



Original Article

Prognostic Value of Serum Soluble Programmed Death-Ligand 1 and Dynamics During Chemotherapy in Advanced Gastric Cancer Patients

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Purpose The soluble form programmed death-ligand 1 (sPDL1) has immunosuppressive properties and is being studied as a candidate biomarker for immuno-oncology drug development. We measured the serum sPDL1 at pre-and post-chemotherapy and evaluated its prognostic implication and dynamics during chemotherapy in advanced gastric cancer (GC).

Materials and Methods We prospectively enrolled 68 GC patients who were candidates for palliative standard first-line chemotherapy, and serially collected blood at baseline and after one cycle of chemotherapy, at the best response and after disease progression. sPDL1 was measured using an enzyme-linked immunosorbent assay. Response to chemotherapy, overall survival (OS), progression-free survival (PFS) and other prognostic factors including neutrophil-lymphocyte ratio (NLR) were obtained. The cut-off value of sPDL1 levels for survival analysis was found using C-statistics.

Results The median baseline sPDL1 was 0.8 ng/mL (range, 0.06 to 6.06 ng/mL). The median OS and PFS were 14.9 months and 8.0 months, respectively. sPDL1 and NLR showed a weak positive correlation (Spearman's rho=0.301, p=0.013). Patients with low levels of sPDL1 at diagnosis (< 1.92 ng/mL) showed a better OS and PFS than patients with a high sPDL1. The baseline sPDL1 before treatment was higher in the progressive disease group than in the stable disease and partial response groups. Patients whose sPDL1 increased after the first cycle of chemotherapy showed worse PFS and OS. Following disease progression, sPDL1 increased compared with the baseline.

Conclusion sPDL1 at prechemotherapy confers a prognostic value for PFS and OS in GC patients under palliative first-line chemotherapy. Dynamics of sPDL1 during chemotherapy correlates with disease progression.

Key words B7-H1 antigen, Stomach neoplasms, Liquid biopsy, Progression-free survival

Introduction

Gastric cancer (GC) is the fifth most common cancer in the world and is the third most common cause of cancer-related death [1]. There is a high incidence in East Asia including Korea, Japan, and China [2].

Early GC can be cured with endoscopic mucosal resection or curative surgery. In patients with advanced GC, chemotherapy improves symptoms and increases overall survival (OS) [3,4]. In addition, trastuzumab and ramucirumab have been approved as targeted therapies for suitable patients [5,6].

Following the development of anticancer immunotherapeutic agents, the anti-programmed death-1 (PD-1) antibody, pembrolizumab received U.S. Food and Drug Administration approval for treatment of GC patients with programmed death-ligand 1 (PD-L1) expression with disease progression on after two or more prior lines of therapy [7]. Nivolumab

showed a survival benefit in a heavily pretreated patient population compared with placebo without consideration of PD-L1 expression [8].

Recently, the most important issue is to select which biomarker is suitable for specific patients who benefited from immunotherapy in GC. Microsatellite instability (MSI) is a proven biomarker, it is also applied to GC [9]. Although tumor mutational burden is presented as a biomarker for immunotherapy in various cancers, little is known about its role in GC [10]. Most biomarkers use tumor tissue, which is usually difficult to obtain. This poses a limitation in clinical practice where repeated tumor biopsies are performed as treatment progresses.

Currently, the soluble form of PD-L1 (sPDL1) can be measured from the blood of cancer patients. The relationship between sPDL1 levels and prognosis of patients has been studied in several cancers. These studies suggest that the

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higher the serum level of sPDL1, the worse the prognosis of the tumor [11-14]. Controversial studies have been published on the level of sPDL1 in the blood of advanced GC patients and their prognosis [15,16]. Furthermore, it is not yet known how the serum level of sPDL1 changes in patients with GC according to disease progression and how they relate to treatment response. Because it is a liquid biopsy-based test, it can overcome the limitation of obtaining tumor tissue samples, and it is relatively easy to obtain samples repeatedly in the course of therapy.

This study was carried out to investigate the role of sPDL1 in GC.

