ORIGINAL PAPER

DOI: http://dx.doi.org/10.15403/jgld-5858

# Programmed Cell Death-ligand 1 as Biomarker of Poor Prognosis in Patients with Gastrointestinal Stromal Tumour

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Received: 23.08.2024
Accepted: 15.01. 2025

## ABSTRACT

**Background & Aims**: To explore the prognostic role of programmed cell death-ligand 1 (PD-L1) in patients with gastrointestinal stromal tumours (GISTs).
**Methods**: PD-L1 expression was detected using immunohistochemistry in tissue microarrays from 96 GISTs samples. Survival of patients was assessed using Kaplan – Meier analysis. Possible risk factors for GISTs were explored using Cox proportional hazards regression models. The role of PD-L1 in GISTs proliferation, invasion, and metastasis was assessed using colony formation assay, scratch, transwell invasion, and migration assays.
**Results**: Survival in GISTs patients was significantly associated with PD-L1 expression ($p < 0.05$). High PD-L1 expression in GISTs resulted in relatively short overall survival ($p=0.016$). Univariate regression analysis showed that PD-L1 was a risk factor for poor prognosis ($p<0.05$). Compared with the control group, knockdown of PD-L1 significantly decreased the colony formation rate, and cell migration and invasion were inhibited compared with the control group. The wound healing ability of PD-L1 knockdown group was significantly weaker than that of the control group.
**Conclusions**: The results suggest that overexpression of PD-L1 is a risk factor for a poor prognosis in patients with GISTs. Knockdown of PD-L1 significantly inhibited the development of GISTs. PD-L1 may serve as a biomarker for the poor prognosis of GISTs and a potential therapeutic target.

**Key words:** gastrointestinal stromal tumours – PD-L1 – biomarker – poor prognosis.

**Abbreviations**: DOG1: discovered on gastrointestinal stromal tumors; GIST: gastrointestinal stromal tumours; IHC: immunohistochemistry; PD-L1: programmed cell death-ligand 1; SMA: smooth muscle actin; VCAM: vascular cell adhesion molecule.

## INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are common neoplasms in the gastrointestinal tract and are generally considered benign [1]. They are most common in the stomach and account for the majority of all GIST cases, approximately 60-70%. The incidence of GISTs is relatively low, with an annual incidence of 10-20% [2]. It has no significant gender preference and usually affects adults over 40 years of age [3]. Although GISTs may not cause overt clinical symptoms at an early stage, patients may experience abdominal discomfort, gastrointestinal bleeding, or gastrointestinal obstruction [4, 5]. Notably, GISTs can develop rapidly and carry a high risk of malignant transformation while not responding well to conventional chemotherapy and radiotherapy. Surgery is the treatment of choice for GISTs, but even so, patients still have a high risk of metastasis and recurrence after surgery, approximately 80% [6]. Currently, imatinib mesylate is the first-line drug for the treatment of GISTs, but patients may soon become resistant to it, resulting in decreased efficacy [7]. Given that the incidence of GISTs has risen in recent years, researchers are actively exploring more effective treatment strategies.

During tumor development, immune escape is a critical biological process, and this phenomenon can be achieved by the characteristics of tumor cells themselves as well as immune cells in the tumor microenvironment [8]. T cells, in particular, play a central role in immune escape. Driven by the inflammatory response, programmed cell death (PD)-1 molecules on the surface of T cells become activated, which helps control the activity of T cells in infected and tumor

tissues to prevent them from causing damage to healthy tissues [9, 10]. Binding of PD-1 to its ligand PD-L1 is an important immunomodulatory mechanism, which inhibits the activity of tumor-specific T cells, blocks signaling of T cell receptors, and reduces the expression of anti-apoptotic proteins and pro-inflammatory factors [11, 12]. This interaction helps tumor cells evade surveillance and attack by the immune system. Therefore, it is particularly critical to identify biomarkers that can guide clinical treatment decisions and reflect treatment outcomes. These markers can help physicians more precisely assess the patient's immune status, develop personalized treatment options, and monitor immune response changes during treatment in order to improve treatment outcomes and reduce unnecessary side effects. Further studies are needed on the role of PD-L1 in GISTs. In this study, we explored the relationship between PD-L1 and the prognosis of GISTs patients and its role in GISTs cells.

## METHODS

### Patients and Samples

In this retrospective study, 96 patients with GISTs who were diagnosed and surgically treated in our hospital between January 2004 and December 2014 were included, and 100 stromal tumor paraneoplastic tissues were used as a negative control group. All patients were diagnosed by pathological examination, the clinicopathologic data were complete, no distant metastasis was found before surgery, and no targeted therapy or radiotherapy was performed before surgery. The study was approved by the Ethics Committee of our hospital, and specimens were collected from patients and their families after written informed consent.

Clinical characteristics were collected from the medical records of 96 patients, including gender, age, tumor size, primary tumor site, depth of tumor invasion, grade according to NIH risk grading criteria [13], and nuclear schistosome count (Table I).

