups.

Infiltration of Inflammatory Cells

The results showed that there were abundant tumor-infiltrating inflammatory cells. Except that the nucleus was reddish brown in Foxp3 IHC staining, the other IHC staining positive cells were all brown on the cell membrane. Representative examples are shown in Fig. 1. The positive cells of the various immune markers were counted according to the method mentioned above. It was found that the infiltrating immune cells in primary PDGFRA-mutant GISTs were mainly $CD3^+$, $CD8^+$, and $CD68^+$ cells, which were diffusely distributed among tumor cells, with a few clustering around the perivascular structure in a nest shape. A small number of immune cells, including $CD4^+$, $CD56^+$, $Foxp3^+$, and $CD20^+$ cells were also observed (Fig. 2).

Relationship Between Inflammatory Cells and the Clinicopathological Features

There were significantly more $CD8^+$ T cells in small GISTs (≤ 5 cm) compared with large tumors (12.5 ± 7.1 vs. 8.0 ± 5.1,

Table 1 Relationship between the patient clinicopathological characteristics and the PDGFRA D842V mutation status

Factors	Total ($n = 42$)	D842V ($n = 26$)	Non-D842V ($n = 16$)	*P* value
Sex				1.000
Male	31 (73.8%)	19 (73.1%)	12 (75.0%)	
Female	11 (26.2%)	7 (26.9%)	4 (25.0%)	
Age(years)	60 (32–76)	59 (32–72)	62 (44–76)	0.290
Tumor size				0.287
≤ 5 cm	29 (69.0%)	20 (76.9%)	9 (56.3%)	
> 5 cm	13 (31.0%)	6 (23.1%)	7 (43.8%)	
Morphology				0.636
Spindle	15 (35.7%)	10 (38.5%)	5 (31.2%)	
Epithelioid and mixed	27 (64.3%)	16 (61.5%)	11 (68.8%)	
Mitotic index				1.000
≤ 5/50 HPF	38 (90.5%)	24 (92.3%)	14 (87.5%)	
> 5/50 HPF	4 (9.5%)	2 (7.7%)	2 (12.5%)	
NIH risk grade				0.072
Very low-low	28 (66.7%)	20 (76.9%)	8 (50.0%)	
Moderate-high	14 (33.3%)	6 (23.1%)	8 (50.0%)	