Materials and Methods

1. Patients and data collection

We prospectively enrolled pathologically diagnosed metastatic or recurrent GC patients who provided informed consent for the biomarker analysis study at Seoul National University Hospital. A total of 68 patients were enrolled. Their blood samples were collected before initiation of palliative first-line chemotherapy, after the first cycle of chemotherapy, at the best response, and after disease progression. First-line chemotherapy was fluorouracil and platinum doublet. Trastuzumab was given to human epidermal growth factor receptor 2 (HER2)-positive patients. The chemotherapy response was evaluated every two cycles. Patients' blood samples were taken every outpatient visit for chemotherapy. The sample of the first registered patient was 2013-11-27. The data censored at May 2018. The number of patients included in the analysis at baseline and after first cycle was 68, 67 at the best response, and 42 after disease progression, respectively.

Data of baseline characteristics, laboratory data including whole blood cell count with neutrophils, lymphocytes, monocytes and platelets, carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA 19-9) were collected. The neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR) were calculated by dividing the neutrophils, monocytes or platelet counts by the lymphocyte counts.

The serum level of sPDL1 was measured using an enzyme-linked immunosorbent assay (PDCD1LG1 ELISA kit, USCN Life Science, Wuhan, China) in each sample according to the manufacturer's instructions [13]. Each sample was analyzed in duplicate.

2. Statistical analysis

A t test was used for comparison of means. ANOVA was used for comparison of the mean between the three groups

Table 1. Baseline characteristics

Characteristic	No. (%)
Ages at diagnosis, median (range, yr)	56 (28-80)
Sex	
Male	43 (63.2)
Female	25 (36.8)
Pathology	
Adenocarcinoma, W/D	2 (2.9)
Adenocarcinoma, M/D	20 (29.4)
Adenocarcinoma, P/D	18 (26.5)
Poorly cohesive carcinoma	20 (29.4)
Mixed	8 (11.8)
HER2 status	
HER2 positive	13 (19.1)
HER2 negative	55 (80.9)
Microsatellite status	
MSI-High	3 (4.4)
MSI-Low	3 (4.4)
MSS	27 (39.7)
Unknown	35 (51.5)
Disease status	
Relapsed after curative surgery	10 (14.7)
Undergone palliative surgery	18 (26.5)
Metastatic cancer	40 (58.8)
Metastatic organ	
Liver	15 (22.1)
Lymph nodes	22 (32.4)
Peritoneum	37 (54.4)
Lung	10 (14.7)
Brain	2 (2.9)
Best response to 1st-line chemotherapy	
Complete response	0
Partial response	34 (50)
Stable disease	29 (42.6)
Progressive disease	5 (7.4)
Post progression treatment	
1st line	32 (47.0)
2nd line	25 (36.8)
3rd line	8 (11.8)
4th line	3 (4.4)
Median OS (95% CI, mo)	14.9 (7.3-22.5)
Median PFS (95% CI, mo)	8.00 (6.0-10.0)

CI, confidence interval; HER2, human epidermal growth factor receptor 2; M/D, moderately differentiated; MSI, microsatellite instability; MSS, microsatellite stable; OS, overall survival; P/D, poorly differentiated; PFS, progression-free survival; W/D, well differentiated.

and Turkey's multiple comparison method was used for post-hoc analysis. OS was defined as the time from day 1 of chemotherapy to the date of death or last follow-up. Median