**Table I.** Clinicopathological characteristics of GIST patients

| Risk factors | Number of patients | % |
|---|---|---|
| Total | 96 | |
| Age | | |
| <55 | 46 | 47.90 |
| ≥55 | 50 | 52.10 |
| Gender | | |
| male | 49 | 51.00 |
| female | 47 | 49.00 |
| Infiltration depth | | |
| Mucosal layer | 43 | 44.80 |
| Muscular layer | 25 | 26.00 |
| Plasma layer | 28 | 29.20 |
| Tumour size | | |
| ≥5 | 46 | 47.90 |
| <5 | 50 | 52.10 |
| Tumour site | | |
| Gastric | 62 | 64.58 |
| Small intestine | 22 | 22.92 |
| Others | 12 | 12.5 |
| Pathological type | | |
| Spindle cell type | 96 | 100.00 |
| Epithelial cell type | 0 | 0.00 |
| Mixed type | 0 | 0.00 |
| Pathological nuclear division | | |
| ≥5 | 14 | 14.60 |
| <5 | 82 | 85.40 |
| Hazard classification | | |
| Low | 57 | 59.40 |
| Moderate | 18 | 18.80 |
| High | 21 | 21.90 |
| CD117 | | |
| negatives(-) | 14 | 14.60 |
| positive (+) | 71 | 74.00 |
| positive (++) | 11 | 11.40 |
| CD34 | | |
| negatives (-) | 15 | 15.60 |
| positive (+) | 77 | 80.20 |
| positive (++) | 4 | 4.20 |
| DOG1 | | |
| negatives (-) | 28 | 29.20 |
| positive (+) | 68 | 70.80 |
| Ki67 | | |
| ≥5 | 25 | 26.00 |
| <5 | 71 | 74.00 |
| Desmin | | |
| negatives (-) | 94 | 97.90 |
| positive (+) | 2 | 2.10 |
| S-100 | | |
| negatives (-) | 93 | 96.90 |
| positive (+) | 3 | 3.10 |
| SMA | | |
| negatives (-) | 86 | 89.60 |
| positive (+) | 10 | 10.40 |
| VCAM | | |
| negatives (-) | 60 | 62.50 |
| positive (+) | 36 | 37.50 |
| Stathmin | | |
| negatives (-) | 52 | 54.20 |
| positive (+) | 44 | 45.80 |
| Cofilin | | |
| negatives (-) | 63 | 65.60 |
| positive (+) | 33 | 34.40 |
| PDL1 | | |
| negatives (-) | 68 | 70.80 |
| positive (+) | 28 | 29.20 |

DOG1: discovered on gastrointestinal stromal tumors; GIST: gastrointestinal stromal tumours; PD-L1: programmed cell death-ligand 1; SMA: smoothie muscle actin; VCAM: vascular cell adhesion protein.

**Follow-up**

Patients were followed and assessed every 6 months after discharge according to the hospital's standardized system. Follow-up assessments were finally received on 31 December 2018. Four patients were lost to follow-up in the middle of presentation, with a follow-up rate of 95.83%. All patients were followed up for 4 to 41 months (mean 24.12 months). Survival was calculated from the date of admission to the time of death or the cut-off date for follow-up.

**Cell culture**

GIST8820 cell line was purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured under constant conditions of 5% $CO_2$ at 37 °C. All cell cultures were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin. Passage digestion was performed using 0.25% trypsin. Passages were performed every 3-5 days.

**Data Acquisition**

Data from The Cancer Genome Atlas (TCGA) were obtained from http://ualcan.path.uab.edu/index.html. After selecting "TCGA and TCGA gene analysis", "gastric adenocarcinoma" was selected in the "TCGA dataset" window for the search of "PDCD1".

**siRNAs and Transfection**

Transfection of GIST8820 cells was performed using OPTI-MEM. GIST8820 cells were plated in 6-well plates, 1500 μL OPTI-MEM transfection solution was added to each well after rinsing the transfected cells twice with serum-free OPTI-MEM (Invitrogen, Carlsbad, CA, USA) transfection solution, and the 6-well plates were returned to the cell incubator for continued incubation. Lipofectamine TM was mixed with OPTI-MEM medium and incubated on a super clean bench for 5 min, and transfection reagent: Lipofectamine 2000 was mixed at a ratio of 1:1 and incubated for 20 min. Incubated transfectants were added to pretreated GIST cells and incubated for 5 h in a cell incubator. After 5 h of transfection, the transfectant was discarded and replaced with normal complete medium, and the cells were returned to the incubator for further incubation. Total protein and RNA were extracted and quantified for later experiments.

**Colony Formation Assay**

Cultures of GIST8820 cells were performed using six-well plates. Following completion of incubation, the medium was removed and 0.1% crystal violet staining, formaldehyde, and 90% methanol fixation/staining solutions were added to the Petri dishes. Cells were then stained for 20 min at room temperature. Stained cells were gently washed three times with isotonic PBS and the number of clones was counted by taking pictures. The total number of cell clones per dish was calculated three times using ImageJ software (64-bit Java 1.8.0-172, NIH, Bethesda, MD, USA).

**Transwell assay**

For cell migration assays, GIST8820 cells in log phase were adjusted to 2 x $10^4$/100 μL cell suspension using serum-free basal medium concentration. Adjusted cells were seeded into upper transwell inserts (Corning, Inc., Corning, NY, USA) at a density of approximately $2 \times 10^4$ cells/well. The chambers were placed in 24-well plates with serum-free cell culture medium in the upper chamber and FBS medium with 10% FBS in the lower chamber. After 24 h of incubation, the upper chamber was removed and migrated cells were swabbed inside the chamber, fixed in 4% paraformaldehyde for 15 min, and stained with crystal violet. Migrated cells were observed under 200x microscope field and the number of migrated cells was counted. For cell invasion assays, prepared Matrigel (Corning, Inc.) gels were applied to the upper chamber prior to cell seeding. The remaining procedures were consistent with those used for the cell migration assay.

