Intratumoural PD-L1 expression is associated with worse survival of patients with Epstein–Barr virus-associated gastric cancer

An Na Seo1,6, Byung Woog Kang2,6, Oh Kyoung Kwon3, Ki Bum Park3, Seung Soo Lee3, Ho Young Chung3, Wansik Yu3, Han Ik Bae1, Seong Woo Jeon4, Hyojeung Kang5 and Jong Gwang Kim*,2

1Department of Pathology, Kyungpook National University Medical Center, Kyungpook National University School of Medicine, 807 Hogukno, Buk-Gu, Daegu 41404, South Korea; 2Department of Oncology/Hematology, Kyungpook National University Hospital, Kyungpook National University Cancer Research Institute, Kyungpook National University, 807 Hogukno, Buk-Gu, Daegu 41404, South Korea; 3Department of Surgery, Gastric Cancer Center, Kyungpook National University Medical Center, Kyungpook National University School of Medicine, 807 Hogukno, Buk-Gu, Daegu 41404, South Korea; 4Department of Gastroenterology, Kyungpook National University Medical Center, Kyungpook National University School of Medicine, 807 Hogukno, Buk-Gu, Daegu 41404, South Korea and 5College of Pharmacy, Institute of Microorganisms and Research Institute of Pharmaceutical Sciences, Kyunpook National University, 80 Daehakno, Buk-Gu, Daegu 41566, South Korea

Background: This study investigated the clinical relevance and prognostic impact of the overall expression of programmed cell death protein ligand-1 (PD-L1) and programmed cell death protein ligand-2 (PD-L2), in patients with Epstein–Barr virus-associated gastric cancer (EBVaGC).

Methods: After reviewing 1318 consecutive cases of surgically resected or endoscopic submucosal dissected gastric cancers, the expression status of PD-L1 and PD-L2 in 120 patients with EBVaGC identified by EBV-encoded RNA in situ hybridisation was retrospectively analysed using immunohistochemistry (IHC). For each IHC marker, positivity was separately in intraepithelial tumour cells (iTu-) and immune cells in the tumour stroma area (str-).

Results: Among 116 eligible patients, 57 (49.1%) and 66 patients (56.9%) were determined as iTu-PD-L1-positive and str-PD-L1-positive, respectively, whereas 23 (21.6%) and 45 patients (38.8%) were determined as iTu-PD-L2 positive and str-PD-L2 positive, respectively. Intraepithelial tumour cell PD-L1 positivity was found to be significantly associated with lymph node (LN) metastasis ($P = 0.012$) and a poor disease-free survival (DFS) ($P = 0.032$), yet not overall survival ($P = 0.482$). In a multivariate analysis, iTu-PD-L1 positivity was independently associated with a poor DFS ($P = 0.006$, hazard ratio = 12.085). In contrast, str-PD-L2-positivity was related to a lower T category ($P = 0.003$), absence of LN metastasis ($P = 0.032$) and perineural invasion ($P = 0.028$). Intraepithelial tumour cell and str-PD-L2 positivity showed a trend towards an improved DFS, although not significant ($P = 0.060$ and $P = 0.073$, respectively).

Conclusions: Intraepithelial tumour cells PD-L1 expression can be used to predict a poor outcome in patients with EBVaGC and can represent a rational approach for PD-1/PD-L pathway-targeted immunotherapy.

*Correspondence: Professor JG Kim; E-mail: jkk21c@knu.ac.kr

6These authors contributed equally to this work.

