



# Clinicopathological features of 22C3 PD-L1 expression with mismatch repair, Epstein–Barr virus status, and cancer genome alterations in metastatic gastric cancer

Akihito Kawazoe<sup>1</sup> · Kohei Shitara<sup>1</sup> · Yasutoshi Kuboki<sup>1</sup> · Hideaki Bando<sup>1</sup> · Takashi Kojima<sup>1</sup> · Takayuki Yoshino<sup>1</sup> · Atsushi Ohtsu<sup>1</sup> · Atsushi Ochiai<sup>2</sup> · Yosuke Togashi<sup>3</sup> · Hiroyoshi Nishikawa<sup>3</sup> · Toshihiko Doi<sup>1</sup> · Takeshi Kuwata<sup>2</sup>

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## Abstract

**Background** Recently, the U.S. Food and Drug Administration approved pembrolizumab for patients (pts) with PD-L1-positive metastatic gastric cancer (MGC) based on 22C3 immunohistochemistry (IHC) assay. However, little is known about detailed clinicopathological features of 22C3 PD-L1 expression in MGC.

**Patients and methods** Pts with histologically confirmed MGC were eligible for this prospective observational study. PD-L1 expression (22C3) on tumor cell (TC) or immune cell (IC) and mismatch repair (MMR) were analyzed by IHC. Epstein–Barr virus (EBV) was detected by in situ hybridization. The expressions of tyrosine kinase receptors (RTKs) and cancer genome alterations were evaluated by IHC or next-generation sequencing.

**Results** A total of 225 pts were analyzed in this study. PD-L1 expression on TC, PD-L1 on IC, MMR-deficient (D-MMR), and EBV positivity were identified in 8.4, 65.3, 6.2, and 6.2% cases, respectively. PD-L1 expression in TC was more frequently observed in pts with D-MMR ( $P < 0.001$ ), *PIK3CA* mutation ( $P = 0.020$ ), and *KRAS* mutation ( $P = 0.002$ ), and PD-L1 on IC was associated with EBV positivity ( $P = 0.034$ ), and lymph-node metastasis ( $P < 0.001$ ). PD-L1 expression on either IC or TC was less frequently observed in pts with peritoneal metastasis and Borrmann Type 4. A significant association was not observed between PD-L1 expression and RTKs expression or presence of other gene alterations. PD-L1 expression on either TC or IC was not prognostic factor.

**Conclusions** 22C3 PD-L1 expression in MGC was associated with distinct clinicopathological features, but was not a prognostic factor.

**Keywords** 22C3 PD-L1 expression · Metastatic gastric cancer · Mismatch repair · Epstein–Barr virus · Cancer genome alterations

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✉ Kohei Shitara  
kshitara@east.ncc.go.jp

<sup>1</sup> Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

<sup>2</sup> Department of Pathology and Clinical Laboratories, National Cancer Center Hospital East, Kashiwa, Japan

<sup>3</sup> Division of Immunology, Research Cancer for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Japan

## Introduction

Recently, blockade of immune checkpoint molecules with monoclonal antibodies has emerged as a promising strategy in several malignancies [1–6]. Programmed death 1 (PD-1), which belongs to the CD28 family of proteins, is a negative costimulatory receptor expressed on the surface of activated T cells [7]. The binding of PD-1 and its ligands, PD-L1 and PD-L2 on tumor or immune cells, can inhibit a cytotoxic T-cell response, which leads tumor cells to escape from immune surveillance [7]. Accordingly, blockade of this interaction restores the antitumor activity of T cells [7]. Clinical trials of anti-PD-1/PD-L1 antibodies have shown high response rates and improved overall survival in several malignancies, leading to the US Food and

Drug Administration (FDA) approvals [1–6]. A phase III trial of nivolumab for patients (pts) with metastatic gastric cancer (MGC) after two or more previous line chemotherapies showed a survival benefit, leading to the approval of nivolumab for MGC in Japan [8].