HPF, high-power fields; *NIH*, National Institutes of Health

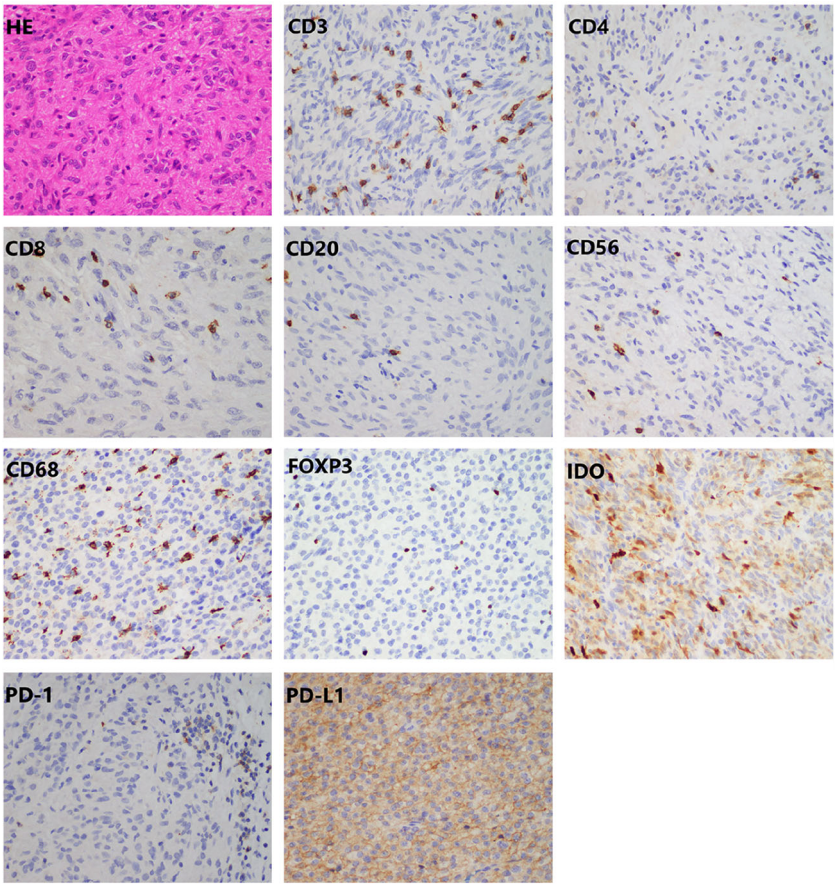

Fig. 1 Representative microphotographs of immunohistochemistry results in PDGFRA-mutant gastrointestinal stromal tumors(X 400). HE, hematoxylin and eosin staining; PD-L1, programmed cell death-ligand 1; IDO, indoleamine-2,3-dioxygenase

$P = 0.047$), and the number of $CD8^+$ T cells was significantly larger in the PDGFRA D842V group as compared with that in the non-D842V group (13.4 ± 7.3 vs. 7.5 ± 3.8, $P = 0.005$). Exon 12 mutation was detected in 5 cases and exon 18 mutation in 37 cases. Besides, point mutation was detected in 31 cases, deletion mutation in 6 cases, and mixed mutation in 5 cases. The proportion of $CD8^+$ T cells in PDGFRA-mutant GISTs with point mutation was higher than that with deletion and mixed mutations together (12.6 ± 7.1 vs.7.0 ± 3.5, $P = 0.017$). There was no significant difference in $CD8^+$ T cell infiltration between the deletion mutation type and other mutation types ($P = 0.137$) or between the exon 12 and 18 mutations ($P = 0.350$). Besides, GISTs with a high mitotic index (> 5/50 HPF) were enriched with $CD3^+$ T cells (58.1 ± 18.8 vs. 34.8 ± 18.5) and $CD68^+$ cells (32.9 ± 14.4 vs. 17.3 ± 9.6) as compared with those with a low mitotic index ($P = 0.022$ and $P = 0.006$). In addition, there was a strong association between the number of $CD4^+$ and $CD20^+$ cells and the tumor histological morphology. The epithelioid and mixed types of GISTs were enriched with $CD4^+$ cells (9.1 ± 5.6 vs. 3.9 ± 2.8) and $CD20^+$ cells (1.8 ± 1.4 vs. 0.9 ± 0.8) as compared with the spindle cell type ($P = 0.002$ and $P = 0.045$). No such significant association was found in other immune cells (Fig. 3).

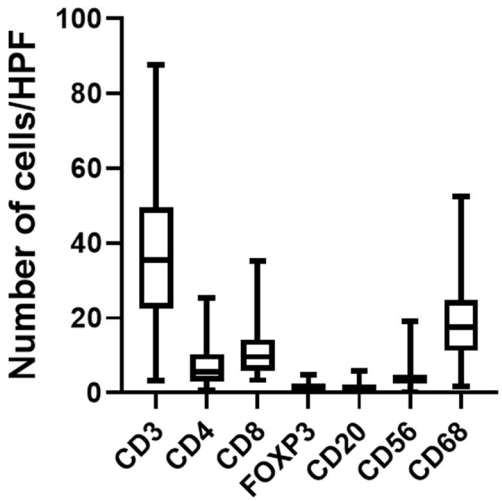

Fig. 2 The number of infiltrated immune cells in PDGFRA-mutant GISTs. HPF, high-power field

Association Between PD-1, PD-L1, IDO Expression, and the Clinicopathological Features

The correlation of protein expression with the clinicopathological features is presented in Table 2. The IHC results showed that PD-1 expression was present in 37 (88.0%) PDGFRA-mutant GISTs. The expression of PD-L1 was variable, of which 18 samples were positive for PD-L1