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Immunotherapy has begun to revolutionise cancer treatment, by introducing therapies such as checkpoint inhibitors that target the host immune system instead of the tumour (Chen and Mellman, 2013). For an anticancer immune response, the programmed cell death protein-1 (PD-1) pathway is considered an important inhibitory mechanism regulating T-cell exhaustion (Jin and Yoon, 2016). PD-1, which belongs to the CD28 family of proteins, is a receptor expressed on a number of immune cells, including T cells, B cells, monocytes, NK cells, and dendritic cells (Park et al, 2016). PD-1 has two ligands, the programmed cell death protein ligand-1 (PD-L1) and programmed cell death protein-ligand-2 (PD-L2). PD-L1 is expressed on T cells, B cells, dendritic cells, macrophages, mesenchymal stem cells, bone marrow-derived mast cells, and some non-haematopoietic cells, whereas PD-L2 is mainly expressed on antigen-presenting cells, including macrophages, dendritic cells, and non-haematopoietic tissues (Jin and Yoon, 2016). Several studies have already demonstrated that PD-L1 or PD-1 is highly expressed on tumour cells in gastric cancers (GCs) (Wu et al, 2006; Takaya et al, 2015; Zhang et al, 2015; Boger et al, 2016; Derks et al, 2016; Dong et al, 2016a; Eto et al, 2016; Kim et al, 2016; Takano et al, 2016; Cho et al, 2017). Interestingly, Wang et al (2016) suggested that GC patients with positive PD-L1 expression had a significantly shorter survival than PD-L1-negative patients, although the prognostic impact remained inconsistent.

The Cancer Genome Atlas data revealed that Epstein–Barr virus (EBV)-associated GC (EBVaGC) closely associated with a PIK3CA mutation, PD-L1/2 overexpression, EBV-CIMP, CDKN2A silencing, and immune cell signalling activation among GC subtypes. As previous studies have suggested that high PD-L1 expression is related to a good responsiveness to anti-PD-1/PD-L1 therapy in human cancers, the EBVaGC subtype, in particular, may be a potential candidate for anti-PD-1/PD-L1 therapy among GC subtypes (Herbst et al, 2014; Taube et al, 2014). Furthermore, accumulating evidence suggests that tumour-infiltrating T cells may also be critical for the good responsiveness to anti-PD-1/PD-L1 treatment (Teng et al, 2015). As EBVaGC is known to be associated with a prominent lymphoid infiltration of the stroma and high density of tumour-infiltrating lymphocytes (TILs), evaluating the PD-1 pathway affecting EBV infection may also be helpful in selecting appropriate targets for therapeutic interventions against EBVaGC. Moreover, this close relationship points to the possibility of PD-L1 and PD-L2 as prognostic markers in EBVaGC patients, suggesting a pivotal role of the immune mechanism in the EBVaGC subset. Several studies have reported PD-L1 expression in 34.4–92.5% of EBVaGC patients (Derks et al, 2016; Dong et al, 2016a; Kawazoe et al, 2016; Ma et al, 2016). Notably, Saito et al (2016) recently reported that PD-L1 expression in tumour cells was associated with poor overall survival (OS) and disease-specific survival in 96 patients with EBVaGC. However, unfortunately, detailed clinicopathologic characteristics of EBVaGC showing PD-L1 have not been fully elucidated and no published study has yet investigated the clinical significance and prognostic impact of PD-L2 expression in EBVaGC.

Accordingly, this study used a relatively large series of EBVaGC to determine the clinical and prognostic significance of PD-L1 and PD-L2 in both tumour cells and immune cells, and examine the association with the density of TILs.

**MATERIALS AND METHODS**

**Patients and samples.** A total of 120 EBVaGC cases were used in this study, all of which were obtained from our previous studies (Kang et al, 2016). The inclusion criteria for patient selection and results have already been reported (Kang et al, 2016). Briefly, all the patients were histologically diagnosed with gastric adenocarcinoma and underwent surgical resection or endoscopic mucosal dissection (ESD) at Kyungpook National University Medical Center (KNUMC) between January 2011 and November 2014. Their status as EBV positive was determined by EBV-encoded RNA in situ hybridisation conducted at our molecular pathology laboratory. The baseline characteristics of the 120 patients are presented in Supplementary Table 1. The clinical and pathologic information was collected based on a review of the patients’ medical records and pathologic reports, respectively. The patients were all staged according to the seventh edition of the American Joint Committee on Cancer staging Manual for Stomach cancer (Edge et al, 2010).