Table 2. Laboratory test result

	Baseline (n=68)		After 1st cycle (n=68)		Best response (n=67)		After progression (n=42)	
	Median (range)	Average (95% CI)	Median (range)	Average (95% CI)	Median (range)	Average (95% CI)	Median (range)	Average (95% CI)
WBC (×1,000/mm ³)	6.81 (3.86-21.07)	7.46 (6.76-8.16)	5.45 (2.64-17.92)	6.47 (5.70-7.24)	4.68 (2.42-23.75)	5.59 (4.81-6.37)	5.52 (2.80-17.00)	5.23 (4.81-6.37)
Hb (g/dL)	11.6 (8.70-14.80)	11.66 (11.26-12.06)	11.45 (8.40-15.2)	11.47 (11.09-11.84)	11.2 (6.50-15.40)	11.2 (10.79-11.61)	10.85 (8.10-14.80)	10.81 (10.32-11.24)
PLT (×1,000/mm ³)	279 (108-559)	295 (270-320)	250 (91-536)	253 (231-276)	205 (54-556)	214 (190-239)	211 (33-597)	225.9 (192-260)
Neutrophil/Lymphocyte ratio	2.38 (0.45-15.00)	3.06 (2.43-3.69)	1.64 (0.49-16.02)	2.46 (1.88-3.03)	1.3 (0.41-7.65)	1.87 (1.48-2.26)	2.19 (0.64-17.56)	3.08 (2.34-4.18)
Monocyte/Lymphocyte ratio	0.3 (0.13-1.24)	0.34 (0.3-0.38)	0.29 (0.13-0.95)	0.36 (0.31-0.40)	0.33 (0.12-0.94)	0.38 (0.34-0.42)	0.35 (0.11-2.15)	0.46 (0.36-0.57)
Platelet/Lymphocyte ratio	162.4 (53.49- 427.66)	174.65 (156.80- 192.50)	136.09 (40.47-354.42)	148.93 (133.70-164.15)	126.01 (31.04-608.98)	131.81 (111.98-151.63)	137.15 (22.24-639.35)	166.11 (130.14-201.19)
CEA (ng/dL)	2.65 (< 0.5-3,867.40)	90.17 (0-212.772)	2.9 (< 0.5-477.50)	21.61 (2.96-40.26)	2.8 (< 0.5-512.7)	20.55 (1.96-39.14)	4.3 (0.60-1,480.30)	63.91 (0-143.78)
CA 19-9 (U/mL)	17 (< 1.0- 7,869.0)	382.17 (76.33-664.56)	14.2 (< 1.0-10,349)	317.14 (0-677.56)	17 (< 1.0-6,300)	209 (0-416.27)	35 (2.00-12,000)	605.09 (0-1,228.02)
sPDL1 (ng/mL)	0.8 (0.06-6.06)	1.31 (0.98-1.63)	0.92 (0.28-6.73)	1.52 (1.16-1.88)	1.01 (0.07-6.29)	1.46 (1.11-1.81)	1.22 (0.23-7.11)	1.45 (1.06-1.84)

CA 19-9, cancer antigen 19-9; CEA, carcinoembryonic antigen; CI, confidence interval; Hb, hemoglobin; PLT, platelet; sPDL1, soluble form programmed death-ligand 1; WBC, white blood cell.

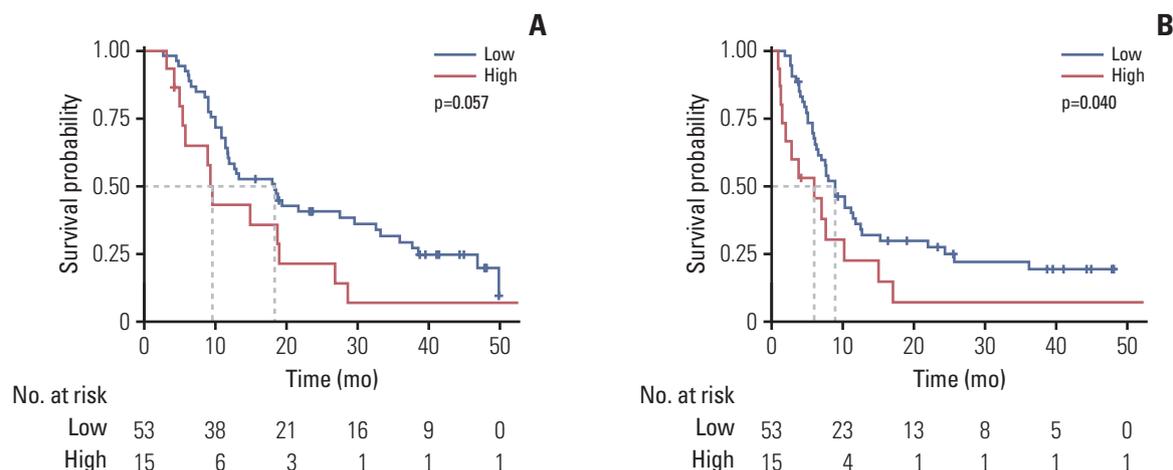


Fig. 1. Comparison of overall survival and progression-free survival according to soluble form programmed death-ligand 1 (sPDL1). (A) The overall survival according to baseline sPDL1 value. (B) The progression-free survival according to baseline sPDL1 value. Patients with lower levels of sPDL1 at baseline showed a better progression-free survival than the patients with a higher level of sPDL1 (median PFS, 8.9 months vs. 6.0 months; $p=0.040$).

