Discordancy and changes in the pattern of programmed death ligand 1 expression before and after platinum-based chemotherapy in metastatic gastric cancer

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Abstract

Background Our goal was to evaluate changes in PD-L1 expression in primary tumours of metastatic gastric cancer before and after chemotherapy.

Methods We evaluated the PD-L1 expression of 72 patients with primary gastric cancer, before and after palliative first-line platinum-based chemotherapy, between January 2015 and March 2017. The PD-L1 ratio was defined as pre-chemotherapy PD-L1 expression divided by the post-chemotherapy PD-L1 expression.

Results In 30 patients with PD-L1 negative pre-chemotherapy, 12 (40%) were positive post-chemotherapy; among the 42 patients with PD-L1 positive pre-chemotherapy, 24 (57.1%) were negative post-chemotherapy. The degree of PD-L1 expression decreased from 58.3% before chemotherapy to 41.7% after chemotherapy ($P=0.046$). Among patients with complete response/partial response (CR/PR), the degree of PD-L1 expression decreased ($P=0.002$), as well as PD-L1 positivity with statistical significance ($P=0.013$) after chemotherapy, but not among patients with stable disease/progressive disease (SD/PD). Higher disease control rates (CR/PR/SD) were observed in patients with an elevated PD-L1 ratio ($P=0.043$). Patients with a high PD-L1 ratio (>1) were found to be associated with a better progression-free survival (HR 0.34, 95% CI 0.17–0.67, $P=0.002$).

Conclusions PD-L1 expression can change during chemotherapy. Moreover, changes in patterns of PD-L1 expression might be associated with patient prognosis and response to chemotherapy.

Keywords Gastric cancer · Programmed cell death ligand 1 · Chemotherapy · Prognosis · Survival

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Background

Gastric cancer (GC) is the fourth most common cancer worldwide and the second leading cause of cancer death [1]. The standard treatment for advanced GC is palliative systemic chemotherapy [2]. Although systemic chemotherapy with target agents such as trastuzumab and ramucirumab show clinical benefit [3, 4], the prognosis of GC remains dismal, indicating a need for new treatment approaches.

Recent understanding of the molecular mechanisms regulating host-response to cancer cells led to the identification of key signalling pathways and checkpoint molecules involved in anticancer immune response [5]. Programmed death-1 (PD-1), which is a member of B7/CD28 family, is a cell membranous co-inhibitory receptor on activated T cells, B cells, and myeloid cells [6]. Programmed death ligand 1 (PD-L1), which is expressed on both immune and non-immune cells within the tumour and stroma of peripheral tissue, is one of the two ligands that bind to PD-1 [6, 7]. Increased PD-L1 expression has been reported in many different types of cancers [7]. Tumours expressing PD-L1 can evade anti-tumour immune surveillance and attack cytotoxic T cells through the PD1/PD-L1 pathway, reducing T cell activation and survival [8]. Recently, PD-1/PD-L1 pathway blockades have shown clinical benefit, including durable response, and have been approved by the Food and Drug Administration for many types of solid cancers, including GC [8–10].

Although the most accurate predictive marker for PD-1/PD-L1 axis inhibitor remains unclear, some trials have shown the usefulness of PD-L1 expression as a predictive marker for immune checkpoint inhibitors [11, 12]. However, PD-L1 expression is affected by inflammatory cytokines, which are released by infiltrative immune cells in the tumour microenvironment [8]. Moreover, homeostasis of the tumour microenvironment can change due to cancer progression or to exogenous factors such as chemotherapy or radiotherapy [13–15].

In this study, we aimed to investigate the impact of discordancy of, and changes in, PD-L1 expression status on primary tumour tissue in metastatic GC (mGC) pre- and post-chemotherapy.