**Wound Healing Assay**

For the wound healing assay, cells at the logarithmic growth stage were digested with 0.25% trypsin, inoculated in pre-labelled 6-well plates for 24h, and observed to reach 90% cell fusion. The cells were then rinsed twice with an isotonic PBS solution to ensure that as many cells as possible remained in the lane, and the culture was continued in DMEM medium with 2% FBS (to reduce cell proliferation factors while ensuring cell viability) and placed in the cell culture incubator. Photographs were taken at 0 h, 12 h, and 24 h to capture images of cells within the marker range. Wound closure distances were measured at three separate wound sites in each group. The wound healing rate was independently calculated using the following formula: healing rate = (0 h scratch width - 24 h/48 h scratch width)/0 h scratch width × 100%.

**Immunohistochemistry and Scoring**

Immunohistochemical (IHC) staining was performed using GISTs and adjacent normal tissue microarrays to detect the expression of CD34, CD117, discovered on gastrointestinal stromal tumors (DOG-1), Ki67, desmin, S-100, smooth muscle actin (SMA), vascular cell adhesion molecule (VCAM), stathmin, cofilin, and PD-L1 (Fig. 1). We used anti-CD34 (ab81289; Abcam, Cambridge, UK; 1:400), anti-CD117 (ab32363; Abcam, 1:400), anti-DOG-1 (ab212744; Abcam; 1:300), anti-Ki67 (ab16667; Abcam; 1:200), anti-desmin (ab227651; Abcam; 1:100), anti-S-100 (ab183979; Abcam; 1:500), anti-SMA (ab244177; Abcam; 1:300), anti-VCAM (ab134047; Abcam; 1:500), anti-stathmin (ab52630; Abcam; 1:200), anti-cofilin (ab124979; Abcam; 1:200), and anti-PD-L1 (ab213524; Abcam; 1:250) antibodies. Two experienced pathologists, who maintained the confidentiality of the patients' clinical information, independently assessed and recorded the IHC results. PD-L1-positive cells were evaluated according to the presence or absence of brown or brownish-yellow granules within the cells, and a semi-quantitative evaluation was performed to determine the rate of positive cell staining. This was done as follows: Based on the intensity of staining of the tumour cells, 0 marks for no staining; 1 mark for light brown; 2 marks for brown; 3 marks for dark brown. Based on the percentage of tumour-positive cells, 0% < positive cell count < 5%, score 0; 5% ≤ positive cell count ≤ 10%, score 1; 10% < positive cell count < 50%, score 2; and 50% ≤ positive cell count ≤ 100%, score 3. The final score = tumour cell staining intensity* percentage of tumour positive cells, with scores of 0 and 1 classified as negative and scores of 2, 3, 4, 6,

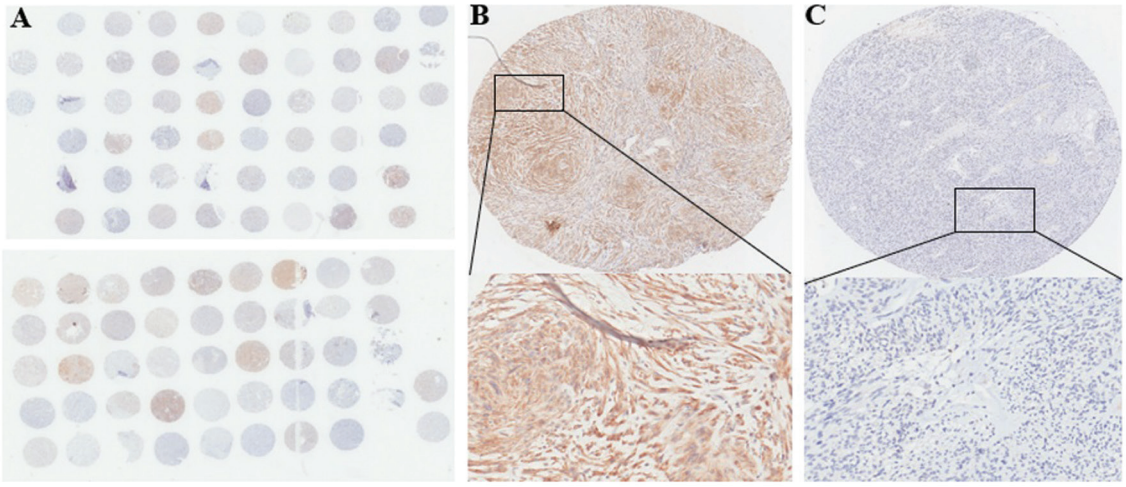


**Fig. 1.** A) Tissue microarray of GIST tissue and adjacent normal tissue. B) Positive expression of PD-L1 in tumor tissues; 200× magnification. C) Negative expression of PD-L1 in tumor tissues; 200× magnification.

and 9 classified as positive, with CD34 and CD117 classified as weakly positive (+) for 2 and 3 and strongly positive for 4, 6, and 9. For Ki67, high expression was defined as Ki67 staining in ≥5% of the nuclei, while low Ki67 expression was defined as Ki67 staining in <5% of the nuclei.

**Statistical Analysis**

Data analysis was performed using SPSS software (version 22.0, SPSS, Inc., Chicago, IL, USA). The Chi-square test was used to assess the relationship between PD-L1 expression and clinicopathological characteristics of GIST patients. Survival analysis was performed using the Kaplan-Meier method with logit test and Cox proportional hazards regression model to explore potential prognostic factors for OS. All statistical tests were two-sided and statistical significance was set at $p<0.05$.