OS was calculated using the Kaplan-Meier method and comparisons of difference between groups were assessed using log-rank tests. Median follow-up duration was calculated using the reverse Kaplan-Meier method. Of the 68 patients, 15 had survived at the time of data-cut off and were therefore censored in the survival analysis. All statistical tests were two-sided, with significance defined as $p < 0.05$.

Cut-off values of each variable (sPDL1, NLR, MLR, and PLR) for OS were found by using the C-statistics [17]. To find the cut-off, we divided the variables into 100 sections and calculate the C-value and the p-value based on the Cox proportional hazard model, and then selected the most significant value.

We performed this analysis using R ver. 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria) and SPSS software ver. 23 (IBM Corp., Armonk, NY).

Results

1. Patient characteristics

A total of 68 patients were prospectively enrolled (Table 1). The median age at diagnosis was 56 years old (range, 28 to 80 years). The median follow-up duration was 44.3 months (95% confidence interval [CI], 41.0 to not applicable), and OS was 14.9 months (95% CI, 7.3 to 22.5 months).

All patients received fluorouracil and platinum doublet (XELOX or FOLFOX) as first-line palliative chemotherapy. Among them, 13 HER2-positive patients received trastuzumab on top of chemotherapy. Three patients had MSI-high tumors, three patients had MSI-low tumors, 27 patients had

microsatellite stable tumors, and the rest were unknown. Ten patients recurred after curative surgery and the rest were metastatic GC patients, 19 of whom underwent palliative surgery. PD-L1 expression was evaluated in 16 patients (23.5%) using immunohistochemistry. Two patients had PD-L1-positive GC. One was in lower level of sPDL1 group and the other was in high level of sPDL1 group. All patients received immune checkpoint inhibitor on the 1st line and 2nd line were excluded. Only two patients received nivolumab in the 3rd and the 4th line, respectively. Both of them belonged to the low baseline sPDL1 group and survived for more than 3 years. Thirty-four patients (50%) had target lesion at baseline.

The best response of first-line chemotherapy was partial response (PR) in 34 patients (50%), stable disease (SD) in 29 patients (42.6%), and progressive disease (PD) in five patients (7.4%).

2. Association between sPDL1 and other variables

The baseline sPDL1 median value was 0.80 ng/mL (range, 0.06 to 6.06 ng/mL) and mean value was 1.31 ng/mL (range, 0.98 to 1.63 ng/mL). Median NLR was 2.38 (range, 0.45 to 15.00) in baseline samples of all patients (Table 2).

The correlation between baseline sPDL1 and other variables in the non-parametric test showed a significant correlation between sPDL1 and NLR, white blood cell count, platelet count, MLR, and PLR (S1 Fig.).

3. Prognostic value of sPDL1

Using C-statistics, the cut-off value of sPDL1 with the most significant C-value and p-value was found to be 1.92 ng/mL