Methods

Study population and design

A total of 79 consecutive patients with mGC and at least one measurable lesion, treated between January 2015 and

March 2017, were included in this study. We performed computed tomography (CT) imaging and gastric endoscopic biopsy of the primary gastric tumour lesion pre-chemotherapy. Patients were also subsequently evaluated for chemotherapy response after 8 ± 4 weeks via CT scan and gastric endoscopy, and additional biopsy was performed. We excluded 7 patients because they showed no tumour cells, such as necrotic tissues at the time of biopsy after chemotherapy.

All diagnoses were confirmed via biopsy of the primary tumour. The tumour site was classified as oesophagogastric (EGJ) and gastric lesion. Lauren classification was classified as intestinal and diffuse/mixed type. Histological types were classified as well/moderate differentiation or poor differentiation/signet ring cell/other adenocarcinoma. HER2 positivity was defined as IHC 3+ or IHC 2+ with fluorescence in situ hybridization (+). Chemotherapy regimen consisted of 5-FU, oxaliplatin, and leucovorin (FOLFOX), or capecitabine and oxaliplatin (CAPOX), for HER2-negative patients, while administering trastuzumab, 5-FU/capecitabine, and cisplatin for HER2-positive patients. Radiological changes were evaluated using the Response Evaluation Criteria in Solid Tumors version 1.1 [16]. Objective response was defined as complete response (CR) or partial response (PR), while disease control was defined as CR, PR, or stable disease (SD). This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital and written informed consent was obtained from all patients.

Immunohistochemistry of PD-L1 expression

Immunostaining was performed manually using a rabbit monoclonal anti-PD-L1 antibody (1:75, E1L3N, Cell Signaling, Danvers, MA, USA). The intensity and percentage of stained cells were evaluated separately by two pathologists. If the two pathologists had conflicting opinions, they had a discussion and performed a re-evaluation to produce consistent results. For the evaluation of the PD-L1 expression in tumour cells, only the membranous staining was evaluated (Supplementary material 1). The following system of immunoreactivity scores (IRSs) was applied [17]: category A was the percentage of immunoreactive cells that were graded as 0 (negative), 1 ($\leq 1\%$ positive), 2 (2–10% positive), and 3 ($> 11\%$); category B was the intensity of immunostaining that was graded as 0 (no immunostaining), 1 (weak), 2 (moderate), or 3 (strong). The sum of category A and B resulted in an IRS ranging from 0 to 6. PD-L1 positivity was defined as ≥ 2 points. For analysis of changes in PD-L1, the PD-L1 ratio was defined as the quotient of pre-chemo PD-L1 IRS + 1 divided by post-chemo PD-L1 IRS + 1.

Statistical analysis

The correlation between pre-chemotherapy PD-L1 status and both clinicopathologic factors and radiologic response were analysed using the Pearson's Chi-square test and linear-by-linear association. Changes in PD-L1 positivity and immunoreactivity scoring pre- and post-chemotherapy were analysed using the Wilcoxon signed-rank test. Overall survival (OS) and progression-free survival (PFS) were calculated from the start date of first-line palliative chemotherapy until the date of death or disease progression, respectively. For survival analyses, living patients or those with no disease progression were censored from the last follow-up date. Univariate analyses for OS and PFS were performed using the Kaplan–Meier method and the log-rank test. Multivariate Cox regression models were used to verify the prognostic values of pre-chemotherapy PD-L1 and the PD-L1 ratio and were adjusted for age, sex, performance status, tumour site, Lauren classification, histology, HER2 status, and the number and location of metastatic organs. All analyses were performed using SPSS software (version 21; SPSS Inc., Chicago, IL), and a two-sided *P* value of <0.05 was considered statistically significant.

Results

Baseline patient characteristics and PD-L1 expression according to clinicopathologic factors

A total of 72 patients were included in the analysis. Patient characteristics are listed in Table 1. No significant association between pre-chemotherapy PD-L1 status and clinicopathologic factors was noted (Supplementary material 2).