Although there have been no definitive biomarkers of anti-PD-1/PD-L1 antibodies, some reports have shown that PD-L1 expression on tumor cell (TC) and immune cell (IC) was associated with better clinical outcomes of anti-PD-1/PD-L1 antibodies in several malignancies [1, 3–5, 9]. Indeed, pembrolizumab is approved for chemo-naïve pts with metastatic non-small-cell lung cancer (NSCLC) whose tumors demonstrate PD-L1 expression in  $\geq 50\%$  of TC or second-line pts with  $\geq 1\%$  PD-L1 on TC, based on PD-L1 immunohistochemistry (IHC) using 22C3 pharmDx assay [3, 4]. FDA also granted accelerated approval to pembrolizumab for MGC pts with PD-L1 expression in  $\geq 1\%$  of TC or IC as determined by 22C3 IHC assay based on a large phase 2 study [5]. Although several reports assessed clinicopathological features of PD-L1 expression in gastric cancer using different anti-PD-L1 antibodies [10–12], information on clinical relevance of PD-L1 expression based on 22C3 PharmDx assay especially in MGC has been limited. In addition, PD-L1 expression especially in IC was frequently discordant across several antibodies in NSCLC [13].

In this study, we investigated clinicopathological features of 22C3 PD-L1 expression as well as mismatch repair (MMR), Epstein–Barr virus (EBV) status, the expressions of tyrosine kinase receptors (RTKs), and cancer genome alterations in MGC pts.

## Methods

### Patients

We have conducted a prospective observational study in our institution to evaluate the frequencies and clinicopathological features of PD-L1 expression with mismatch repair, EBV status, the expressions of RTKs including HER2, EGFR, and MET, and cancer genome alterations in MGC. Principal inclusion criteria were as follows: histologically proven MGC; planned to or receive systemic chemotherapy for advanced or recurrent disease; and the presence or ability to newly obtain adequate clinical samples for histological analysis.

Between September 2015 and January 2017, we analyzed 225 pts with MGC who met the inclusion criteria. Pts characteristics such as age, gender, site of primary lesion, macroscopic type, histology, site of metastases, and treatment history were collected. All participants provided written informed consent for the biomarker analyses. This study was approved by the institutional review boards of National

Cancer Center and was conducted in accordance with ethics guidelines, including the Declaration of Helsinki.

### Immunohistochemistry (IHC)

Primary antibodies used for immunohistochemistry (IHC) were anti-PD-L1 (22C3) rabbit monoclonal antibody (PD-L1 IHC 22C3 pharmDX; Agilent Technologies, Carpinteria, CA, USA), the Ventana PATHWAY anti-HER2/neu (4B5) rabbit monoclonal antibody (Ventana, Tucson, AZ), CONFIRM anti-EGFR (3C6) mouse monoclonal antibody (Ventana), CONFIRM anti-Total c-MET (SP44) rabbit monoclonal antibody (Ventana), anti-mutLhomolog 1 (MLH1; ES05) monoclonal antibody, anti-mutS homolog 2 (MSH2; FE11) monoclonal antibody, anti-postmeiotic segregation increased 2 (PMS2; EP51) monoclonal antibody, and anti-mutShomolog 6 (MSH6; EP49) monoclonal antibody (Dako). Formalin-fixed, paraffin-embedded blocks (FFPE) were cut into 4- $\mu$ m serial sections. PD-L1, MLH1, MSH2, PMS2, and MSH6 IHC were performed with Dako's autostainer according to the manufacturer's instructions. HER2, EGFR, and MET IHC were performed with a fully automated Ventana Benchmark ULTRA automated slide processing system (Ventana) according to the manufacturer's instructions.

### Evaluation of PD-L1 expression

All tissues immunohistochemically stained with anti-PD-L1 antibody were evaluated by TK and AK. PD-L1 positivity on TC or IC was defined by the presence of  $\geq 1\%$  of TC or IC with membrane staining.

### Evaluation of MMR status

Tumors were considered negative for MLH1, MSH2, PMS2, or MSH6 expression when there was a complete absence of nuclear staining of tumor cells, while positive staining was confirmed in normal epithelial and lymphocytes as inner control. Tumors lacking either MLH1, MSH2, PMS2, or MSH6 expression were considered MMR-deficient (D-MMR), whereas tumors that maintained expression of MLH1, MSH2, PMS2, and MSH6 were considered MMR proficient (P-MMR).