## RESULTS

**Clinicopathological features of patients with GIST**

Detailed clinicopathological characteristics of the 96 patients are shown in Table I. Approximately half of the patients were ≥ 55 years old of age. The depth of invasion included 43 cases of mucosal invasion, 25 cases of muscular invasion, and 28 cases of serosal invasion. Tumor size was ≥ 5 cm in 46 patients and < 5 cm in 50 patients. All cases in this study were spindle cells. Mitotic image counts were < 5/50 HPFs and ≥ 5/50 HPFs in 82 and 14 cases, respectively. According to the NIH risk classification criteria, 57 very low-risk and low-risk patients were included in the low-risk group, 18 in the intermediate-risk group, and 21 in the high-risk group.

Immunohistochemistry staining results showed that CD117 was negative in 14 cases, positive (+) in 71 cases and positive (++) in 11 cases and CD34 was negative in 15 cases, positive (+) in 77 cases, and positive (++) in 4 cases. DOG1 was negative in 28 cases and positive in 68 cases. For Ki67, high expression was defined as Ki67 staining in ≥5% of the nuclei, while low Ki67 expression was defined as Ki67 staining in <5% of the nuclei. Ki67 expression was positive in 25 cases and negative in 71 cases. Desmin expression was positive in 2 cases and negative in 94 cases. S-100 expression was positive in 3 cases and negative in 93 cases. SMA expression was positive in 10 cases and negative in 86 cases. VCAM expression was positive in 36 cases and negative in 60 cases. Stathmin expression was positive in 44 cases and negative in 52 cases. Cofilin expression was positive in 33 cases and negative in 63 cases. PD-L1 expression was positive in 28 cases and negative in 68 cases (Table I).

**PD-L1 Expression and Clinicopathological Characteristics of GIST Patients**

According to statistics, 29.20% (28/96) of GISTs tissue samples showed positive expression of PD-L1, indicating that the protein was relatively commonly expressed in GISTs tissues. The results of the study on the relationship between PD-L1 expression levels in GISTs tissues and clinicopathological features of GIST showed that PD-L1 expression in GISTs tissues was not significantly correlated with gender, age, tumour size, CD117, DOG1, desmin, S-100, SMA, cofilin, and Ki67 expression (all $P > 0.05$; Table II). In contrast, PD-L1 expression correlated with depth of tumour invasion, CD34 expression, VCAM expression, and stathmin expression ($p < 0.05$; Table II).

**Table II.** Relationships between PDL1 expression and clinicopathological

| Risk factors | PDL1 positive (n=28) | PDL1 negative (n=68) | χ2 or Z | p |
|---|---|---|---|---|
| Age | | | 1.180 | 0.277 |
| <55 | 11 | 35 | | |
| ≥55 | 17 | 33 | | |
| Gender | | | 0.101 | 0.750 |
| male | 15 | 34 | | |
| female | 13 | 34 | | |
| Infiltration depth | | | 5.782 | 0.014 |
| Mucosal layer | 10 | 33 | | |
| Muscular layer | 5 | 20 | | |
| Plasma layer | 13 | 15 | | |

**Table I** (continued)

| | | | | |
|---|---|---|---|---|
| Tumour size | | | 2.590 | 0.107 |
| ≥5 | 17 | 29 | | |
| <5 | 11 | 39 | | |
| Tumour site | | | 1.549 | 0.213 |
| Gastric | 15 | 47 | | |
| Small intestine | 8 | 14 | | |
| Others | 5 | 7 | | |
| Pathological nuclear division | | | 0.138 | 0.491 |
| ≥5 | 3 | 11 | | |
| <5 | 25 | 57 | | |
| Hazard classification | | | 5.429 | 0.066 |
| Low | 12 | 45 | | |
| Moderate | 6 | 12 | | |
| High | 10 | 11 | | |
| CD117 | | | 0.243 | 0.622 |
| negatives (-) | 5 | 9 | | |
| positive (+) | 20 | 51 | | |
| positive (++) | 3 | 8 | | |
| CD34 | | | 6.200 | 0.013 |
| negatives (-) | 9 | 6 | | |
| positive(+) | 18 | 59 | | |
| positive(++) | 1 | 3 | | |
| DOG1 | | | 0.007 | 0.934 |
| negatives(-) | 8 | 20 | | |
| positive(+) | 20 | 48 | | |
| Ki67 | | | 1.920 | 0.166 |
| ≥5 | 10 | 15 | | |
| <5 | 18 | 53 | | |
| Desmin | | | 0.017 | 0.896 |
| negatives (-) | 28 | 66 | | |
| positive (+) | 0 | 2 | | |
| S-100 | | | 0.651 | 0.420 |
| negatives (-) | 26 | 67 | | |
| positive (+) | 2 | 1 | | |
| SMA | | | 0.094 | 0.759 |
| negatives (-) | 26 | 60 | | |
| positive (+) | 2 | 6 | | |
| VCAM | | | 4.356 | 0.037 |
| negatives (-) | 13 | 47 | | |
| positive (+) | 15 | 21 | | |
| Stathmin | | | 25.324 | <0.001 |
| negatives (-) | 4 | 48 | | |
| positive (+) | 24 | 20 | | |
| Cofilin | | | 0.031 | 0.859 |
| negatives (-) | 18 | 45 | | |
| positive (+) | 10 | 23 | | |

For abbreviations see Table I.