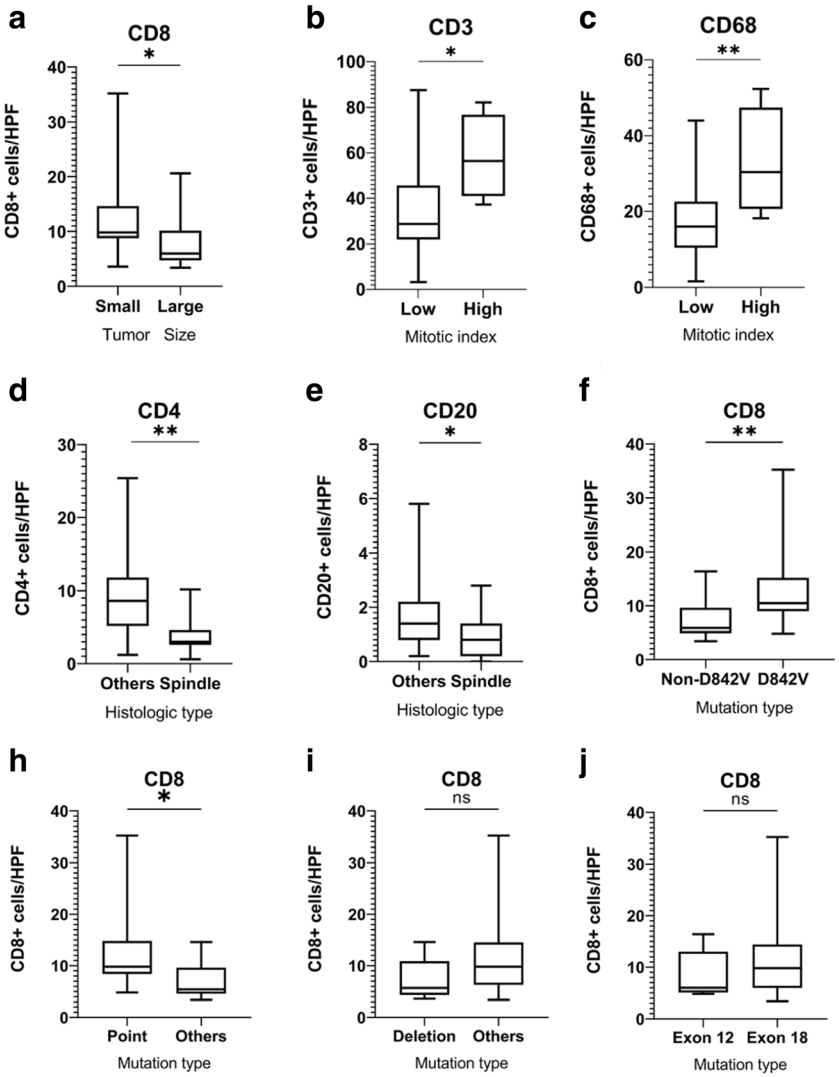

Fig. 3 Relationship between inflammatory cells and clinicopathological features. **a** A higher $CD8^+$ T cell count was associated with smaller tumor size(≤ 5 cm) (12.5 ± 7.1 vs. 8.0 ± 5.1, respectively; $P = 0.047$). **b** and **c** GISTs with a high mitotic index (> 5/50 HPF) were enriched with $CD3^+$ T cells (58.1 ± 18.8 vs. 34.8 ± 18.5) and $CD68^+$ cells (32.9 ± 14.4 vs. 17.3 ± 9.6) as compared with those with a low mitotic index ($P = 0.022$ and $P = 0.006$). **d** and **e** Epithelioid and mixed type of GISTs were enriched with $CD4^+$ T cells (9.1 ± 5.6 vs. 3.9 ± 2.8) and $CD20^+$ B cells (1.8 ± 1.4 vs. 0.9 ± 0.8) as compared with spindle cell type ($P = 0.002$ and $P = 0.045$). **f** $CD8^+$ T cell infiltration was almost doubled in GISTs bearing PDGFRA D842V mutations as compared with those bearing other PDGFRA mutations (13.4 ± 7.3 vs. 7.5 ± 3.8, $P = 0.005$). **h** The proportion of $CD8^+$ T cells in PDGFRA-mutant GISTs with point mutation was higher than that with deletion and mixed mutations together (12.6 ± 7.1 vs. 7.0 ± 3.5, $P = 0.017$). **i** and **j** There was no significant difference in $CD8^+$ T cell infiltration between the deletion mutation type and other mutation types ($P = 0.137$) or between the exon 12 and 18 mutations ($P = 0.350$). HPF, high-power field; D842V, PDGFRA exon 18 D842V mutation. $^*P < 0.05$; $^{**}P < 0.01$; ns, no significance

