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Efficacy of Cytotoxic Agents After Progression on Anti-PD-(L)1 Antibody for Pre-treated Metastatic Gastric Cancer

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Abstract. *Background/Aim: The efficacy of treatment using the anti-programmed cell death-1 (anti-PD-1) antibody for metastatic gastric cancer (mGC) has been established previously. Exploratory analyses in various types of tumours suggest that prior exposure to immune checkpoint inhibitors can enhance the efficacy of subsequent cytotoxic chemotherapy (CTx). Our aim is to evaluate the efficacy and safety of CTx for mGC after progression on anti-PD-(ligand) 1 [anti-PD-(L)1] antibody. Patients and Methods: We retrospectively evaluated patients with mGC who underwent CTx. The patients received CTx after progression on anti-PD-(L)1 antibody (cohort A) or as a third-line treatment without prior exposure to anti-PD-(L)1 antibody (cohort B). We evaluated: i) clinical characteristics, ii) efficacies, iii) prognoses, and iv) adverse events (AEs). Results: In cohorts A and B, 16 and 68 patients fulfilled the criteria, respectively. In the univariate analysis, the overall response rate was significantly higher in cohort A compared to cohort B (31% vs. 10%, respectively; Odds Ratio:3.96, 95% Confidence Interval:1.06-14.8, p=0.040). The multivariate analysis showed a similar trend. Immune-related AEs did not worsen and were manageable, while new immune-related AEs were not observed. Conclusion: CTx after progression on anti-PD-(L)1 antibody demonstrated a favourable efficacy in intensively treated patients with mGC.*

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Globally, gastric cancer is the third leading cause of cancer-related deaths annually. In Japan, the mortality rate associated with gastric cancer is the second and third highest among men and women, respectively. Chemotherapy (CTx) prolongs the survival of patients with metastatic gastric cancer (mGC); however, the prognosis of this disease remains poor. The use of immune checkpoint inhibitors (ICI) targeting the programmed cell death-1 (PD-1) or PD-ligand 1 (PD-L1) have improved patients' survival compared to standard treatment options in various types of solid tumours (1, 2). The ATTRACTION-2 trial compared the efficacy and safety of nivolumab *versus* placebo as a third- or later-line for mGC. The results of the trial have revealed that the nivolumab group exhibits significantly longer survival compared to the placebo group (3). Consequently, nivolumab was approved in Japan in 2017 for previously treated gastric cancer.

Retrospective studies in various types of tumours have demonstrated potential improvements in the overall response rate (ORR) to CTx after exposure to ICI. In metastatic melanoma, it has been reported that patients who have failed previous anti-PD-1 therapy appear to benefit from subsequent systemic treatments compared to the historical control (26% objective response). In advanced non-small cell lung cancer (NSCLC), patients who received CTx immediately after treatment with anti-PD-1 antibody had a 39-53.4% ORR (4, 5). Also, the efficacy of targeted therapy after PD-1/PD-L1 blockade has been investigated in metastatic renal cell carcinoma. Median time to treatment failure (TTF) on subsequent targeted therapy was 6.6 months (range: from 0.2+to 23.0), while 1- and 2-year overall survival (OS) from the initiation of subsequent targeted therapy was in 58% and 36% of these patients, respectively (6). In patients with recurrent/metastatic squamous cell carcinoma of the head and neck, the ORR to salvage CTx

after progression on ICI was 30%, while median progression-free survival and median OS were 3.6 and 7.8 months, respectively (7).

Importantly, it is necessary to consider the safety of subsequent CTx after anti-PD-1 therapy. For example, in epidermal growth factor receptor (EGFR) mutation-positive lung cancer, it has been suggested that the use of EGFR tyrosine kinase inhibitors (TKI) increases the risk of interstitial pneumonia after treatment with anti-PD-1 antibody (8).

Currently, there is no information regarding the efficacy and safety of CTx for mGC after progression on anti-PD-(L)1 antibody. Therefore, we aimed to evaluate the efficacy and safety of CTx in this setting.

Patients and Methods

Patients. In this retrospective cohort study, we reviewed the: i) clinical characteristics, ii) efficacies, iii) prognoses, and iv) adverse events (AEs) of patients with mGC who underwent CTx at the Aichi Cancer Centre Hospital (Nagoya, Japan). In cohort A, patients underwent CTx after progression on anti-PD-(L)1 antibody. Patients who received the anti-PD-(L)1 antibody as a third- or later-line treatment were included in this group. In cohort B, patients received CTx as a third-line treatment without prior exposure to the anti-PD-(L)1 antibody. In both cohorts, patients received CTx between April 2014 and August 2017.