To explore the role of PD-L1 expression in GIST, we analysed the transcriptome data of patients with GIST extracted from TCGA. The expression levels of PD-L1 were significantly higher in patients with GIST compared to those in controls (Fig. 2). In our study, after 4–41 months of follow-up, 29.17% (28/96) of the patients died. The Kaplan–Meier method and log-rank test used to analyse overall survival (OS) in 96 patients showed a relatively shorter OS in patients with PD-L1-positive tumours than in those with PD-L1-negative tumours (p=0.016) (Fig. 3). Therefore, we further analysed different NIH risk classifications in relation to OS in patients with GISTs. Overall survival was significantly shorter in patients with PD-L1-positive tumours in both the low- and medium-high NIH risk classification groups (p = 0.011 and p = 0.012, respectively) (Fig. 3). Univariate and multivariate Cox hazard regression models were used to assess the risk factors affecting OS (Table III). Univariate regression analysis showed that the depth of tumour infiltration [hazard ratio (HR)=1.570, 95% confidence interval [CI]: 1.011–2.437, p=0.044], stathmin expression (HR=3.751, 95%CI: 1.592–8.835, p=0.002), cofilin expression (HR=3.242, 95%CI: 1.516–6.933, p=0. 002), PD-L1 expression (HR=2.827, 95%CI: 1.346–5.939, p=0.006) and Hazard classification (HR=1.692, 95% CI: 1.115-2.569, p=0.013) were risk factors. Multivariate regression analysis showed that cofilin expression (HR=3.238, 95%CI: 1.350-7.765, p=0.008) was a significant factor for poor prognosis (Table III).

PD-L1 Knockdown Reduced the Proliferation of GIST Cells

We subsequently explored the effect of PD-L1 on GIST8820 cell proliferation. Knockdown of PD-L1 significantly inhibited the proliferation rate of GIST8820 cells (Fig. 4). Colony formation assay was also performed to assess the effect of PD-L1 on GIST8820 cell proliferation. Knockdown of PD-L1 using siRNA showed that GIST8820 cells showed a significant reduction in colony formation numbers upon knockdown of PD-L1 (Fig. 5). These results showed that siRNA-mediated knockdown of PD-L1 in GIST8820 cells significantly decreased the rate of cell colony formation compared with control cells (p<0.0001).

**PD-L1Knockdown Reduced the Migration, Invasion, and Wound Healing Abilities of GIST Cells**

To investigate the effect of PD-L1 on the invasion and migration of GIST8820 cells after knockdown of PD-L1, we performed transwell chamber and wound healing assays. The average numbers of invasive cells in PD-L1 knockdown and control groups were 356 and 457, respectively, and the average numbers of migrating cells were 329 and 534, respectively (p<0.0001) (Fig. 6). Cell migration and invasion were inhibited in the PD-L1 knockdown group compared with those in the control group. The inhibition rates in the PD-L1 knockdown group were 38.39% and 22.1%, respectively. In wound healing experiments, the mean scratch areas of PD-L1 knockdown and control groups were 906618 $\mu m^2$ and 927957 $\mu m^2$ at 0–12 h, 657077 $\mu m^2$ and 351945 $\mu m^2$ at 12–24 h, and 401616 $\mu m^2$ and 157770 $\mu m^2$ at 0–24 h, respectively (p<0.01) (Fig. 7). The wound-healing ability of the PD-L1 knockdown group was much weaker than that of the control group, with a 27.29% decrease in scratch-healing ability, indicating a significant migratory ability of the cells.

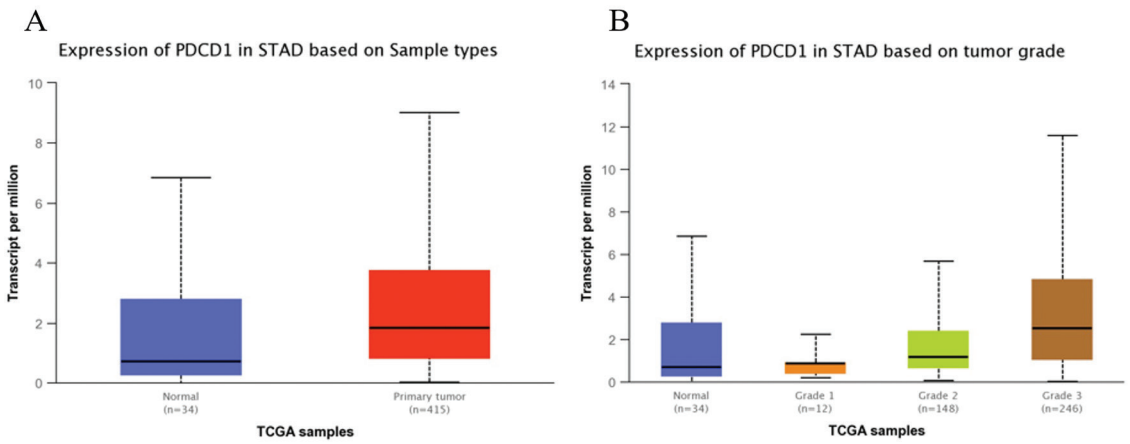


**Fig. 2.** A) PD-L1 was overexpressed in GIST tissue. B) Analysis of PD-L1 levels in GIST tissues compared to those in normal samples and at different disease stages, using TCGA databases.