Table 3. Univariate and multivariate analysis for survival

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age \geq 60 yr (vs. < 60 yr)	1.082 (0.612-1.915)	0.785	-	-
ECOG PS > 2 (vs. \leq 2)	1.851 (0.436-7.861)	0.443	-	-
Relapsed disease (vs. unresectable)	1.496 (0.750-2.987)	0.253	-	-
Peritoneal seeding (vs. without seeding)	1.874 (1.085-3.236)	0.024	1.340 (0.726-2.697)	0.315
No. of metastasis organ > 2 (vs. \leq 2)	1.706 (0.793-3.669)	0.171	-	-
Undergone resection (vs. no)	0.503 (0.284-0.891)	0.019	0.603 (0.321-1.134)	0.117
1st line therapy (vs. further line)	0.794 (0.456-1.383)	0.415	-	-
HER2-positive (vs. -negative)	1.094 (0.561-2.132)	0.792	-	-
With anemia (vs. without anemia)	0.892 (0.502-1.582)	0.675	-	-
CEA > 5 ng/dL (vs. \leq 5)	1.422 (0.793-2.548)	0.237	-	-
CA 19-9 > 37 U/mL (vs. \leq 37)	1.315 (0.742-2.331)	0.349	-	-
sPDL1 > 1.92 ng/mL (vs. \leq 1.92)	1.837 (0.975-3.458)	0.061	1.448 (0.714-2.935)	0.305
NLR > 3.51 (vs. \leq 3.51)	3.975 (2.044-7.731)	< 0.01	2.381 (1.127-5.031)	0.023
MLR > 0.30 (vs. \leq 0.30)	2.498 (1.416-4.408)	< 0.01	1.565 (0.816-3.005)	0.178
PLR > 135.8 (vs. \leq 135.8)	2.180 (1.218-3.903)	< 0.01	1.525 (0.740-3.319)	0.252

CA 19-9, cancer antigen 19-9; CEA, carcinoembryonic antigen; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; sPDL1, soluble form programmed death-ligand 1.

Table 4. Dynamics of sPDL1 according to response groups

Best response	PR	SD	PD	Total	p-value
Baseline sPDL1	n=34	n=29	n=5	n=68	
Median (range)	0.74 (0.22-6.06)	0.90 (0.06-4.73)	2.77 (0.25-5.03)	0.80 (0.06-6.06)	
Mean (95% CI)	1.19 (0.77-1.61)	1.17 (0.73-1.61)	2.91 (1.20-4.62)	1.31 (0.98-1.63)	0.018
After 1st cycle	n=34	n=29	n=5	n=68	
Median (range)	0.86 (0.28-6.73)	0.84 (0.31-5.44)	1.43 (0.32-2.45)	0.92 (0.28-6.73)	
Mean (95% CI)	1.45 (0.95-1.95)	1.61 (1.04-2.18)	1.47 (0.76-2.18)	1.52 (1.16-1.88)	0.912
At best response	n=33	n=29	n=5	n=67	
Median (range)	1.01 (0.07-4.85)	0.97 (0.27-6.29)	1.85 (0.29-7.11)	1.01 (0.07-7.11)	
Mean (95% CI)	1.34 (0.95-1.73)	1.37 (0.86-1.88)	2.80 (0.47-5.13)	1.46 (1.11-1.81)	0.097
After progression	n=17	n=20	n=5	n=42	
Median (range)	1.24 (0.29-2.89)	0.97 (0.23-3.46)	1.85 (0.29-7.11)	1.22 (0.23-7.11)	
Mean (95% CI)	1.36 (1.02-1.70)	1.19 (0.78-1.60)	2.80 (0.47-5.13)	1.45 (1.06-1.84)	0.029

CI, confidence interval; PD, progressive disease; PR, partial response; SD, stable disease; sPDL1, soluble form programmed death-ligand 1.

(S2 Fig.). Patients were divided into two groups according to baseline sPDL1, and OS was compared (Fig. 1A). Patients with lower levels of sPDL1 at baseline showed a better OS and progression-free survival (PFS) than the patients with higher levels of sPDL1 (OS: 18.3 months vs. 9.5 months, $p=0.057$; PFS: 8.9 vs. 6.0 months, $p=0.040$) (Fig. 1).

In the univariate analysis for OS, high baseline sPDL1 being associated with shorter OS (hazard ratio [HR], 1.837; 95% CI, 0.975 to 3.458; $p=0.061$) (Table 3). However, in multivariate analysis, no significant effect of baseline sPDL1 on

OS was found (HR, 1.408; 95% CI, 0.685 to 2.895; $p=0.352$). The history of resection surgery, no peritoneal seeding, and lower baseline NLR, MLR, PLR were favor prognostic factors in the univariate analysis. But, In the multivariate analysis, lower baseline NLR was the only favor prognostic factors. Other tumor markers (CEA, CA 19-9) showed no prognostic role in this study.

