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Title: Clinical significance of programmed cell death-ligand expression in small bowel adenocarcinoma is determined by the tumor microenvironment

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Reviewer #1:
Specific Comments to Authors: This study systematically investigated the expression of programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2) in SBA, aiming to clarify the clinical significance of their expression and its relationship with infiltrating lymphocytes in tumor environment. Despite immunoediting, the beneficial effect of the adaptive immunity may persist throughout tumor progression. So, the original intention of this study has important clinical implications and helps to guide and improve the prognosis of SBA patients. However, there are still some questions that need to be addressed： 1. This study found that patients with PD-L2 CPS≥10 had a worse prognosis in the low FoxP3/ cd8 group (P = 0.088), but the difference was not significant. It is generally believed that a high ratio of FoxP3+ to CD8+ T cells is associated with poor clinical outcomes in digestive system cancers[29-31]. In addition to the number and types of lymphocytes infiltrated in the tumor microenvironment, there is still a very broad space for research on predictors of immunotherapy efficacy, such as tumor mutation burden and peripheral blood circulation markers, which are expected to screen more suitable patients to receive immunotherapy. The study did not examine tumor mutational burden. How was it considered?

RESPONSE TO REVIEWER #1:

In this study, we did not consider the use of TMB. High tumor mutation burden (TMB-high) has been proposed as a predictive biomarker for response to immune checkpoint inhibitors (ICIs) based on the assumption that increasing the number of mutant proteins will create antigenic peptides, allowing for enhanced immunogenicity. We have added a new sentence and references. Page 16, lines 1–16:

Recent studies have demonstrated that the tumor mutation burden (TMB) could be a biomarker in patients with cancer treated with immune checkpoint inhibitors (ICIs), in which high TMB is significantly correlated with better survival[1]. Although the efficacy of ICIs in patients with cancer with TMB-high or MSI-high/dMMR has been confirmed, patients with MSI-high have a favorable prognosis, and generally, only a small number of patients require chemotherapy. In fact, none of the patients with dMMR died in our cohort, and only two of seven patients received chemotherapy. Most patients with SBA have microsatellite stable (MSS)/pMMR or TMB-low, and it is necessary to identify useful biomarkers for ICI therapy in patients with MSS/pMMR. Previous studies have shown that TMB-high tumors are immunogenic and that TMB-high tumors are also present in SBA[2]. In small bowel cancer, TMB-high has been reported to be associated with dMMR, CD8-high, and PD-L1 expression, but it has been shown that TMB-high cases are also present in cases of pMMR [3, 4]. Our results indicate that PD-L2 positive and FoxP3/CD8-low patients with pMMR may benefit from ICI therapy. Although we did not examine the TMB in this study, it may stratify the outcomes of patients with SBA with PD-L2 positive and FoxP3/CD8-low.

1 **Li DD**, Tang YL, Wang X. Challenges and exploration for immunotherapies targeting cold colorectal cancer. *World J Gastrointest Oncol* 2023; **15**: 55-68 [PMID: 36684057 DOI: 10.4251/wjgo.v15.i1.55]
2 **Chan TA**, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, Peters S. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann Oncol* 2019; **30**: 44-56 [PMID: 30395155 DOI: 10.1093/annonc/mdy495]

3 **Wirta EV**, Szeto S, Hänninen U, Ahtiainen M, Böhm J, Mecklin JP, Aaltonen LA, Seppälä TT. Prognostic Value of Immune Environment Analysis in Small Bowel Adenocarcinomas with Verified Mutational Landscape and Predisposing Conditions. *Cancers (Basel)* 2020; **12** [PMID: 32718028 DOI: 10.3390/cancers12082018]
4 **Pedersen KS**, Foster NR, Overman MJ, Boland PM, Kim SS, Arrambide KA, Jaszewski BL, Bekaii-Saab T, Graham RP, Welch J, Wilson RH, McWilliams RR. ZEBRA: A Multicenter Phase II Study of Pembrolizumab in Patients with Advanced Small-Bowel Adenocarcinoma. *Clin Cancer Res* 2021; **27**: 3641-3648 [PMID: 33883178 DOI: 10.1158/1078-0432.CCR-21-0159]

2. Other treatment information, such as chemotherapy, may affect prognosis, which was not available in the study.

We apologize for this confusion. These characteristics have been added to Table 1 for clarity. Twenty-three patients with stages III and IV disease received chemotherapy after surgery.

3. In this study, PD-L1 and PD-L2 CPS were not correlated with prognosis, and patients with PD-L2 CPS≥10 had worse prognosis in the low FoxP3/ CD8 group (P = 0.088), but the difference was not significant. Given the study's small sample size, the basis for the conclusion is weak (Immune checkpoint inhibitors may improve the prognosis of SBA patients with low FoxP3/CD8 ratio and PD-L2 expression.).

We agree with the reviewer's comment that the basis of this conclusion is weak. Although patients with low FoxP3/CD8 group are highly immunogenic, other biomarkers can be used to measure immunogenicity, such as TMB. Therefore, we have added the following sentence on page 16, lines 13–16: Our results indicate that PD-L2 positive and FoxP3/CD8-low patients with pMMR may benefit from ICI therapy. Although we did not examine the TMB in this study, it may stratify the outcomes of patients with SBA with PD-L2 positive and FoxP3/CD8-low.

Reviewer #2:

Specific Comments to Authors: The authors of this manuscript try to investigate the clinicopathological significance of PD-L1/2 expression according to the status of TILs in the TME in small bowel adenocarcinoma. The authors performed immunohistochemical analysis for PD-L1, PD-L2, CD8, FoxP3, and DNA mismatch repair (MMR) proteins. The authors demonstrated that the status of the tumor microenvironment affects the clinical significance of PD-L1 and PD-L2. Although the preliminary hypothesis of the present paper could be of some interest, some methodological issues deserve certain attention. For example, the age and gender distribution of the cases enrolled were not described. The figures should not be put into context.

RESPONSE TO REVIEWER #2:

We apologize for this confusion. These characteristics were added to Table 1 for clarity to avoid misleading the readers.