Discordancy and changes in patterns of PD-L1 status pre- and post-chemotherapy

The discordancy rates of PD-L1 status pre- and post-chemotherapy are shown in Table 2. Among the 30 patients with negative PD-L1 expression before chemotherapy, 12 (40%) were positive after chemotherapy. Meanwhile, among the 42 patients with PD-L1 positivity before chemotherapy, 24 (57.1%) were negative post-treatment. The total rates of discordancy were 50.0% (36/72).

Changes in PD-L1 expression after chemotherapy were also evaluated (Fig. 1, Supplementary material 3). The IRS decreased after chemotherapy (*P*=0.003) and the PD-L1-positive rate decreased from 58.3 to 41.7% after chemotherapy for all patients (*P*=0.046). In particular, PD-L1 positivity decreased significantly in both the diffuse/mixed-type (*P*=0.018) and the well-to-moderately differentiated (*P*=0.035) types. There was no significant association

Table 1 Baseline characteristics

	<i>N</i> =72
Age, median (range)	59 (21–84)
<60	38 (52.8%)
≥60	34 (47.2%)
Sex	
Female	33 (45.8%)
Male	39 (54.2%)
ECOG PS	
0, 1	49 (68.1%)
2	23 (31.9%)
Tumour site	
EGJ	7 (9.7%)
Gastric	65 (90.3%)
Lauren classification	
Intestinal	30 (41.7%)
Diffuse/mixed	42 (58.3%)
WHO classification	
Well to moderate differentiated	22 (30.6%)
Poorly differentiated/Signet ring cells/others	50 (69.4%)
HER2 status	
Negative	58 (80.6%)
Positive	14 (19.4%)
Number of metastatic organ	
0–1	43 (59.7%)
≥2	29 (40.3%)
PD-L1 status	
Negative	30 (41.7%)
Positive	42 (58.3%)

ECOG PS eastern cooperative oncology group performance status, *EGJ* esophagogastric junction, *HER2* human epidermal growth factor receptor 2, *PD-L1* programmed death-ligand 1, *WHO* world health organization

Table 2 Discordancy between pre- and post-chemotherapy PD-L1 status

	Post-chemo PD-L1 status		<i>P</i>
	Negative	Positive	
Pre-chemo PD-L1 status			0.808
Negative	18 (60.0%)	12 (40.0%)	
Positive	24 (57.1%)	18 (42.9%)	

PD-L1 programmed death-ligand 1

between pre-chemotherapy PD-L1 status and radiologic response (Supplementary material 4). The association between PD-L1 expression change post-chemotherapy and radiologic response was also evaluated (Supplementary material 5 and Table 3). Among patients with CR/PR, the both the PD-L1 IRS (*P*=0.002) and the PD-L1 positivity (*P*=0.013) decreased post-chemotherapy, but not among

Fig. 1 Changing pattern of PD-L1 status between pre- and post-chemotherapy. **a** The IRS of PD-L1 decreased after chemotherapy ($Z=-2.971$, $P=0.003$). **b** The positive rate of PD-L1 before chem-

otherapy was 58.3% in all patients, which decreased to 41.7% after chemotherapy ($P=0.046$). *IRS* immunoreactive score, *PD-L1* programmed death-ligand 1, *TC* tumour cell

Table 3 Relationship between PD-L1 change and radiologic response between chemotherapy

	Patients with objective response				Patients with disease control			
	Pre-chemotherapy PD-L1 Median (Mean, SD)	Post-chemotherapy PD-L1 Median (Mean, SD)	<i>Z</i>	<i>P</i>	Pre-chemotherapy PD-L1 Median (Mean, SD)	Post-chemotherapy PD-L1 Median (Mean, SD)	<i>Z</i>	<i>P</i>
PD-L1 IRS	3.0 (2.86, 2.09)	1.0 (1.66, 1.61)	-3.093	0.002	3.0 (2.65, 1.95)	1.0 (1.58, 1.60)	-3.464	0.001
PD-L1 positivity	1.0 (0.66, 0.48)	0 (0.40, 0.50)	-2.496	0.013	1.0 (0.63, 0.49)	0 (0.37, 0.49)	-2.887	0.004