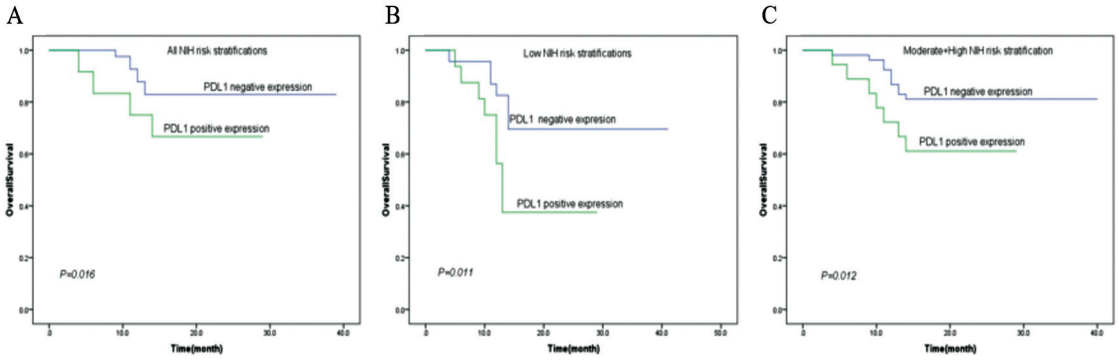


**Fig. 3.** A-C) Kaplan–Meier curve analysis for the overall survival (OS) of all NIH risk stratifications (A), low NIH risk stratifications (B) and moderate+High NIH risk stratification (C) patients according to PD-L1 expression.

**Table III.** Univariate and multivariate Cox regression analyses of risk factors for GIST

| Variable | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | HR(95%CI) | p | HR(95%CI) | p |
| Age(≥55y vs.<55y ) | 1.679(0.775-3.640) | 0.18 | | |
| Gender | 1.559(0.730-3.329) | 0.251 | | |
| Infiltration depth(Mus vs.Plasma) | 1.570(1.011-2.437) | 0.044 | 1.051(0.597-1.852) | 0.863 |
| Tumour site(Gastrointestinal vs. Others) | 1.795(0.682-4.722) | 0.236 | | |
| Tumour size(< 5cm vs. ≥ 5cm) | 2.093(0.966-4.536) | 0.061 | | |
| Pathological nuclear division(≥5 vs.<5 ) | 1.640(0.665-4.046) | 0.283 | | |
| Hazard classification(Low vs.Moderate+High) | 1.692(1.115-2.569) | 0.013 | 1.112(0.641-1.931) | 0.705 |
| CD117(positive vs. negative) | 1.529(0.754-3.101) | 0.24 | | |
| CD34(positive vs. negative) | 0.489(0.215-1.111) | 0.087 | | |
| DOG1 (positive vs. negative) | 0.531(0.249-1.134) | 0.102 | | |
| Ki67(≥5 %vs.<5% ) | 1.359(0.614-3.006) | 0.449 | | |
| Desmin (positive vs. negative) | 0.048(0.00-1806.428) | 0.572 | | |
| S-100(positive vs. negative) | 0.048(0.00-1806.429) | 0.572 | | |
| SMA(positive vs. negative) | 0.323(0.044-2.378) | 0.267 | | |
| VCAM(positive vs. negative) | 1.862(0.886-3.914) | 0.101 | | |
| Stathmin(positive vs. negative) | 3.751(1.592-8.835) | 0.002 | 2.154(0.795-5.837) | 0.131 |
| Cofilin(positive vs. negative) | 3.242(1.516-6.933) | 0.002 | 3.238(1.350-7.765) | 0.008 |
| PDL1(positive vs. negative) | 2.827(1.346-5.939) | 0.006 | 2.249(0.834-6.070) | 0.109 |

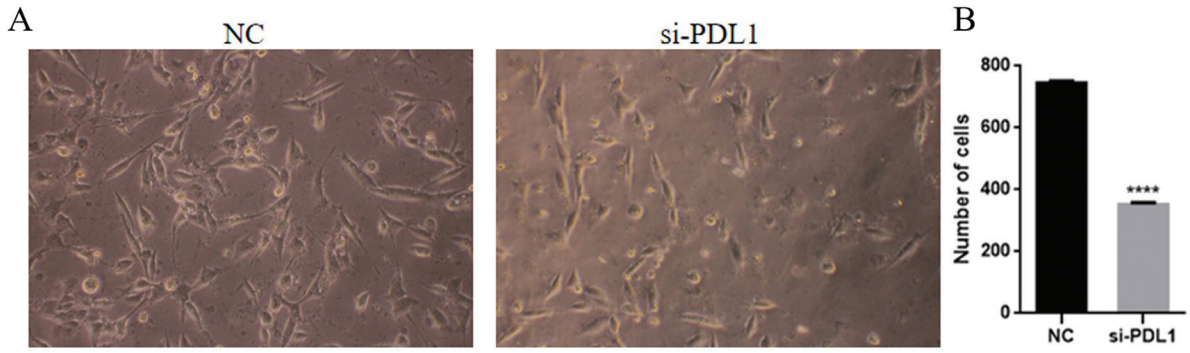


**Fig. 4**. A, B) Effect comparison of the PD-L1 knockdown on tumour cell proliferation. ****: p<0.0001.

## DISCUSSION

In this study, we investigated the role of PD-L1 in patients with GISTs by using 96 clinical patient samples and clinical information. We found that there was a significant difference in PD-L1 expression between GIST and adjacent normal tissues. PD-L1 expression was significantly correlated with the depth of invasion and CD34, VCAM and stathmin expression in tumor tissues. Patients with PD-L1-positive tumors had shorter OS than controls.