### EBV in situ hybridization

Chromogenic in situ hybridization for EBV-encoded RNA (EBER) was performed using fluorescein-labeled oligonucleotide probes (INFORM EBER Probe, Ventana). The visualization system used was the BenchMark ULTRA with enzymatic digestion (ISH Protease 3, Ventana) and the iViewBlue detection kit (Ventana).

## Evaluation of RTKs' expression

Evaluation and scoring of the HER2, EGFR, and MET IHC expressions were performed according to Hofmann's criteria [14]. HER2 gene amplifications were examined in SRL, Inc. Briefly, fluorescence in-situ-hybridizations (FISH) were performed with PathVysion HER-2 DNA Probe Kit (Abbott, IL, USA) according to the manufacturer's instruction. Analysis and interpretation of the FISH samples were conducted based on the previous report [15]. HER2 positivity was defined as IHC 3+ or IHC 2+ plus FISH positivity [15]. In EGFR and MET, we defined an IHC score of 2+ and 3+ as positive.

## Genomic analysis

DNA and RNA were extracted from FFPE tumor samples and were analyzed with the OncoPrint™ Comprehensive Assay version 3 (Thermo Fisher Scientific, Waltham, MA, USA) which allows to detect gene mutations, copy number variants, and fusions across multiple genes (Supplementary Table S1). The detected genomic variant data were classified according to whether genetic drivers of cancer include gain- and loss-of-function mutations or single-nucleotide variants based on the OncoPrint Knowledgebase.

## Statistical analysis

Comparisons of categorical variables were tested using the Chi-square test or Fisher's exact, as appropriate. We performed survival analysis in pts treated with systematic chemotherapy. Pts treated with anti-PD-1/PD-L1 antibodies were excluded from survival analysis. OS was defined from the date of initiation of the first-line chemotherapy until death from any cause. Pts who were alive were censored at the last follow-up date. OS rate was estimated using the Kaplan–Meier method, and the difference among the groups according to PD-L1 expression was identified using Cox proportional hazards models and presented as hazard ratios (HR) with 95% confidence intervals (CI). Statistical analyses were performed using IBM® SPSS® Statistics version 21 (IBM Corporation, Armonk, NY, USA). All tests were two-sided, and differences were considered significant when  $P < 0.05$ .

## Results

### Prevalence of PD-L1 expression with MMR and EBV status

Prevalence of PD-L1 expression, MMR, and EBV status is shown in Fig. 1. All samples were obtained from primary

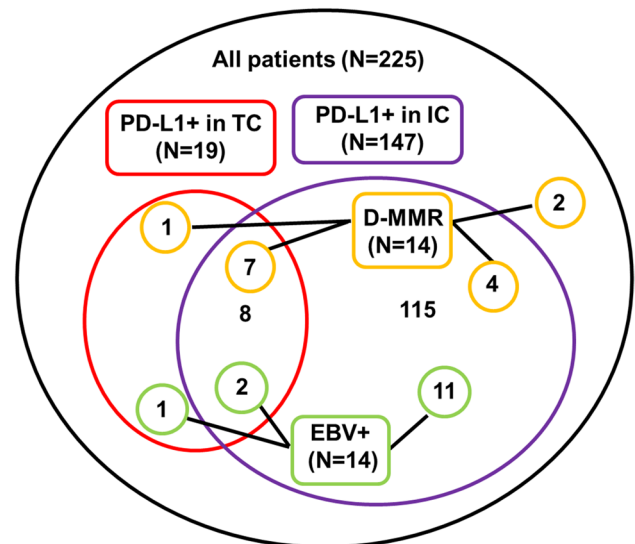


Fig. 1 Prevalence of PD-L1 expression with MMR and EBV status

tumors. Samples of 189 (84%) pts were obtained from biopsy. PD-L1 expression on TC and IC was positive in 19 (8.4%) and 147 pts (65.3%), respectively. Most of the pts who were PD-L1 positive on TC demonstrated PD-L1 positivity on IC as well, leading to the observation that 66.2% of pts had positive PD-L1 expression on either TC or IC.