The Institutional Review Boards of Kyungpook National University Medical Center (IRB No.: KNUMC 2016-05-012) approved this study. The Reporting Recommendations for Tumour marker Prognostic Studies criteria were followed when reporting the results of this study (McShane et al, 2005).

**Pathologic quantification of TILs.** The evaluation of the percentage of intratumoural TILs (iTu-TILs) and stromal TILs (str-TILs) used a modified version of the TIL scoring recommendations of the International TILs Working Group 2014 on breast cancer (Dieci et al, 2014). The detailed quantification definition has been previously described (Kang et al, 2016). Specifically, the iTu-TILs were defined as the percentage of mononuclear inflammatory cells within an intraepithelial tumour cell nest and in direct contact with tumour cells. Meanwhile, str-TILs were defined as the percentage of tumour stroma of invasive carcinoma to be occupied by mononuclear inflammatory cells. The percentage of TILs was evaluated in 10% increments; if the percentage of iTu-TILs and str-TILs was ≤10%, 1%, or 5% criteria were used on full sections of haematoxylin and eosin-stained slides, and evaluated by two pathologists (ANS and HIB). The quantification of iTu-TILs and str-TILs was identified based on our previously published data (Kang et al, 2016).

**Immunohistochemistry and interpretation.** All the tissue slides were stained using an automatic immunohistochemistry (IHC) staining instrument (BenchMarkXT, Ventana Medical Systems, Tucson, AZ, USA) according to the manufacturer’s instructions. The following primary antibodies were used: PD-L1 (E1L3N; 1:100; Cell Signaling Technology, Danvers, MA, USA) and PD-L2 (clone 176611; 1:150; R&D Systems, Minneapolis, MN, USA). In brief, a representative formalin-fixed, paraffin-embedded tissue sample for each case was cut into 4 μm-thick sections, transferred to poly-L-lysine-coated adhesive slides, and dried in a microwave at 60 °C for 1 h. Therefore, the sections were deparaffinised using an EZ Prep solution (Ventana Medical Systems). The cell-conditioning solution 1 (CC1) standard (pH 8.4 buffer containing Tris/borate/EDTA) was then used for antigen retrieval, followed by blocking with inhibitor D (3% H2O2) at 37 °C for 4 min. Next, the slides were incubated with the primary antibodies at 37 °C for 60 min. The staining was optimised using a positive and negative control for each marker according to the recommendation of the supplier’s data sheet. All the sections were visualised with an UltraView Universal DAB detection kit (Ventana Medical Systems).

The expression of PD-L1 and PD-L2 was evaluated by two experienced gastrointestinal pathologists (ANS and HIB) with no prior knowledge of the clinical data. The stained slides were semi-quantitatively scored for the intensity and percentage of membranous and/or cytoplasmic staining of the tumour cells and tumour stoma area (immune cells), respectively. The interpretation of each marker was based on the following criteria: 0, no expression or expression in <1%; 1+, weak/faint expression in ≥1% to 1%; 2+, moderate expression in ≥1% to 10%; 3+, strong expression ≥1% of iTu- or immune cells in tumour str-. The cases were then classified
into dichotomous covariates: negative (0 and 1 +) and positive (2 + and 3 +).

Statistical analyses. The descriptive statistics are reported as proportions and medians. The categorical variables were evaluated using a χ²-test, Fisher’s exact test, or Pearson’s correlation (R) test, as appropriate. Disease-free survival was measured from the time of surgery or ESD to the time of tumour recurrence or death from any cause. Overall survival was calculated from the date of surgery or ESD to death from any cause. Data were censored if patients were free of recurrence or alive at the last follow-up. The patient subgroups were compared with respect to disease-free survival (DFS) and OS with the use of Kaplan–Meier curves, log-rank test, and multivariate survival analysis based on the Cox proportional hazard regression model. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated for each factor. All the tests were two-sided and statistical significance was set at P < 0.05. All the statistical analyses were performed using SPSS for Windows (version 19.0, SPSS Inc., Chicago, IL, USA).