Cox analysis showed that depth of invasion, stathmin, cofilin, and PD-L1 positive expression were risk factors for a poor prognosis. In cell assays, knockdown of PD-L1 significantly inhibited the proliferation rate of GISTs cells, and significantly inhibited cell migration and invasion, and wound healing was significantly weaker in the PD-L1 knockdown group than in the control group.

Immunotherapy targeting immune checkpoints has emerged as a promising strategy for cancer treatment. With the discovery of the PD-1/PD-L1 receptor and its interaction

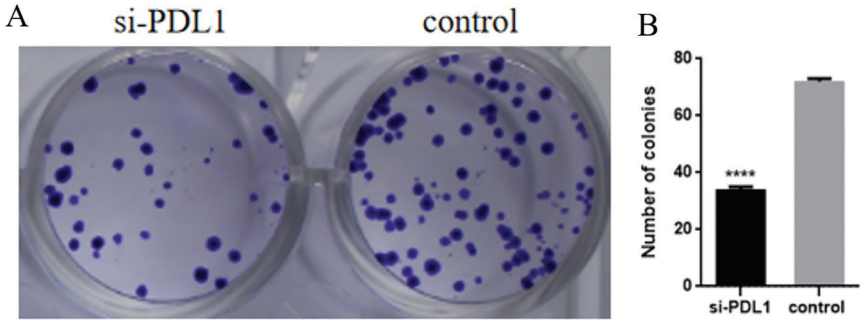


**Fig. 5.** A, B) Effect of PD-L1 on the clonal formation of GIST cells. ****: P < 0.0001.

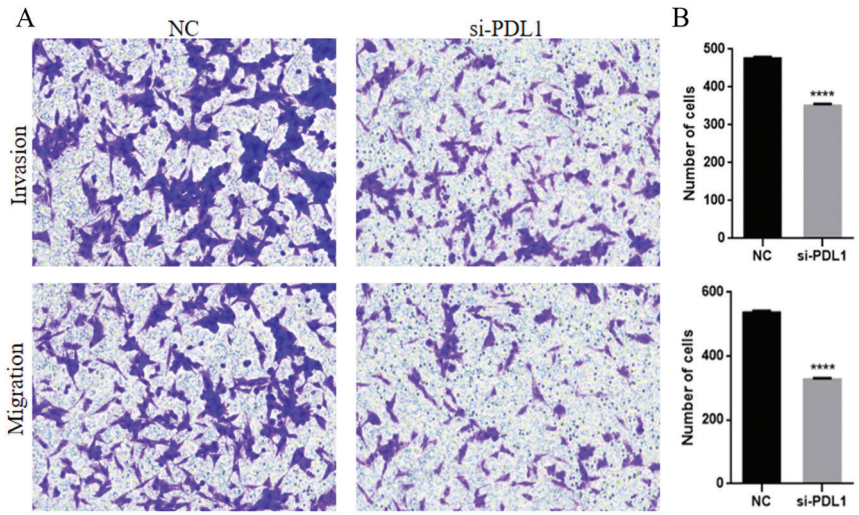


**Fig. 6.** A, B) Effect of the PD-L1 on cell invasion and migration of GIST cells. ****: p<0.0001.

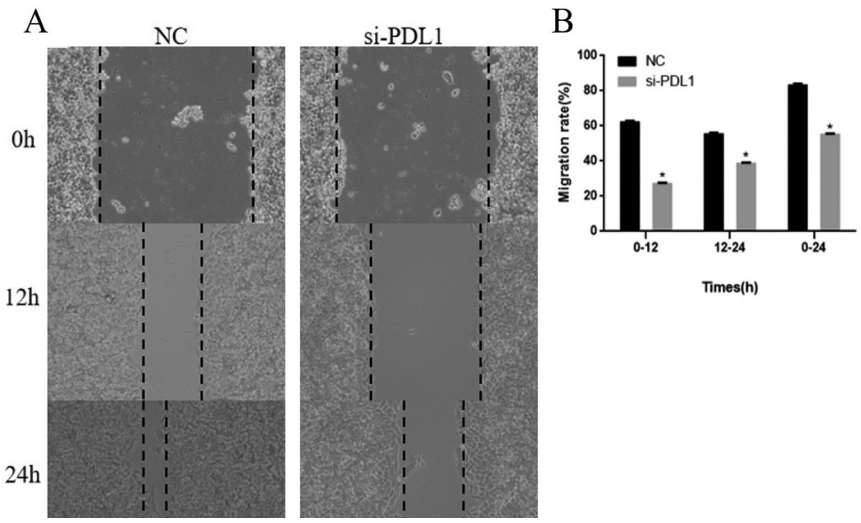


**Fig. 7.** A, B) Effect of PD-L1 knockdown on the wound healing of GIST cells. *: p<0.01.

between tumor cells and the immune system, inhibition of the interaction between PD-1 and PD-L1 has been shown to enhance T-cell responses and mediate antitumor activity. Several clinical trials have demonstrated the antitumor efficacy of blocking the PD-1/PD-L1 pathway in patients with solid tumors and hematological malignancies [14, 15]. Several studies have shown that PD-L1 is hardly expressed in normal tissues of the gastrointestinal tract, but highly expressed in tumor cells. Elfishawy et al. [16] analyzed 60 patients with colon cancer and found that PD-L1 was expressed in 25% of tumor cells and 38.3% of stromal lymphocytes, which was significantly higher than that in adjacent normal tissues. In this study, we found that the expression rate of PD-L1 in 96 GIST patients was 29.2%, which was consistent with the previously reported PD-L1 expression rate in colon cancer. The results of this study showed that PD-L1 expression was positive in 10 patients with tumor infiltration into the mucosa, 5 patients in the muscular layer, and 13 patients in the plasma layer, and the difference was statistically significant. In addition, depth of invasion was associated with PD-L1 expression and risk factors for poor prognosis in GIST patients. Although GISTs are common benign tumors of the gastrointestinal tract, metastases to other organs of the abdominopelvic cavity, such as the ovaries [17] and liver [18], have been reported in the literature. The depth of invasion may be associated with GIST metastasis, but the mechanism remains unclear and needs to be further explored in prospective studies with larger sample sizes.