expression (TPS ≥ 1%), including 10 (23.8%) with low PD-L1 expression and 8 (19.1%) with high PD-L1 expression. The remaining 24 samples (57.1%) were negative for PD-L1 expression (TPS < 1%). Univariate analysis showed no significant relation of PD-L1 expression with patient age, sex, or the type of oncogene mutation. However, it was significantly correlated with tumor size, histological morphology, and NIH risk grade (Table 2). The significant factors in univariate analysis were subjected to multivariate analysis. Knowing that the tumor risk stratification is a combination of the tumor size and mitotic count, we only used tumor size for multivariate analysis. The result showed that PD-L1 expression was higher in small tumors (OR: 0.057, 95% CI: 0.006–0.531, $P = 0.012$), and in the epithelioid and mix cell types than that in the spindle cell type (OR: 5.234, 95% CI: 1.027–26.678, $P = 0.046$) (Table 4), suggesting that PD-L1 expression may be associated with a better prognosis. Twenty-six (61.9%) and sixteen (38.1%) specimens presented high and low expression of IDO. There were no statistically significant correlations between IDO expression and any of the above clinicopathological

Table 2 Univariate analysis of protein expression and clinicopathological features

Factors	PD-L1		*P* value	IDO		*P* value
	Negative	Positive		Low	High	
Sex			0.577			0.344
Male	19	12		10	21	
Female	5	6		6	5	
Age (years)	58 (32–72)	62 (45–76)	0.313	63 (41–76)	56 (32–72)	0.104
Tumor size			*0.002*			1.000
≤ 5 cm	12	17		11	18	
> 5 cm	12	1		5	8	
Morphology			*0.026*			0.130
Spindle	12	3		8	7	
Epithelioid and mixed	12	15		8	19	
Mitotic index			0.069			0.571
≤ 5/50HPF	20	18		15	23	
> 5/50HPF	4	0		1	3	
NIH risk grade			*0.008*			0.822
Very low-low	12	16		11	17	
Moderate-high	12	2		5	9	

All of our variables with *p* values less than 0.05 are in italics

PD-L1, programmed cell death-ligand 1; *IDO*, indoleamine-2,3-dioxygenase; *HPF*, high-power fields; *NIH*, National Institutes of Health

characteristics. However, univariate analysis showed that the expression of IDO was associated with the number of infiltrating inflammatory cells (Table 3). Multivariate analysis showed that the number of $CD4^+$ cells in the specimens with high IDO expression was significantly higher (OR: 5.612, 95% CI: 0.458–68.789, $P = 0.012$) (Table 4).

Discussion

The type of gene mutation in GISTs is very important for tumor cell proliferation and disease progression. However, the degree of progression and prognosis of the same mutated gene type varies. One study [22] suggested that some minute GISTs needed additional stimulation to make progress. In this process, the tumor immune microenvironment plays a key role.[23] Tumor-infiltrating inflammatory cells and the associated immune checkpoint are vital parts of the tumor immune microenvironment and maybe a new therapeutic target. To the best of our knowledge, this is the first study to describe the heterogeneity of tumor immune cells and immune checkpoint in PDGFRA-mutated GISTs.

Few studies have addressed infiltrating inflammatory cells in GISTs. Some studies have shown a high proportion of macrophages and T cells in GIST samples; in contrast, B cells,

Table 3 Univariate analysis of protein expression and immune cells

Cell type	PD-L1		*P* value	IDO		*P* value
	Negative	Positive		Low	High	
$CD3^+$ T cell	35.8 ± 21.0	38.7 ± 18.0	0.645	30.6 ± 15.2	41.0 ± 21.2	0.097
$CD4^+$ T cell	6.2 ± 5.3	8.6 ± 5.3	0.162	3.1 ± 1.5	9.8 ± 5.3	*<0.001*
$CD8^+$ T cell	11.0 ± 7.6	11.4 ± 5.8	0.842	10.4 ± 7.8	11.6 ± 6.3	0.594
$Foxp3^+$ T cell	1.6 ± 0.9	1.9 ± 1.2	0.282	1.1 ± 0.5	2.1 ± 1.1	*<0.001*
$CD20^+$ B cell	1.5 ± 1.3	1.4 ± 1.3	0.907	0.9 ± 0.8	1.8 ± 1.4	*0.015*
$CD56^+$ NK cell	4.1 ± 2.9	4.6 ± 4.3	0.648	3.2 ± 1.7	5.1 ± 4.2	*0.048*
$CD68^+$ macrophages	18.5 ± 12.6	19.2 ± 8.6	0.832	20.8 ± 13.3	17.6 ± 9.6	0.372

All of our variables with *p* values less than 0.05 are in italics.