The patients fulfilled the following criteria: i) histological diagnosis of unresectable gastric adenocarcinoma, ii) Eastern Cooperative Oncology Group performance status (ECOG PS) 0-2, iii) adequate bone marrow, hepatic, and renal function, and iv) CTx, including fluoropyrimidines (FU), platinum, and taxane or irinotecan as prior treatment. Written informed consent was provided by all patients prior to the initiation of treatment. This retrospective study was approved by the institutional review board (approval no. 2019-1-487).

Treatments and safety. The anti-PD-(L)1 antibody was administered as a single agent. The content of the anti-PD-(L)1 drug was nivolumab in 8 cases, pembrolizumab in 6 cases, and avelumab in 2 cases. CTx consisted of single-agent or combination regimens, including cytotoxic agents and molecular targeted drugs approved in Japan, but excluding the anti-PD-(L)1 antibody.

AEs, including immune-related AEs (irAEs), were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (9). IrAEs were evaluated during anti-PD-(L)1 therapy and subsequent CTx.

Evaluation of treatment and statistical analysis. Among those with measurable lesions, the ORR was defined as the portion of patients with a complete or partial response, according to the Response Evaluation Criteria in Solid Tumours, version 1.1 (10). The disease control rate (DCR) was defined as the portion of patients with a i) complete response, ii) partial response, or stable disease, according to the evaluation of best tumour response. TTF was defined as the time from the date of the first administration of CTx to the date of treatment discontinuation for any reason, including disease progression, treatment toxicity, or death. OS was defined as the time from the first administration of subsequent CTx until death from any cause or censored at the last follow-up date.

Differences in patient characteristics and AEs between cohorts A and B were evaluated using Fisher's exact test. The median TTF and OS were estimated using the Kaplan–Meier method. Differences in ORR, DCR, and survival (TTF and OS) were also evaluated through univariate and multivariate analyses using logistic regression and the Cox proportional hazards model, respectively. In the univariate analyses for ORR and DCR, the following variables were included: i) age (<65 *vs.* ≥65 years), ii) sex (male *vs.* female), iii) ECOG PS (0-1 *vs.* 2), iv) histology (intestinal *vs.* diffuse), v) prior gastrectomy (no *vs.* yes), vi) human epidermal growth factor receptor 2 (HER2) status (negative or not evaluated *vs.* yes), vii) number of metastatic sites (1-2 *vs.* ≥3), viii) peritoneum metastatic (no *vs.* yes), ix) liver metastatic (no *vs.* yes), x) lung metastatic (no *vs.* yes), xi)_alkaline phosphatase levels [<upper limit of the normal (ULN) *vs.* ≥ULN iu/l], xii) lactate dehydrogenase levels (<ULN *vs.* ≥ULN iu/l), xiii) carcinoembryonic antigen levels (<ULN *vs.* ≥ULN ng/ml), xiv) albumin levels (<4.0 *vs.* ≥4.0 g/dl), xv) number of prior regimens (2 *vs.* ≥3), xvi) CTx regimen (FU + oxaliplatin or taxane *vs.* irinotecan), xvii) time from the first-line CTx (≥6 months *vs.* <6 months), and xviii) cohort (B *vs.* A). In the multivariate analyses, variables with $p \leq 0.1$ in the univariate analysis were selected, whereas those with a possible multicollinearity were excluded. Two-sided p-Values<0.05 denoted statistical significance.

All statistical analyses were performed using EZR (Saitama Medical Centre, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, EZR is a modified version of R commander designed to add statistical functions frequently used in biostatistics (11).