4. Dynamics of sPDL1

Table 4 summarizes the sPDL1 values at baseline, after the

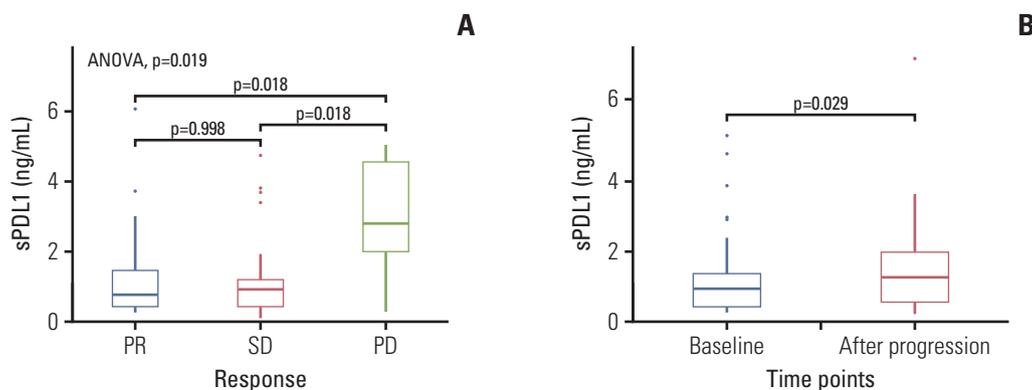


Fig. 2. Dynamics of soluble form programmed death-ligand 1 (sPDL1). (A) Baseline sPDL1 per response. The baseline sPDL1 before treatment was higher in the progressive disease (PD) group than in the stable disease (SD) and partial response (PR) groups (mean, 2.91, 1.17, 1.19 ng/mL; $p=0.019$). (B) Dynamics of sPDL1 after progression in patients who have matched sample. The sPDL1 of after progression was higher than baseline. (mean, 1.45 ng/mL vs. 1.24 ng/mL; $p=0.029$).

first cycle, at the best response, and after disease progression by dividing patients according to the best response to chemotherapy. Baseline sPDL1 was the highest in the PD group than in the SD and PR groups (mean, 2.91, 1.17, 1.19; $p=0.019$) (Fig. 2A).

In all patients who had matched sample, sPDL1 value increased after progression compared with baseline (Fig. 2B). In the PR group and the SD group, the level of sPDL1 was increased with disease progression.

Discussion

Currently, there is no reliable biomarker to predict the response of chemotherapy. Previous studies have examined whether sPDL1 might have a prognostic implication of advanced cancers [11-14]. Our previous studies have examined the value of sPDL1 as a biomarker in biliary tract cancer and pancreatic cancer patients [13,18,19]. The current prospective study examines the role of sPDL1 as a biomarker. In this study, we found that higher pre-chemotherapy sPDL1 is associated with shorter OS and PFS in GC patients, indicating that sPDL1 has a prognostic role in patients with metastatic or recurrent GC. There are conflicting results of sPDL1 value and prognosis in GC [15,16]. In one study, sPDL1 did not play a role as a prognostic factor, meanwhile the subgroup of patients with high sPDL1 had a better prognosis [15]. In another study, higher sPDL1 predicted a worse prognosis [16]. We suggested the association of high baseline sPDL1 with poor prognosis of GC patients through this prospective study.

The PD-1/PD-L1 pathway inhibits T-cell-mediated immune responses. These PD-L1/PD-1 interactions are known to

be the mechanism by which cancer cells avoid host immune responses [20]. This is the main mechanism to inhibit the inflammatory response that kills cancer cells. Although sPDL1 may be secreted from immune cells during the inflammatory process, it may also be secreted from tumor cells. Studies have shown that there are subtypes of sPDL1 depending on the cells that secrete [21,22]. A recent study that summarizes the previous studies on sPDL1 showed several different forms of PD-L1 measured in serum [22]. These include monomeric shed forms, monomeric and dimeric splice variants, microvesicles and exosomes, and their origin may be tumor cells or normal immune cells. This diversity of origins and forms of sPDL1 may be the reason for conflicting conclusions from previous studies analyzing the prognosis of GC patients and sPDL1 [15,16]. Furthermore, although other studies have examined how sPDL1 is secreted and how it acts during this inflammatory reaction, the mechanism remains unclear [21,23,24]. For sPDL1 to establish itself as a biomarker, additional research is needed on the origin of sPDL1 and its mechanism of action in tumors.