D-MMR and EBV was detected in 14 (6.2%) and 14 pts (6.2%) of all cases, respectively. Six pts with D-MMR, eight pts with EBV+, and 52 pts with P-MMR and EBV- were treated with anti-PD-1/PD-L1 antibodies. Expression status of each MMR protein was shown in supplementary Table S2.

### Patients' characteristics according to PD-L1 expression

Baseline patient characteristics are shown in Table 1. The median age was 66 years (range 23–85 years), and there were 136 male pts (60.4%). PD-L1 expression on IC was more frequently observed in pts with lymph-node metastasis than in those without (72.7 vs. 38.8%,  $P < 0.001$ ). In contrast, PD-L1 expression on IC was less frequently observed in pts with peritoneal metastasis than in those without (53.4 vs. 78.0%,  $P < 0.001$ ). Borrmann Type 4 showed lower PD-L1 on both TC and IC than in those with non-Type 4 (TC 0 vs. 11.2%,  $P = 0.009$ ; IC 41.8 vs. 72.9%,  $P < 0.001$ ).

### Association between PD-L1 expression and RTKs expression or gene alterations

Among the RTKs evaluated pts, HER2, EGFR, and MET were positive in 35 (17%) out of 225, 64 (36%) out of 177, and 54 (36%) out of 149 pts, respectively (Table 2).

**Table 1** Patients characteristics according to PD-L1 expression

n (%)	All (n = 225)	PD-L1 in TC			PD-L1 in IC		
		Negative (n = 206)	Positive (n = 19)	P	Negative (n = 78)	Positive (n = 147)	P
<b>Age<sup>a</sup></b>							
< 65	95 (42)	86 (42)	9 (47)	0.809	36 (46)	59 (40)	0.398
≥ 65	130 (58)	120 (58)	10 (53)		42 (54)	88 (60)	
<b>Sex</b>							
Male	136 (60)	121 (59)	15 (79)	0.09	41 (53)	95 (65)	0.087
Female	89 (40)	85 (41)	4 (21)		37 (47)	52 (35)	
<b>Peritoneal metastasis</b>							
Absent	109 (48)	100 (49)	9 (47)	1.000	24 (31)	85 (58)	<0.001
Present	116 (52)	106 (51)	10 (43)		54 (69)	62 (42)	
<b>Lymph-node metastasis</b>							
Absent	49 (22)	45 (22)	4 (21)	1.000	30 (39)	19 (13)	<0.001
Present	176 (78)	161 (78)	15 (79)		48 (61)	128 (87)	
<b>Liver metastasis</b>							
Absent	166 (74)	150 (73)	16 (84)	0.415	63 (81)	103 (70)	0.111
Present	59 (26)	56 (27)	3 (16)		15 (20)	44 (30)	
<b>Lung metastasis</b>							
Absent	209 (93)	192 (93)	17 (90)	0.632	76 (97)	133 (91)	0.060
Present	16 (7)	14 (7)	2 (10)		2 (3)	14 (9)	
<b>Histology</b>							
Intestinal	70 (31)	65 (32)	5 (26)	0.798	19 (24)	51 (35)	0.131
Diffuse	155 (69)	141 (68)	14 (74)		59 (76)	96 (65)	
<b>Borrmann classification</b>							
Type 4	55 (24)	55 (27)	0 (0)	0.009	32 (41)	23 (16)	<0.001
Non-type 4	170 (76)	151 (73)	19 (100)		46 (59)	124 (84)	

<sup>a</sup>Median age 66 (range 23–85)