IRS Immunoreactivity scoring system, *PD-L1* programmed death-ligand 1, *SD* standard deviation

patients with SD/progressive disease (PD). Among patients with CR/PR/SD, the IRS ($P=0.001$) and PD-L1 positivity ($P=0.004$) decreased post-chemotherapy, but not among patients with PD. We also assessed the association between the PD-L1 ratio and radiologic response (Supplementary material 6). The PD-L1 ratio was not associated with the objective response rate (56.8 and 40.0% for PD-L1 ratio >1 and ≤ 1 , respectively). However, a higher disease control rate was observed in patients with an increased PD-L1 ratio (89.2 and 68.6% for PD-L1 ratio >1 and ≤ 1 , respectively; $P=0.043$). Relationship of efficacy of four groups categorized by pre-chemotherapy and post-chemotherapy PD-L1 status was also assessed (Supplementary material 7), and there was no statistically significant association.

Prognostic impact of status of and changes in PD-L1 expression

OS and PFS were assessed according to pre-chemotherapy PD-L1 status (Supplementary material 8). The median OS and PFS were 10.97 and 6.40 months, respectively. OS

($P=0.311$) and PFS ($P=0.133$) were not significantly different based on pre-chemotherapy PD-L1 status. Also, assessment of OS and PFS of four groups according to PD-L1 status of pre-chemotherapy and post-chemotherapy was done (Supplementary material 9); however, no statistically significant differences were observed. We also performed a multivariate analysis of baseline characteristics and pre-chemotherapy PD-L1 status (Supplementary material 10). The only prognostic factor for both OS ($P=0.043$) and PFS ($P=0.034$) was the number of metastatic organs. Pre-chemotherapy PD-L1 status was not associated with prognosis (OS, $P=0.133$; PFS, $P=0.057$). The survival times according to the PD-L1 ratio were assessed (Fig. 2). OS was not significantly associated the PD-L1 ratio ($P=0.120$). However, longer PFS was observed in the PD-L1 ratio >1 group than in the PD-L1 ≤ 1 group ($P=0.021$). The multivariate analysis of the baseline characteristics and pre-chemotherapy PD-L1 ratio is shown in Table 4. The number of metastatic organs was found to be a prognostic factor of OS and PFS (OS, HR 2.29, 95% CI 1.10–4.48, $P=0.027$; PFS, HR 2.77, 95% CI 1.40–5.47, $P=0.003$). A high PD-L1 ratio

Fig. 2 Survival times according to PD-L1 ratio. **a** PD-L1 ratio didn't show OS difference (PD-L1 ratio > 1 group, 11.03 months; PD-L1 ≤ 1 group, 8.90 months, $P=0.120$). **b** Prolonged PFS was observed in PD-L1 ratio > 1 group than PD-L1 ≤ 1 group (PD-L1 ratio > 1 group,

6.90 months; PD-L1 ≤ 1 group, 4.43 months, $P=0.021$). *OS* overall survival, *PD-L1* programmed death-ligand 1, *PFS* progression-free survival

Table 4 Multivariate analysis of clinicopathologic factors and PD-L1 ratio for OS and PFS