The advantages of our study is to serially collect the data of sPDL1 and to examine the serial change of sPDL1 through entire chemotherapy in patients with GC. To our knowledge, our study is the first to show dynamics of sPDL1 during treatment. Our study showed a significant trend toward increase of sPDL1 as tumor progressed, suggesting increase of sPDL1 during chemotherapy could reflect tumor progression. The prognostic or predictive roles of sPDL1 in cancer have been controversial because there are the different kinds of sources of sPDL1 and it depends on several clinical situations [20]. To overcome these issues, we evaluated serial change of sPDL1 as well as baseline sPDL1 and showed significant change according to disease progression, suggesting that sPDL1

could be a biomarker of chemotherapy. Furthermore, we further sought to find the relationship between tumor burden and sPDL1. We hypothesized that the sPDL1 levels of PR group patients who are responding to chemotherapy will be decreased. However, the sPDL1 levels of PR group patients increased after 1st cycle and at the best response. Elevation of sPDL1 levels in patients responding to treatment were found in previous studies of immunotherapy in patients with melanoma [21]. And, patients of the PD group who did not respond to chemotherapy, their sPDL1 levels were initially high and rather decreased after 1st cycle and at the best response. This may be the result of not distinguishing the origin of sPDL1 between sPDL1 derived from membrane-bound PD-L1 expressed in tumors and sPDL1 derived from tumor-promoting inflammation or inflammation from cancer cell death [22]. Further studies are needed to examine the dynamics of sPDL1 in GC and the mechanisms involved. In this case, serial follow-up is easy according to the courses of treatment, and it is less invasive than repetitive tumor biopsy.

The sPDL1 values were associated with NLR, MLR, and PLR. This is probably because these variables are all related to the inflammatory response. Inflammation is known to cause carcinogenesis and cancer progression. Moreover, gastrointestinal cancer is known to be associated with inflammatory reactions [25]. It is suggested that inflammatory markers such as white blood cell count, NLR, and PLR are correlated with sPDL1 as the chronic inflammatory reaction continues. NLR is a prognostic factor in almost all carcinomas and is a simple but powerful [26-28]. In our previous study, NLR was also to be found as a significant prognostic factor in advanced GC [29]. This study is the first to demonstrate the correlation between NLR and sPDL1 in GC patients, which is of great clinical significance.

This study is the first prospective study to investigate the role of sPDL1 as a biomarker in GC patients. Further stud-

ies that confirm the importance of sPDL1 and the effectiveness of immunotherapy may be necessary to predict which patients will benefit from immunotherapy in the future. In addition, in order for sPDL1 to be used as a biomarker, the immunological role of sPDL1 and the relationship with other inflammatory factors should be further studied.

In conclusion, sPDL1 in the serum of metastatic or recurrent GC patients can be a prognostic factor for palliative chemotherapy. This study suggests the role of sPDL1 as a biomarker in the area of liquid biopsy.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (<https://www.e-crt.org>).

Ethical Statement

The study protocol was reviewed and approved by the Institutional Review Board of Seoul National University Hospital (IRB No. 1807-102-960). All studies were conducted according to the declaration of Helsinki.

Author Contributions

Conceived and designed the analysis: Park W, Oh DY.

Collected the data: Park W, Oh DY.

Contributed data or analysis tools: Park W, Bang JH, Nam AR, Jin MH, Seo H, Kim JM, Oh KS, Kim TY, Oh DY.

Performed the analysis: Park W, Kim TY, Oh DY.

Wrote the paper: Park W, Kim TY, Oh DY.

Conflicts of Interest

Conflicts of interest relevant to this article was not reported.

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