**Table 2** Association between PD-L1 expression and RTKs expression

n (%)	All	PD-L1 in TC			PD-L1 in IC		
		Negative	Positive	P	Negative	Positive	P
<b>HER2 (n = 225)</b>							
Negative	190 (84)	172 (84)	18 (95)	0.321	67 (86)	123 (84)	0.704
Positive	35 (17)	34 (16)	1 (5)		11 (14)	24 (16)	
<b>EGFR (n = 177)</b>							
Negative	113 (64)	105 (64)	8 (61)	1.000	34 (58)	79 (67)	0.224
Positive	64 (36)	59 (36)	5 (39)		25 (42)	39 (33)	
<b>MET (n = 149)</b>							
Negative	95 (64)	89 (65)	6 (55)	0.528	38 (69)	57 (61)	0.378
Positive	54 (36)	49 (35)	5 (45)		17 (31)	37 (39)	

A significant association was not observed between PD-L1 expression and RTKs expression.

Genomic analysis was conducted in 155 pts (Table 3). Gene alterations detected in more than 10 pts were *TP53* mutation (n = 68, 44%), *PIK3CA* mutation (n = 20, 13%), *KRAS* mutation (n = 13, 8%), and *CCNE1* amplification (n = 15, 10%), respectively. PD-L1 expression on TC was

more frequently observed in pts with *PIK3CA* mutation than in those with *PIK3CA* wild-type (25.0 vs. 6.7%,  $P = 0.020$ ) and *KRAS* mutation than in those with *KRAS* wild-type (38.4 vs. 6.3%,  $P = 0.002$ ). No other significant difference was observed between PD-L1 expression and presence of other gene alterations.

**Table 3** Association between PD-L1 expression and gene alterations

n (%)	All (n = 155)	PD-L1 in TC		P	PD-L1 in IC		P
		Negative (n = 141)	Positive (n = 14)		Negative (n = 53)	Positive (n = 102)	
<i>TP53</i> mutation							
Negative	87 (56)	79 (56)	8 (57)	1.000	25 (47)	62 (61)	0.126
Positive	68 (44)	62 (44)	6 (43)		28 (53)	40 (39)	
<i>PIK3CA</i> mutation							
Negative	135 (87)	126 (89)	9 (64)	0.020	45 (85)	90 (88)	0.616
Positive	20 (13)	15 (11)	5 (36)		8 (15)	12 (12)	
<i>KRAS</i> mutation							
Negative	142 (92)	133 (94)	9 (64)	0.002	48 (91)	94 (92)	0.765
Positive	13 (8)	8 (6)	5 (36)		5 (9)	8 (8)	
<i>CCNE1</i> amplification							
Negative	140 (90)	127 (90)	13 (93)	1.000	46 (87)	94 (92)	0.390
Positive	15 (10)	14 (10)	1 (7)		7 (13)	8 (8)	

### Clinicopathological features of D-MMR and EBV + MGC

PD-L1 expression on TC was more frequently observed in pts with D-MMR than in those with P-MMR and EBV− (57.1 vs. 3.8%,  $P < 0.001$ ), while that on IC was more frequently observed in pts with EBV+ than in those with P-MMR and EBV− (92.9 vs. 62.4%,  $P = 0.034$ ) (Table 4; Fig. 2). D-MMR was significantly associated with absence of liver metastasis ( $P = 0.050$ ), intestinal type ( $P = 0.014$ ), HER2 negative status ( $P = 0.043$ ), *PIK3CA* mutation ( $P < 0.001$ ), and *KRAS* mutation ( $P < 0.001$ ). EBV+ was significantly associated with diffuse type ( $P < 0.001$ ), HER2 negative status ( $P = 0.043$ ), and *PIK3CA* mutation ( $P < 0.001$ ) (Table 4).

### Survival analysis

Survival analysis of 173 pts who were not treated with anti-PD-1/PD-L1 antibodies is shown in Fig. 3. PD-L1 expression on either TC or IC was not prognostic factor [HR 1.05 (95% CI 0.71–1.67);  $p < 0.791$ ].

### Discussion

In this study, we evaluated clinical relevance of 22C3 PD-L1 expression in MGC. To our knowledge, this study is the first to provide information on clinicopathological features of PD-L1 expression determined by 22C3 PharmDx assay in MGC including its association with MMR, EBV status, RTKs' expression, and cancer genome alterations.