RESULTS

PD-L1 and PD-L2 expression. PD-L1 and PD-L2 IHC results were available for 116 of the 120 cases (4 cases were excluded due to loss of tissue or poor IHC staining). Immunohistochemical expression of PD-L1 and PD-L2 was observed in tumour cells (iTu-) and/or the immune cells in tumour stromal area (str-) in EBVaGC tissues (Figure 1). Intraepithelial tumour cell PD-L1 positivity was detected in 57 of the 116 EBVaGC cases (49.1%) (2 + in 39 and 3 + in 18 cases), whereas str-PD-L1 positivity was observed in 66 cases (56.9%) (2 + in 65 cases and 3 + in 1 case). In addition, EBVaGC exhibiting both iTu-PD-L1 and str-PD-L1 was observed in 26 cases (22.4%). Meanwhile, iTu-PD-L2 positivity was detected in 25 cases (21.6%) (2 + in all cases), whereas str-PD-L2 positivity was observed in 45 cases (38.8%) (2 + in all cases). In addition, EBVaGC exhibiting both iTu-PD-L2 and str-PD-L2 was observed in 14 cases (12.1%).

Association with clinicopathologic features and TILs. As described in Table 1, iTu-PD-L1 positivity was significantly associated with the presence of lymph node (LN) metastasis (P = 0.012), whereas str-PD-L1 positivity was related to categories associated with dense TILs including str-TIL positivity (P = 0.003), iTu-TIL positivity (P = 0.004), and gastric carcinoma with lymphoid stroma (GCLS) (P = 0.024). In contrast, str-PD-L2 positivity were correlated with a lower pT category (P = 0.003) and the absence of LN metastasis (P = 0.0032) and perineural invasion (P = 0.028), str-TIL positivity (P < 0.001), iTu-TIL positivity (P < 0.001), GCLS (P < 0.001), and a non-conventional adenocarcinoma subclassification (P < 0.001). However, iTu-PD-L2 positivity showed no association with any clinicopathologic characteristics.

To determine the relationship between PD-L1 and PD-L2 expression in EBVaGC, we analysed the correlation between the levels of PD-L1 and PD-L2 expression (Table 2 and Supplementary Figure 1). The iTu expression of PD-L1 and PD-L2 was correlated with the str expression of PD-L1 and PD-L2 with each other, respectively (iTu-PD-L1 positivity and str-PD-L1: R = −0.224, P = 0.016; iTu-PD-L1 positivity and str-PD-L1: R = 0.185, P = 0.047).

Patient outcomes. At the time of the last follow-up (April 2016), the median follow-up duration for the survival analyses was 36.2 months (range 6.4–66.6). The probability of OS and DFS at 3 years was 92.2% and 88.5%, respectively. During the analyses, 13 (10.8%) patients experienced recurrence and 13 (10.8%) died. In the univariate analysis, iTu-PD-L1 positivity was significantly associated with a worse DFS (P = 0.032) (Figure 2A), yet not with OS (P = 0.482). In the multivariate analysis using the Cox proportional hazard model adjusted for conventional clinical covariates, iTu-PD-L1 positivity was an independent worse prognostic factor for DFS (HR = 12.085, 95% CI = 2.013–72.559, P = 0.006) (Table 3). Meanwhile, both iTu-PD-L2 positivity and str-PD-L2 positivity tended to show a favourable DFS (iTu-PD-L2: P = 0.060; str-PDL-2: P = 0.073), yet without statistical significance in the univariate analysis (Figure 2B and C).