	Multivariate analysis							
	OS				PFS			
	HR	95% CI		<i>P</i>	HR	95% CI	<i>P</i>	
Age								
< 60	1				1			
≥ 60	0.875	0.433	1.768	0.709	0.891	0.494	1.606	0.701
Sex								
Female	1				1			
Male	0.715	0.369	1.388	0.322	0.406	1.324	0.303	0.372
ECOG PS								
0, 1	1				1			
2	1.599	0.800	3.199	0.184	1.746	0.949	3.210	0.073
Tumour site								
EGJ					1			
Gastric	0.611	0.152	2.453	0.487	0.428	0.126	1.453	0.174
Lauren classification								
Intestinal	1				1			
Diffuse/mixed	0.936	0.501	1.747	0.835	0.999	0.563	1.773	0.997
WHO classification								
Well to moderate differentiated	1				1			
Poorly differentiated/Signet ring cells/others	1.408	0.612	3.241	0.421	0.770	0.398	1.488	0.436
HER2								
Negative	1				1			
Positive	0.623	0.219	1.769	0.374	0.852	0.389	1.864	0.688
Number of metastatic organ								
0–1	1				1			
≥ 2	2.292	1.099	4.779	0.027	2.771	1.404	5.469	0.003
PD-L1 ratio								
≤ 1	1							
> 1	0.520	0.264	1.028	0.060	0.339	0.172	0.671	0.002

CI confidence interval, *ECOG PS* eastern cooperative oncology group performance status, *EGJ* esophago-gastric junction, *HER2* human epidermal growth factor receptor 2, *HR* hazard ratio, *OS* overall survival, *PFS* progression-free survival, *PD-L1* programmed death-ligand 1, *WHO* world health organization

(> 1) was associated with better PFS (OS, HR 0.52, 95% CI 0.26–1.03, $P=0.060$; PFS, HR 0.34, 95% CI 0.17–0.67, $P=0.002$).

Discussion

In this study, we examined changes in PD-L1 expression pre- and post-chemotherapy, as well as the relationship between this change and patient outcomes. The PD-1/PD-L1 pathway has emerged as an attractive target for cancer treatment. Although it is not clear that PD-L1 is the most robust marker for immune checkpoint inhibitors [18], some reports have suggested that PD-L1 expression may be useful in this regard [19, 20].

Meanwhile, homeostasis of the tumour microenvironment can change due to cancer progression and to exogenous factors such as chemotherapy or radiotherapy [13, 15, 18]. Currently, several issues regarding the clinical implication of PD-L1 expression remains unsolved in terms of diagnostic assessment, dynamic changes, and prognostic effects. To our knowledge, our study is the first to report a discordancy and changes in PD-L1 expression pre- and post-chemotherapy for mGC.

Our results showed the discordancy of PD-L1 expression in tumour tissue pre- and post-chemotherapy, which is of important clinical significance. In clinical practice, old tissue acquired before chemotherapy are often used for analysing predictive markers, such as PD-L1 expression, because it is difficult to obtain new biopsy specimens when determining the use of immune checkpoint inhibitors after chemotherapy. Herein, we assessed whether old archival tissues were appropriate for evaluating PD-L1 expression. Our results suggest that it would be more ideal to analyse PD-L1 status by obtaining new tumour samples when using immune checkpoint inhibitors. Although it is unclear whether a change in PD-L1 is caused by a chemotherapeutic agent or a result of the natural disease course, it is assumed that additional biopsies would improve prognosis as it allows the precise analysis of predictive markers.

In some studies, PD-L1 expression is hypothesised to be associated with more aggressive progression and is expected to increase the incidence of drug resistance from chemotherapy [21–23]. However, our results showed that PD-L1 expression decreased after chemotherapy and this was associated with better treatment response and prognosis. This is consistent with Lim et al., whom showed that PD-L1 expression decreased after neoadjuvant chemotherapy in oesophageal cancer [14]. Jin et al. also showed that patients who responded to neoadjuvant chemotherapy showed significantly reduced PD-L1 expression in non-small cell lung cancer [24]. Although the exact mechanism causing these results remains unclear, considering that PD-L1 is