In our patient cohort, a total of 66.2% of pts had positive PD-L1 expression on either TC or IC, comparable to the rate seen in Phase II trial of pembrolizumab for MGC [5]. In this

trial, the objective response rate (ORR) in the third-line setting was 22.7% for pts with PD-L1 expression in  $\geq 1\%$  of TC or IC as determined by 22C3 IHC assay, while the ORR was 8.6% for those with PD-L1-negative tumors, leading to the FDA approval of pembrolizumab for PD-L1-positive MGC and PD-L1 22C3 IHC as a companion diagnostic assay [5, 16].

It has been reported that lymph-node metastasis in pts with NSCLC were more sensitive to nivolumab than other organs [17]. In MGC, peritoneal metastasis in the pts treated with anti-PD-1/PD-L1 monotherapy has been reported to be associated with worse clinical outcomes [18]. Interestingly, our study revealed the higher PD-L1 positivity in pts with lymph-node metastasis and the lower positivity in those with peritoneal metastasis and Borrmann Type 4, which might be one of the explanations for the association between clinical characteristics and response to anti-PD-1/PD-L1 antibodies in the previous reports.

Recently, FDA also approved pembrolizumab for pts with D-MMR solid tumors based on promising efficacies in several phase II trial [19]. It has been well known that D-MMR colorectal cancers had higher mutation loads compared with P-MMR, leading to high infiltration of CD8+ T cells presumably due to recognition of a high number of tumor neoantigens and its corresponding expression of immune checkpoints in the tumor microenvironment [20]. Our study also showed that PD-L1 positivity on TC was significantly higher in MGC cases with D-MMR compared with P-MMR. In agreement with TCGA report showing the elevation of PD-L1 gene amplification in EBV + GC [21], PD-L1 expression on TC was more frequently observed in EBV + MGC than in EBV- in our analysis (21 vs. 4%). Importantly, Panda et al. reported that a patient with EBV + MGC showed durable response from treatment with the anti-PD-L1 antibody avelumab, although this tumor had low mutation burden

**Table 4** Clinicopathological features of D-MMR and EBV + MGC

<i>n</i> (%)	D-MMR ( <i>n</i> = 14)	EBV+ ( <i>n</i> = 14)	P-MMR and EBV– ( <i>n</i> = 197)	<i>P</i>
Age				
≥ 65	9 (64)	6 (43)	115 (55)	0.439
Sex				
Male	10 (71)	11 (79)	115 (55)	0.220
Site of metastasis				
Peritoneal	8 (57)	6 (43)	102 (49)	0.754
Lymph nodes	13 (93)	11 (79)	152 (73)	0.472
Liver	0 (0)	3 (21)	56 (27)	0.050
Lung	2 (14)	1 (7)	13 (6)	0.573
Histology				
Intestinal	8 (57)	1 (7)	61 (31)	0.014
Diffuse	6 (43)	13 (93)	136 (69)	
PD-L1 in TC				
Positive	8 (57)	3 (21)	8 (4)	<0.001
PD-L1 in IC				
Positive	11 (79)	13(93)	123 (62)	0.034
HER2				
Positive	0 (0)	0 (0)	35 (18)	0.043
EGFR				
Positive	2 (20)	1 (11)	61 (39)	0.137
MET				
Positive	3 (38)	1 (17)	50 (37)	0.618
<i>TP53</i> mutation				
Positive	3 (30)	3 (30)	62 (46)	0.446
<i>PIK3CA</i> mutation				
Positive	6 (60)	5 (50)	9 (7)	<0.001
<i>KRAS</i> mutation				
Positive	7 (70)	0 (0)	6 (4)	<0.001
<i>CCNE1</i> amplification				
Positive	0 (0)	0 (0)	15 (11)	0.261