Figure 1. Representative features of immunohistochemical expression of PD-L1 and PD-L2 in EBV-associated gastric cancers. (A) Representative photographs of intraepithelial tumour cells (iTu-) or immune cells in tumour stroma area (str-) PD-L1 negativity; (B) iTu-PD-L1 positivity; (C) str-PD-L1 positivity. (D) Representative picture of iTu- or str-PD-L2 negativity; (E) iTu-PD-L2 positivity; (F) str-PD-L2 positivity.
As str-TILs were also associated with DFS, the patients were further categorised into three groups (iTu-PD-L1 negativity and str-TIL positivity, iTu-PD-L1 positivity and str-TIL negativity, and others). In univariate analysis, the patient groups with iTu-PD-L1 positivity and str-TIL negativity, further categorised into three groups (iTu-PD-L1 negativity and str-TIL positivity, and iTu-PD-L1 positivity and str-TIL negativity, and others). In univariate analysis, the patient groups with iTu-PD-L1 positivity and str-TIL negativity, further categorised into three groups (iTu-PD-L1 negativity and str-TIL positivity, and iTu-PD-L1 positivity and str-TIL negativity, and others).

DISCUSSION

Accumulating data have revealed that PD-L1 expression can be a potential prognostic and therapeutic predictor for anti-PD-1/PD-L1 immunotherapy, therefore, understand the implications of PD-L1 expression in patients with EBVaGC is of critical importance (Jin and Yoon, 2016). However, as the detailed implications of PD-L1 and PD-L2 expression, two main ligands of PD-1, remain unclear in patients with EBVaGC, the present study investigated the clinicopathologic significance and prognostic value. As a result, the following key points were identified: iTu-PD-L1 positivity was significantly correlated with LN metastases and a poor prognosis, whereas str-PD-L2 positivity was inversely related to tumour invasion and LN metastasis. Notably, iTu-PD-L1 positivity was also identified as an independent prognostic factor of worse DFS. Furthermore, when combining iTu-PD-L1 and str-TILs, the patient with iTu-PD-L1 positivity and str-TIL negativity was associated with worse DFS when compared with the other patients with EBVaGC. Therefore, these findings support the concept that increased PD-L1 expression in tumours and intense lymphocytic infiltration around tumours can act as an immune modulator, promoting escape from immune surveillance in patients with EBVaGC.

The PD-1 pathway is considered a critical inhibitory mechanism regulating T-cell exhaustion (Jin and Yoon, 2016). In tumour beds, T cells must pass through numerous barriers with immune regulators (Palucka and Coussens, 2016). The PD-1 pathway is involved in suppressing autoimmunity during T-cell activation, promoting escape from immune surveillance in patients with EBVaGC.
allowing for immune tolerance of PD-L1- or PD-L2-expressed cells, with a blockade leading to T-cell rescue in subsequent response activation (Pardoll, 2012). Interestingly, PD-L1 mRNA and protein can be upregulated by cytokines produced by infiltrating immune cells (Dong et al., 2016b). Plus, oncogenic signalling pathways in tumour cells can activate PD-L1 expression (Dong et al., 2016b). Fang et al. (2014) already reported that PD-L1 expression could be increased by exogenous and endogenous induction of Latent membrane protein 1 (LMP1)-induced PD-L1 and was suppressed by knocking down LMP1 in EBV-positive cell lines. Therefore, these mechanisms influence the regulation of virus immunity, as well as the cancer immune reaction in EBVaGC. To date, several studies have already shown that PD-L1 expression is associated with a worse prognosis in GC. According to a recent meta-analysis by Zhang et al. (2016) of 1901 GC patients based on 10 studies, PD-L1 expression was identified as a valuable predictor of poor OS with a final HR for OS of 1.64. Recently, low PD-L1 expression and high CD8+ TILs were associated with better OS and DFS in 392 patients with surgically resected GC. Although this study included only 25 patients with EBVaGC, group with increased PD-L1 expression and high CD8+ TILs was closely correlated with EBV infection (Koh et al., 2017). However, there is still insufficient evaluation of PD-L1 expression in patients with EBVaGC. Furthermore, to the best of our knowledge, there is no previously reported evaluation of PD-L2 in EBVaGC. So far, the association between PD-L1 expression and prognosis has been inconsistent across various studies (Table 4). These conflicting results could be due to different patient ethnicities and different antibody clones and interpretation. Indeed, previous studies have only focused on the frequency of PD-L1 expression and not the impact on the survival outcomes of EBVaGC. In consistent with the current study, Saito et al. (2016) reported that the PD-L1-positive EBVaGC patients exhibited a deeper invasion of the tumour and poorer prognosis than the PD-L1-negative patient. These authors also demonstrated that PD-L1-amplified cells corresponded to PD-L1-positive cells, showing high-intensity immunohistochemical cells. In contrast, another recent study found no association between PD-L1 expression and patient outcomes (Dong et al., 2016a). Consequently, the present findings need to be validated through further studies, in order to clarify the