expressed on tumour cells, a decrease in the absolute number of tumour cells due to chemotherapy can be a major cause. In fact, 7 patients in our study were excluded from the analysis because no tumour cells were found in the additional biopsy tissues following chemotherapy, and these 7 patients showed good response to chemotherapy. Our results may also be attributed to tumour sampling time. Tumour sampling was performed in the present study after 2–4 cycles of chemotherapy. If tumour sampling was performed at the time of disease progression where drug resistance typically occurs and tumour cells regrow, not first response evaluation, change pattern of PD-L1 expression may be different. Unfortunately, we were unable to perform a tumour biopsy at the time of disease progression. In addition, the characteristics of the chemotherapeutic agent or cancer types are potential topics. Many chemotherapeutic agents exert immunomodulatory effects on the host system, in addition to their cytotoxicity [15]. Zhang et al. reported that paclitaxel, etoposide, and 5-fluorouracil, can induce PD-L1 surface expression in breast cancer, thereby increasing PD-L1-mediated T-cell apoptosis [25]. In contrast, Ghebeh et al. reported that doxorubicin is down-regulated on the cell surface for PD-L1 [26]. These differences were attributed to the heterogeneity among different malignancies and chemotherapeutics agents. Further research on the effects of other chemotherapeutic agents and various cancer types on PD-L1 expression are warranted.

In terms of prognostic role, it is unclear whether PD-L1 expression has significance in GC. Boger et al. reported that high PD-L1 expression on tumour cells is an independent favourable prognostic factor in Western patients with stage I–IV GC [17]. Kim et al. showed that positive PD-L1 status resulted in better disease-free survival and OS in 243 patients with curatively resected GC [27]. However, some previous studies reported that high PD-L1 expression in tumours was associated with poor prognosis and adverse clinicopathological findings in GC [28] and other cancers [29, 30]. In our study, clinicopathological factors and prognosis were not associated with PD-L1 expression before chemotherapy. These results can be attributed to different reasons. The small patient number and different cut-off values for interpreting PD-L1 expression or different PD-L1 antibodies are possible causes. Currently, no definite cut-off value of PD-L1 expression or antibody has been established. Moreover, our study included only stage IV GC patients. This may cause a difference from other studies of patients with stage I–IV GC, since the role of PD-L1 could be different during the cancer progression.

Our study has some limitations. First, the association between infiltrative immune cell and PD-L1 expression was not analysed. Thompson et al. showed that high CD8 + T cell densities in GC were statistically related with high PD-L1 expression within both tumour and stroma, and

high CD8 + T cell and PD-L1 expression was associated with a worse survival [31]. Other studies reported that the GC patient survival is significantly associated with intra-tumoural and peri-tumoural infiltrative immune cells, such as CD3 + T cells, CD8 + T cells, and macrophages [32]. Comprehensive approaches with immune cell and PD-L1 expression in tumour cells should be considered in future studies. Second, tumour heterogeneity in PD-L1 expression cannot be evaluated [11, 33]. A small endoscopic biopsy sample is insufficient to reflect whole-tumour PD-L1 status. Studies using techniques that can overcome tumour heterogeneity such as liquid biopsy are needed. Third, although we observed discordancy in PD-L1 expression and changes pre- and post-chemotherapy, we did not determine the intrinsic mechanism of chemotherapy-induced alteration of PD-L1 expression. Further studies are warranted to determine such mechanisms. Finally, the number of patients included in the study was small; therefore, the results should be interpreted with caution. A study with a larger sample size is required to confirm our findings.

Overall, we found that PD-L1 expression can change over time and during chemotherapy. Therefore, clinicians should consider the possibility of discordancy and changes in predictive markers such as PD-L1 expression. In addition, the usefulness of this predictive marker for immunotherapy should be evaluated using the most recently obtained serial tissue biopsy samples. This approach may lead to help select patients with mGC who will benefit from novel immunotherapeutic agents. Moreover, changes in PD-L1 expression patterns might be associated with patient response to chemotherapy and prognosis. Further studies are needed to confirm our findings.

Supplementary information is available at the Gastric Cancer's website.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Informed consent Informed consents were obtained from all patients for their inclusion in the study.

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