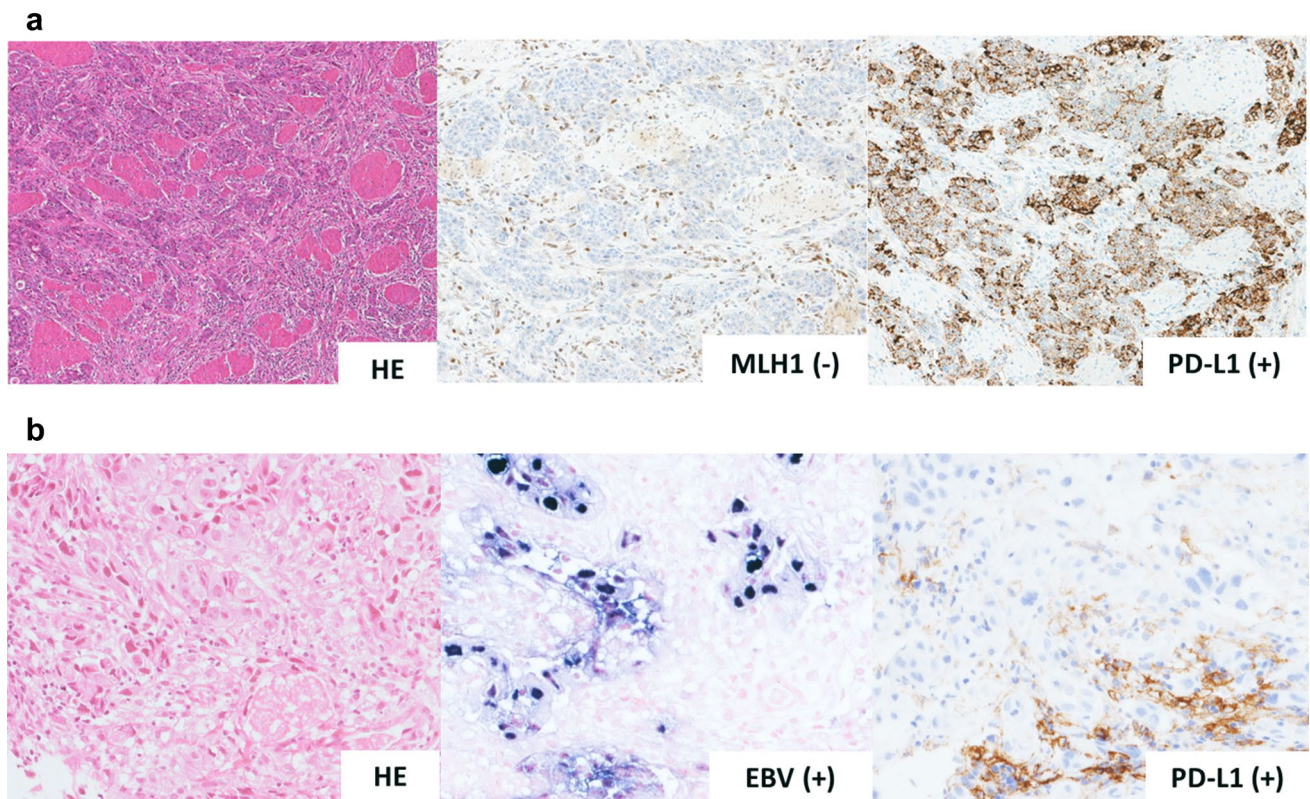
[22]. It has also been reported that EBV+ gastric cancer showed enriched infiltration of immune cells and higher expression of immune checkpoint pathway genes [22]. Considering these findings, the screening of MMR and EBV status as well as PD-L1 expression and mutation burden might be useful to predict clinical benefit of anti-PD-1/PD-L1 antibodies in MGC.

It has been considered that constitutive oncogenic signaling induces PD-L1 expression on TC [7]. Recently, Coelho et al. reported that oncogenic RAS signaling in human lung and colorectal tumors can upregulate tumor cell PD-L1 expression through a mechanism involving increases in PD-L1 mRNA stability [23]. Our study also revealed the higher PD-L1 positivity on TC in pts with *KRAS* mutation and *PIK3CA* mutation. However, these mutations were frequently observed in D-MMR or EBV + MGC in our analysis. Thus, whether cancer genome alterations are associated with PD-L1 expression or not warrants further evaluations.