| iTu-PD-L1 | Str-PD-L1 | iTu-PD-L2 | Str-PD-L2 |
|----------|----------|----------|----------|
| R (P-value) | R (P-value) | R (P-value) | R (P-value) |
| iTu-PD-L1 | — | 0.224 (0.016) | 0.114 (0.224) | 0.146 (0.119) |
| Str-PD-L1 | — | — | 0.094 (0.315) | 0.086 (0.361) |
| iTu-PD-L2 | 0.114 (0.224) | — | — | 0.185 (0.047) |
| Str-PD-L2 | 0.146 (0.119) | 0.086 (0.361) | 0.185 (0.047) | — |

Abbreviations: iTu = intratumoural; PD-L1 = programmed cell death protein ligand-1; PD-L2 = programmed cell death protein ligand-2; R = Pearson’s correlation; str = stromal.
TILs, yet not iTu-PD-L2 expression. Ohigashi et al (2016) positively correlated with dense iTu- and/or str-
et al (2016). Interestingly, when combining the iTu-PD-L1 expression in EBVaGC (Kawazoe et al, 2016). Several studies have already demonstrated a correlation between TILs and PD-L1 expression in EBVaGC (Kawazoe et al, 2016; Ma et al, 2016; Saito et al, 2016). Interestingly, when combining the iTu-PD-L1 expression and TIL score, the patient group with iTu-PD-L1 negativity and str-TIL positivity was associated with a better DFS and OS, although not significant in the multivariate analysis. This finding supports that TILs exhibit a host cellular immune response against tumours, indicating that immunotherapy may have a potential role in patients with EBVaGC (Kang et al, 2015). Several studies have shown a stimulatory role for PD-L2, whereas others suggest that PD-L2 expression inversely correlated with a favourable DFS and OS, although not significant in the multivariate analysis. This finding supports previous report, where iTu-PD-L2 expression was induced in the absence of PD-L1 co-expression tumours, in which case PD-L2 expression was less likely to possess PD-L1+, PD-L2+ expression in EBVaGC (Kang et al, 2016). This finding supports that TILs exhibit a host cellular immune response against tumours, indicating that immunotherapy may have a potential role in patients with EBVaGC. TILs could be used to identify a subgroup of patients with excellent outcomes (Chang et al, 2014) and our previous study also showed an independent association between a high density of TILs and str-TIL positivity was associated with a better DFS than the other EBVaGC patients. This result creates a very meaningful subgroup that can provide more information for tailored therapy for each group of patients. In particular, this finding can provide a novel strategy for subgroups with decreased immunoediting via the PD-1 pathway and a strengthened immune response by TILs, and vice versa, considering that the state of the association between PD-L1 expression and the prognosis of EBVaGC.

Table 3. Multivariate analysis for disease-free survival

| Variables          | Category               | P       | HR     | 95% CI     |
|--------------------|------------------------|---------|--------|------------|
| Age, years         | < 62 vs ≥ 62           | 0.584   | 0.613  | 0.106–3.534|
| Gender             | Female vs male         | 0.250   | 3.879  | 0.385–39.115|
| pTNM stage         | I vs II and III        | 0.014   | 13.863 | 1.688–113.847|
| WHO classification  | GCCL vs non-GCCL       | 0.695   | 0.551  | 0.028–10.859|
| Lymphatic invasion | Negative vs positive   | 0.026   | 0.139  | 0.024–0.788|
| Venous invasion    | Negative vs positive   | 0.027   | 0.055  | 0.004–0.715|
| Perineural invasion| Negative vs positive   | 0.096   | 4.702  | 0.762–29.034|
| Str-TILs           | ≥ 25 vs < 25           | 0.647   | 1.982  | 0.106–36.975|
| iTu-PD-L1          | Negative vs positive   | 0.006   | 12.085 | 2.013–72.559|

Abbreviations: CI = confidence interval; GCCL = gastric carcinoma with lymphoid stroma; R = hazard ratio; iTu = intratumoural; PD-L1 = programmed cell death protein ligand-1; str = stromal; TILs = tumour-infiltrating lymphocytes; WHO = World Health Organization.