PD-L1, programmed cell death-ligand 1; *IDO*, indoleamine-2,3-dioxygenase; *Bon*, Bonferroni correction

Table 4 Multivariate analysis of protein expression and the related factors

Factors	PD-L1 (negative vs. positive)		IDO (low vs. high)	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Tumor size		*0.012*	-	-
≤ 5 cm	1		-	-
> 5 cm	0.057 (0.006–0.531)		-	-
Morphology		*0.046*	-	-
Spindle	1		-	-
Epithelioid and mixed	5.234 (1.027–26.678)		-	-
$CD4^+$ T cell	-	-	1 5.612 (0.458–68.789)	*0.013*
$Foxp3^+$ T cell	-	-	1 2.152 (1.174–3.994)	0.177
$CD20^+$ B cell	-	-	1 1.499 (0.336–6.697)	0.596
$CD56^+$ NK cell	-	-	1 1.511 (0.841–2.714)	0.168

All of our variables with *p* values less than 0.05 are in italics.

PD-L1, programmed cell death-ligand 1; *IDO*, indoleamine-2,3-dioxygenase; *OR*, odds ratio; *CI*, confidence interval

NK cells, and dendritic cells are often much less common or even absent.[8, 24] Van Dongen et al. [25] found that infiltrating macrophages were mainly of the type M2, which was associated with regulatory T cells; the number of M2 macrophages in the metastatic foci of GISTs was twice that of primary GISTs, which may promote tumor progression. The number of $CD68^+$ macrophages was found to be negatively correlated with tumor metastasis and size, but it was positively correlated with the risk of tumor recurrence and prognosis.[26] Cameron et al. [27] found that the number of $CD3^+$ T cells and $CD20^+$ B cells in metastatic lesions was greater than that in primary lesions, and a high risk of recurrence GISTs was more than a low risk of recurrence GISTs. Rusakiewicz et al. [8] demonstrated that the number of $CD3^+$ T cells was negatively correlated with the tumor size and recurrence rate and positively correlated with progression-free survival (PFS) rate. Besides, the density of NK cell infiltration was found to be an independent prognostic factor for GISTs and related to the risk grade and gene mutation type. It was found in our study that immune cells including lymphocytes (mostly $CD3^+$, $CD8^+$, and $CD4^+$ T lymphocytes) were most abundant in inflammatory cells in PDGFRA-mutant GISTs, while $CD20^+$ and $Foxp3^+$ T cells only accounted for a small proportion in lymphocytes (Fig. 2). In recent years, more attention has been paid to the ratio of different lymphocyte subsets, especially the ratio of $CD8^+/Foxp3^+$ T cells, knowing that the ratio of $CD8^+$ to $Foxp3^+$ T cells reflects the balance between immune activation and immunosuppression. A high $CD8^+/Foxp3^+$ T cell ratio is associated with good tumor pathological features, treatment response, and patient survival in many solid tumors, including ovarian cancer, cervical cancer, and rectal cancer.[28–32] A common feature of the 42 samples in our series is a much higher density of $CD8^+$ T cells than $FOXP3^+$ T cells (Fig. 2), which may be related to the better prognosis of PDGFRA-mutant GISTs.

Besides, like other studies, our results also showed a strong correlation between inflammatory cell infiltration and the clinicopathological features of PDGFRA-mutant GISTs. Recently, one study reported RNA sequencing of 75 GIST tumors. Using IHC bioinformatics and flow cytometry, they found that PDGFRA-mutant GISTs had more immune cells and higher cytolytic activity than KIT-mutant GISTs.[33] However, they only included 24 PDGFRA-mutant GISTs and did not mention the difference in infiltration in the PDGFRA-mutant subgroup. Our results showed that a higher $CD8^+$ T cell count was associated with PDGFRA D842V mutation and point mutation, but there was no significant difference in $CD8^+$ T cell infiltration between the deletion mutation type and other mutation types or between the exon 12 and 18 mutations. $CD8^+$ T cell infiltration in tumors is generally accepted as a sign of immune recognition. Based on the above results, we anticipate that the differences in immune infiltration between different PDGFRA mutation types could be used to guide the therapeutic decisions of immunotherapy in the future.