Results

Patient characteristics. In cohort A, we identified 48 patients who received anti-PD-(L)1 as a third- or later-line treatment. Of those, we evaluated 16 patients who received subsequent CTx after progression on treatment with anti-PD-(L)1. In cohort B, 104 patients received CTx as a third-line treatment. Of those, we analysed 86 who received CTx without prior exposure to anti-PD-(L)1 (Figure 1). Median age was 67 *versus* 63 years in cohorts A and B, respectively. The portions of patients with a diffuse type and a peritoneal metastasis were higher in cohort B compared to cohort A (65% *vs.* 31%, $p=0.024$ and 58% *vs.* 25%; $p=0.027$, respectively). In contrast, cohort A included a higher number of patients who had a previous history of treatment with irinotecan (75% *vs.* 16%, respectively, $p<0.010$) and involved a higher median number of prior regimens (4 *vs.* 2, respectively, $p<0.010$) compared to cohort B. Moreover, the median time from the first-line treatment was longer in cohort A compared to cohort B (33.4 *vs.* 12.1 months, respectively, $p<0.010$). In cohorts A and B, the administered CTx comprised: i) FU + oxaliplatin (62% *vs.* 31%, respectively), ii) taxane (19% *vs.* 14%, respectively), and iii) irinotecan (19% *vs.* 55%, respectively). In cohort A, CTx was administered as a i) fourth- (19%), ii) fifth- (56%), and iii) sixth-line (25%) of treatment (Table I).

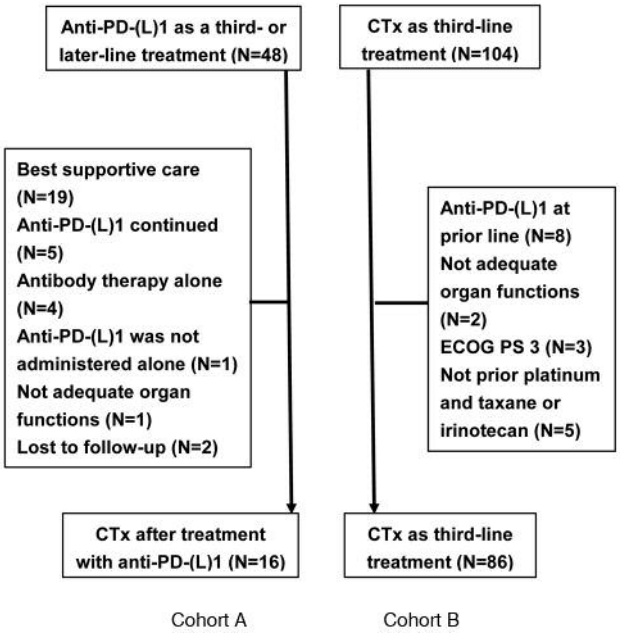

Figure 1. *Study flow chart. CTx: Chemotherapy; ECOG PS: Eastern Cooperative Oncology Group performance status.*

Efficacy. Among the 16 patients with measurable lesions included in cohort A, none presented a complete response, five showed partial response, and eight had stable disease (Figure 2). There was no relationship between the ORR to treatment with anti-PD-(L)1 and that of sequential CTx [odds ratio (OR)=3.00, 95% Confidence Interval (CI)=0.29-31.6, p=0.36]. The subsequent CTx showed effectiveness regardless of the response to treatment with anti-PD-(L)1. Two of the partial response cases, one of the stable disease cases, and two of the PD cases treated with anti-PD-L1 responded to subsequent CTx. Three patients who did not respond to treatment with anti-PD-(L)1 responded to CTx. Among the 68 patients with measurable lesions in cohort B, complete response, partial response, and stable disease were observed in one, six, and 20 patients, respectively. In the univariate analysis, the ORR was significantly higher in cohort A compared to cohort B (31% *vs*. 10%, respectively, OR=3.96, 95%CI=1.06-14.8, p=0.040). In the multivariate analysis, there was no significant difference between cohorts A and B in ORR (adjusted OR=1.93, 95%CI=0.41-9.02, p=0.40); however, we observed a similar tendency to that noted in the univariate analysis. In the univariate analysis, the DCR was also significantly higher in cohort A compared to cohort B (81% *vs*. 40%, respectively, p<0.010). In the multivariate analysis, the DCR was significantly better in cohort A (adjusted OR=5.96, 95%CI=1.49-23.8, p=0.012) (Table II).

The median TTF in cohorts A and B was 3.4 months (95%CI:1.6-3.9 months) and 1.9 months (95%CI=1.6-2.3 months), respectively. There was no statistically significant difference (hazard ratio:0.79, 95%CI:0.46-1.35; p=0.39); however, cohort A showed a tendency for longer TTF compared to cohort B. The median OS in cohorts A and B was 7.0 months (95%CI:4.2-8.4 months) and 4.9 months (95%CI:3.8-7.2 months), respectively. There was no statistically significant difference (hazard ratio=0.89, 95%CI=0.49-1.63, p=0.71) (Figure 3).