Table 4. Frequency and prognostic value of PD-L1 expression in EBVaGC

| References       | Tumour type | Sample size | Country | PD-L1 expression | Criteria (cut-off) | Antibody | Prognostic role |
|------------------|-------------|-------------|---------|------------------|-------------------|----------|-----------------|
| Saito et al (2016)| EBVaGC      | 96          | Japan   | 34.4%*           | > 5%              | Clone E1L3N, Cell Signaling | OS and DSS* |
| Derks et al (2016)| EBVaGC      | 32          | USA     | 50%*             | > 5%              | Clone 405.9A11 | Not reported* |
| Dong et al (2016a) | EBVaGC      | 59          | China   | 92.5%*           | > 9.0*            | Ab58810, Abcam | No significant |
| Kawazoe et al (2016)| EBVaGC     | 25          | Japan   | 52.0%*           | ≥ 1%              | SP142, Ventana   | Not reported |
| Ma et al (2016)   | EBVaGC      | 7           | USA     | 71.4%*           | ≥ 5%              | SP263, Ventana   | Not reported |
| Current study     | EBVaGC      | 116         | South Korea | 47.5%*          | ≥ 1%              | Clone E1L3N, Cell Signaling | DFS |

Abbreviations: DFS = disease-free survival; DSS = disease-specific survival; D-L1 = programmed cell death protein ligand-1; EBVaGC = Epstein–Barr virus-associated gastric cancer; IFN-γ = interferon-γ; IHC = immunohistochemistry; OS = overall survival.

*PD-L1 expression evaluated in tumour epithelial cells.
**Univariate analysis.
***The study demonstrated that EBVaGC had high IFN-γ response gene expression.
****IHC staining was evaluated using the immunoreactive score (0–12) obtained by multiplying the staining intensity and percentage of positive tumour cells.
immune equilibrium is a critical factor for therapeutic success (Chen and Mellman, 2013; Palucka and Coussens, 2016). In fact, alterations of TILs have already been implicated in immune homeostasis, as well as immune response in the PD-1 pathway (Menon et al., 2016).

Although the present data identified a significant prognostic role of PD-L1 expression in operable EBVaGC, these results should be cautiously interpreted due to potential limitations. The current study is a retrospective evaluation, and the evaluation and interpretation of PD-L1 and PD-L2 within tumours and/or a tumour microenvironment have not yet been standardised in GCs (Manson et al., 2016). Moreover, a standardised cutoff value for PD-L1- and PD-L2 positivity has not yet been clearly established (Festino et al., 2016), plus PD-L1 and PD-L2 expression cannot be used to predict treatment response to anti-PD-1/PD-L1 therapy in patients with EBVaGC. Nevertheless, the present study has several important differences with previous studies as follows: (1) a relatively large-scale cohort of EBVaGC patients with a Korean homogeneous ethnic identity (Jin and Yoon, 2016), (2) equivalent treatment application, (3) minimal loss to follow-up, (4) assessment of TILs, and (5) comprehensive interpretation of expression in cancer cells and immune cells as separate microenvironments of cancer cells.

CONCLUSION

In conclusion, str-PD-L1- and str-PD-L2 positivity was shown to be associated with dense iTu- and str-TILs in patients with EBVaGC. The current study also revealed significant prognostic impact of iTu-PD-L1 positivity by demonstrating an independent association with a poor DFS. Therefore, these findings support the concept that PD-L1 can be a prognostic indicator for predicting patient outcomes and a rationale for therapeutic targeting in EBVaGC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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