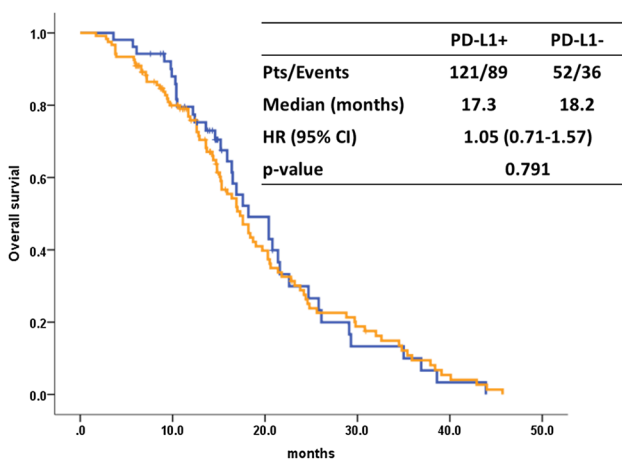
Impact of PD-L1 expression on prognosis remains controversial in several malignancies [10–12, 24, 25]. In gastric cancer, two previous studies showed that high PD-L1 expression in tumors was associated with poor prognosis [11, 12], while another one showed a better prognosis [10]. In our study, no association between PD-L1 expression and prognosis was observed, although which should be evaluated in a larger cohort. These wide ranges of reported outcomes might be influenced by patient cohort (sample size and clinical stages) examined and evaluation criteria of PD-L1 expression.

A limitation of our study is that we did not analyzed RTKs' expressions and cancer genome alterations in all the pts enrolled in this study, which warrants further evaluations in a larger cohort.

In conclusion, 22C3 PD-L1 expression in MGC had distinct clinicopathological features including D-MMR, EBV-positive status, and gene alternations. We also showed that



**Fig. 2** **a** Representative image of a case with D-MMR with PD-L1+ on TC. **b** Representative image of a case with EBV+ with PD-L1+ on IC



**Fig. 3** Kaplan–Meier plots of overall survival according to PD-L1 expression

PD-L1 expression was not a prognostic factor in MGC. Impact of these characteristics on efficacy of anti-PD-1/PD-L1 antibodies warrants further evaluation.

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## Compliance with ethical standards

**Conflict of interest** KS has received personal fees from Astellas, Bristol-Myers Squibb, Takeda, Pfizer, Novartis, Abbvie and Yakult; grants and personal fees from Eli Lilly and Ono Pharma; grants from Sumitomo Dainippon Pharma, MSD, Daiichi-Sankyo, Taiho Pharma, and Chugai Pharma. All remaining authors have declared no conflicts of interest. YK has received personal fees from Taiho Pharma, Bayer, and Eli Lilly; grants from Takeda, AstraZeneca, Daiichi-Sankyo, and Incyte. TY has received personal fees from Eli Lilly; grants from GlaxoSmithKline K.K and Nippon Boehringer Ingelheim Co., Ltd. AO has received grants from Bristol-Myers Squibb. YT has received personal fees and grants from Ono Pharma, personal fees from MSD, Chugai Pharmaceutical Co., Ltd, AstraZeneca, Boehringer Ingelheim Japan Inc, Novartis Pharma, and Eli Lilly Japan. HN has received grants from Ono Pharma, Kyowa Hakko Kirin, Sysmex, Taiho Pharma, Zenyaku-kogyo, and Daiichi-Sankyo. TD has received personal fees from Amgen; grants and personal fees from Eli Lilly, Chugai Pharma, Kyowa Hakko Kirin, MSD, and Daiichi Sankyo; grants from Taiho Pharma, Novartis, Merck Serono, Astellas Pharma Janssen, Boehringer Ingelheim, Takeda, Pfizer and Sumitomo Group, Celgene and Bristol Myers Squibb, Abbvie, and Quintiles. TK has received personal fees from Chugai Pharma and grants from government. All remaining authors have declared no conflicts of interest.

**Human rights statement** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

**Informed consent** Informed consent or substitute was obtained from all patients for being included in the study.

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