The subsequent treatment was administered as follows. In cohort A, six patients received another CTx, four patients chose best supportive care, and six patients were receiving ongoing therapy at the time of cut-off. In cohort B, 22 patients received another CTx, nine patients received treatment with anti-PD-(L)1, 51 patients received best supportive care, and four patients were receiving ongoing therapy. Excluding the cases of ongoing treatment, subsequent treatment was performed in 60% and 38% of patients in cohorts A and B, respectively.

AEs. CTx-related grade (G) 3-4 AEs observed in cohorts A and B were: i) neutropenia (56% *vs*. 30%, respectively), ii) anaemia (12% *vs*. 22%, respectively), iii) fatigue (6% *vs*. 2%, respectively), and iv) peripheral sensory neuropathy (6% *vs*. 5%, respectively). There was no significant difference observed between the cohorts in AEs (Table III).

In cohort A, three patients developed irAEs at the initiation of CTx. Two patients developed G 1 or 2 rash; one of them (G1) was managed only through follow-up, while the other (G2) was managed *via* the administration of prednisolone (10 mg) and application of steroid ointment. The remaining one patient developed both rash (G1) and pemphigoid (G1) prior to CTx, and was managed by administration of prednisolone (5 mg) and application of steroid ointment. These irAEs did not worsen and were manageable with the same treatment performed prior to CTx; there were no new irAEs observed during CTx (Table IV).

Discussion

To the best of our knowledge, this is the first study to investigate the efficacy and safety of CTx after ICI for mGC, proposing three important findings. Firstly, the ORR and DCR were higher in the patients who underwent CTx after ICI compared to those who received a third-line treatment without pre-ICI, despite intensive pre-treatment. Secondly, it was difficult to predict the effect, including the effect of pre-ICI therapy, based on patients' background. Thirdly, the rate of AE occurrence did not increase. In addition, irAEs that were present at the initiation of CTx did not worsen and were manageable, whereas new irAEs did not develop during CTx.

Table I. *Patient characteristics*.

	Cohort A, N=16 (%)	Cohort B, N=86 (%)	*p*-Value
Age			
Median (range)	67 (46-83)	63 (26-83)	0.11
<65 years/≥65 years	7 (44)/9 (56)	49 (57)/37 (43)	0.42
Gender			
Male/Female	13 (81)/3 (19)	58 (67)/28 (33)	0.38
ECOG PS			
0-1/2	14 (88)/2 (12)	75 (87)/11 (13)	1.0
Histology			
Intestinal/Diffuse	11 (69)/5 (31)	30 (35)/56 (65)	0.024
Prior gastrectomy			
No/Yes	7 (44)/9 (56)	47 (55)/39 (45)	0.59
HER2 status			
Negative/Positive/Unknown	9 (56)/7 (44)/0 (0)	62 (72)/23 (27)/1 (1)	0.23
Measurable lesions			
No/Yes	0 (0)/16 (100)	18 (21)/68 (79)	0.068
No. of metastatic sites			
1-2/≥3	9 (56)/7 (44)	57 (66)/29 (34)	0.57
Metastatic lesions			
Peritoneum (No/Yes)	12 (75)/4 (25)	36 (42)/50 (58)	0.027
Liver (No/Yes)	7 (44)/9 (56)	61 (71)/25 (29)	0.045
Lung (No/Yes)	12 (75)/4 (25)	74 (86)/12 (14)	0.27
Laboratory data			
ALP, IU/L (<ULN/≥ULN)	5 (31)/11 (69)	47 (55)/39 (45)	0.11
LDH, IU/L (<ULN/≥ULN)	6 (37)/10 (63)	56 (65)/30 (35)	0.051
CEA, ng/ml (<ULN/≥ULN)	3 (19)/13 (81)	32 (37)/54 (63)	0.25
Alb, g/dl (≥4.0/<4.0)	2 (12)/14 (88)	8 (9)/78 (91)	0.66
No. of prior regimen			
Median (range)	4 (3-5)	2 (2)	<0.010
2/3/4/5	0 (0)/3 (19)/9 (56)/4 (25)	86 (100)/0 (0)/0 (0)/0 (0)	<0.010
Prior CTx			
FU (No/Yes)	0 (0)/16 (100)	0 (0)/86 (100)	1.0
Platinum (No/Yes)	0 (0)/16 (100)	0 (0)/86 (100)	1.0
Taxane (No/Yes)	1 (6)/15 (94)	13 (15)/73 (85)	0.69
Irinotecan (No/Yes)	4 (25)/12 (75)	72 (84)/14 (16)	<0.010
CTx regimen			
FU + Ox	10 (62)	27 (31)	0.019
Taxane	3 (19)	12 (14)	
Irinotecan	3 (19)	47 (55)	
Time from the first-line CTx, months			
Median (range)	33.4 (12.7-68.1)	12.0 (3.8-56.3)	<0.010
≥6/<6	16 (100)/0 (0)	77 (90)/9 (10)	0.35