In addition to inflammatory cell infiltration, an immune checkpoint also plays a key role in immunotherapy. The PD-1/PD-L1 axis is known as the canonical checkpoint, probably via the mechanism that binding of PD-L1 to PD-1 expressed in activated T cells attenuates the proliferation of $CD8^+$ T cells and T cells receptor signaling.[9] To explore the potential of

using PD-1/PD-L1 for the treatment of GISTs, it is necessary to identify ideal biomarkers. The national comprehensive cancer network (NCCN) guidelines recommend PD-L1 testing for non-small cell lung cancer (NSCLC) patients, and Keytruda (pembrolizumab) requires PD-L1 expression ≥ 50% for first-line NSCLC treatment and ≥ 1% for second-line treatment.[34] A review [35] critically evaluated several predictive parameters of PD-1/PD-L1 blockade therapy in NSCLC trials and suggested that the most convincing predictor of PD-1/ PD-L1 treatment was the IHC expression of PD-L1 in cancer cells. CARBOGNIN et al. [36] showed that the overall response rate of PD-L1-positive patients was higher than that of PD-L1-negative patients when PD-L1 inhibitors were used in advanced melanoma, NSCLC, and genitourinary cancer (34.1% vs. 19.9%).

Our results demonstrated the expected proportions with 57.1% negative cases, 23.8% of cases with low PD-L1 expression, and 19.1% cases with high expression. In addition, PD-L1 expression in small tumors was higher than that in large tumors (> 5 cm). Bertucci et al. [14] analyzed PD-L1 expression in a large series of clinical samples of 139 surgically treated localized GISTs without imatinib treatment and proved that PD-L1 expression was an independent factor affecting the prognosis of localized GISTs. Also, it has been demonstrated that imatinib combined with PD-1/PD-L1 blockade in vivo could increase the $CD8^+$ T cell effector function and enhance the anti-tumor effect in GISTs.[15] It is worth noting that the most abundant immune cells belonged to $CD8^+$ T lymphocytes in our study. Therefore, according to the results above, the expression of PD-L1 was heterogeneous in PDGFRA-mutant GISTs, which might inhibit tumor growth and be associated with a better prognosis. PD-1/PD-L1 immunotherapy may be effective in PDGFRA-mutant GISTs.

IDO is a rate-limiting enzyme in human tryptophan metabolism and plays an important role in the metabolic pathway of tryptophan decomposition.[37] IDO exerts its immunomodulatory effect by inhibiting the effector function of T cells.[38, 39] In a murine GIST model, combined imatinib with IDO inhibitors increased the anti-tumor efficacy by activating $CD8^+$ T cells and inducing apoptosis of regulatory T cells.[40] Our results showed that IDO expression was positive in all 42 patients, with high expression in 26 samples (61.9%) and low expression in 16 samples (38.1%). It is worth noting that high IDO expression was correlated with high immune cell infiltrates, in particular $CD4^+$ cells. Given the close correlation between IDO expression and infiltration of inflammatory cells, it could be concluded that IDO inhibitors may play an important role in the immunotherapy of PDGFRA-mutant GISTs.

This study has certain limitations. Due to the extremely low incidence of PDGFRA-mutant GISTs, the number of patients included in this study is not large enough, but it is the largest study sample size at present. Besides, it was a retrospective study and further research is needed to confirm the results. The mechanism of heterogeneity of tumor immune cells and immune checkpoint in PDGFRA-mutated GIST remains unclear.

Conclusion

Our study provides a detailed immune microenvironment profile in PDGFRA-mutant GISTs. The results demonstrated that the infiltrating immune cells were mainly $CD3^+$ T lymphocytes, $CD8^+$ T lymphocytes, and $CD68^+$ macrophages. PD-1 expression was present in most GISTs and nearly half of the tumors expressed PD-L1 protein, and IDO expression was seen in all samples. PD-1/PD-L1 checkpoint therapy and IDO-targeted therapy may be beneficial to the treatment of PDGFRA-mutant GISTs, but the effectiveness of immunotherapy needs to be confirmed in future clinical trials.

Authors' Contributions X.S., J.S., and W.Y. designed the work and wrote the manuscript. X.G., M.F., A.X., H.L., P.S., and Y.F. analyzed and interpreted the patient data. W.Y. and Y.H. performed the immunohistochemistry examinations. Y.H., K.S., J.S., J.Q., and X.Q. revised the manuscript. X.S., J.S., and W.Y. were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

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

Ethical Approval This study was approved by the Clinical Research Ethics Committee of Zhongshan Hospital, Fudan University.

Informed Consent Informed consent was acquired from all patients for the acquisition of clinical and pathological information and the use of surgical specimens.

Code Availability Not applicable.

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