An increasing number of studies have shown that PD-L1 can be used as a predictor of tumor prognosis, but the role of PD-L1 in different tumor prognoses is controversial. Songül et al. [19] showed that high PD-L1 expression was associated with poor prognosis in non-small cell lung cancer. Boger et al. [20] showed that gastric cancer patients with high PD-L1 expression had a better prognosis, and PD-L1 may be an independent prognostic factor for survival. The prognosis of GIST patients after surgery varies widely because GIST is a clinically and morphologically heterogeneous tumor with very different biological behaviors. The risk level of GISTs is an important clinical guide to assess the risk of recurrence and metastasis and predict the prognosis of patients [21]. In a study including 539 GIST patients, risk classification of GIST patients was found to be an independent factor for prognosis, with mean OS times of 57.0, 56.9, and 53.6 months for low-risk, intermediate-risk, and high-risk patients, respectively [22]. The grading method used in this study is the AFIP grading criteria, where the risk stratification of GIST patients is mainly based on factors such as tumor size, mitotic count, and tumor location. In contrast, our study found that positive PD-L1 expression was associated with a GIST risk grade, suggesting that the higher positive PD-L1 expression, the higher GIST risk grade, and the worse the prognosis of GIST patients.

In this study, we found no significant correlation between the degree of PD-L1 expression and the expression of CD117, DOG1, Ki67, desmin, S-100, SMA, and cofilin in GIST patients. However, it was positively correlated with CD34, VCAM-1, and stathmin expression. The positive expression of CD34 was 84.3%, and the positive expression of PD-L1 correlated with the positive expression of CD34, a transmembrane phosphoglycoprotein that is considered to be the most effective immunomarker until antibodies to CD117 were found. The overall positive expression of CD34 in some CD117-negative GIST tissues can reach 70% [23], and the positive expression of VCAM-1 is 37.5%, which is correlated with the positive expression of PD-L1.VCAM-1, a member of the immunoglobulin superfamily, is a class of adhesion molecules formed by glycoproteins after shedding from the cell surface in a lytic state and is involved in a series of pathophysiological processes, including cell recognition, activation, signal transduction, coagulation, immune response and wound healing [24]. Moreover, Stathmin positive expression was 45.8%, which was associated with PD-L1 positive expression. Stathmin is a small cytosolic phosphoprotein that belongs to the highly conserved Stathmin-like microtubule-binding protein family and plays an important role in regulating microtubule dynamics, cell cycle progression, proliferation, and differentiation [25]. The degree of PD-L1 expression was closely related to the positive rates of CD34, VCAM-1, and stathmin, suggesting that PD-L1 may be an important immune marker for auxiliary diagnosis of GIST and evaluation of tumor cell progression. Using

Cox regression models, we found that the depth of tumor invasion, risk score classification, and positive expression of stathmin, cofilin, and PD-L1 were associated with a shorter patient survival and were risk factors for prognosis. Among them, cofilin is an independent risk factor for the prognosis of GIST patients. The above findings suggest that PD-L1 expression is closely related to the depth of invasion and its risk grade of GISTs, suggesting that PD-L1 may be involved in the occurrence, development, and metastasis of GISTs and is expected to be a predictor of GIST occurrence, development, and metastasis, assessing its prognosis and may guide the selection and efficacy of GIST treatment options.

The strength of this study lies in analyzing samples from 96 GISTs patients to investigate the expression of PD-L1 in GIST and its association with patient prognosis. The results indicate that a high expression of PD-L1 is significantly correlated with a poor prognosis in GIST patients, and cell experiments have shown that knockdown of PD-L1 can inhibit the proliferation, migration, and invasion of GIST cells. These findings provide evidence for PD-L1 as a prognostic marker and potential therapeutic target for GIST. However, there are some limitations in the study, such as regional and population bias in sample sources, short follow-up time, and insufficient in-depth research on the mechanisms of PD-L1 action. Future studies need to expand the sample sources, extend the follow-up time, and further explore the specific mechanisms of PD-L1 in GISTs, in order to provide more comprehensive and accurate guidance for the diagnosis and treatment of GISTs.

## CONCLUSIONS

In summary, PD-L1 plays a crucial role in the mechanism of tumour immune escape, and the mechanism of GIST development is complex. In this study, we detected the PD-L1 expression in GIST tissues and analyzed the relationship between clinicopathological factors to provide a basis for the diagnosis and individualized treatment of GIST patients.

**Conflicts of interest:** None to declare.

**Authors' contributions** D.Y. conceived the study and designed the methodology. All the authors collected and analysed the data, and participated in drafting and revising the manuscript. All authors read and approved the final manuscript.

**Acknowledgments:** The study was funded by the Natural Science Foundation of Xinjiang Uygur Autonomous Region (No: 2018D01C115).

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