Alb: Albumin; ALP: alkaline phosphatase; CEA: carcinoembryonic antigen; CTx: chemotherapy; ECOG PS: Eastern Cooperative Oncology Group performance status; FU: fluoropyrimidines; HER2: human epidermal growth factor receptor 2; LDH: lactate dehydrogenase; Ox: oxaliplatin; Taxane, paclitaxel, nab-paclitaxel, or docetaxel; ULN: upper limit of the normal.

Use of CTx after treatment with ICI has been retrospectively reported for NSCLC, melanoma, head and neck cancer, and renal cell carcinoma. The clinical outcome of this study was consistent with those previously reported, stating that the ORR and DCR were better in the CTx after ICI group compared to the control group. In this study, patients receiving third-line CTx without pre-ICI formed the control group. The JAVELIN Gastric 300 trial compared avelumab with CTx in the third-line treatment of mGC; the ORR was 4.3% (95%CI=1.9-8.3) in the CTx group (12). Data obtained from retrospective analyses showed that the ORR to irinotecan therapy in the third-line treatment was 1.9-18.4% (13-15). In our study, the ORR of the control group was 9%. Therefore, it was considered equivalent to those previously reported, and the selection of the control group in this study was appropriate.

Several mechanisms through which CTx promotes tumour immunity have been investigated (16). CTx induces

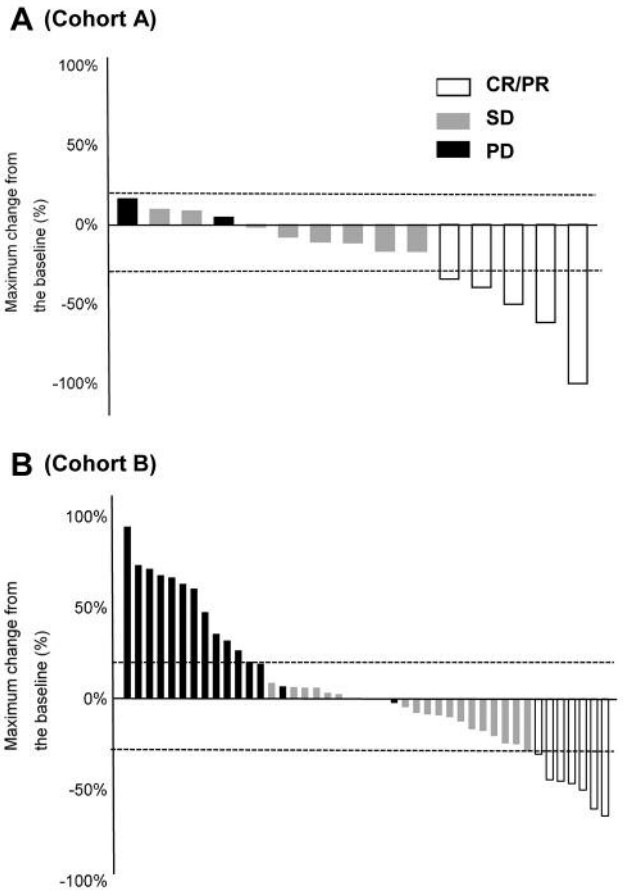

Figure 2. *Best tumor response according to cohorts A (A) and B (B). Waterfall plot for best tumor response in the patients with measurable lesions, according to the RECIST criteria, version 1.1. CR: complete response; PD: progressive disease; PR: partial response; SD: stable disease.*

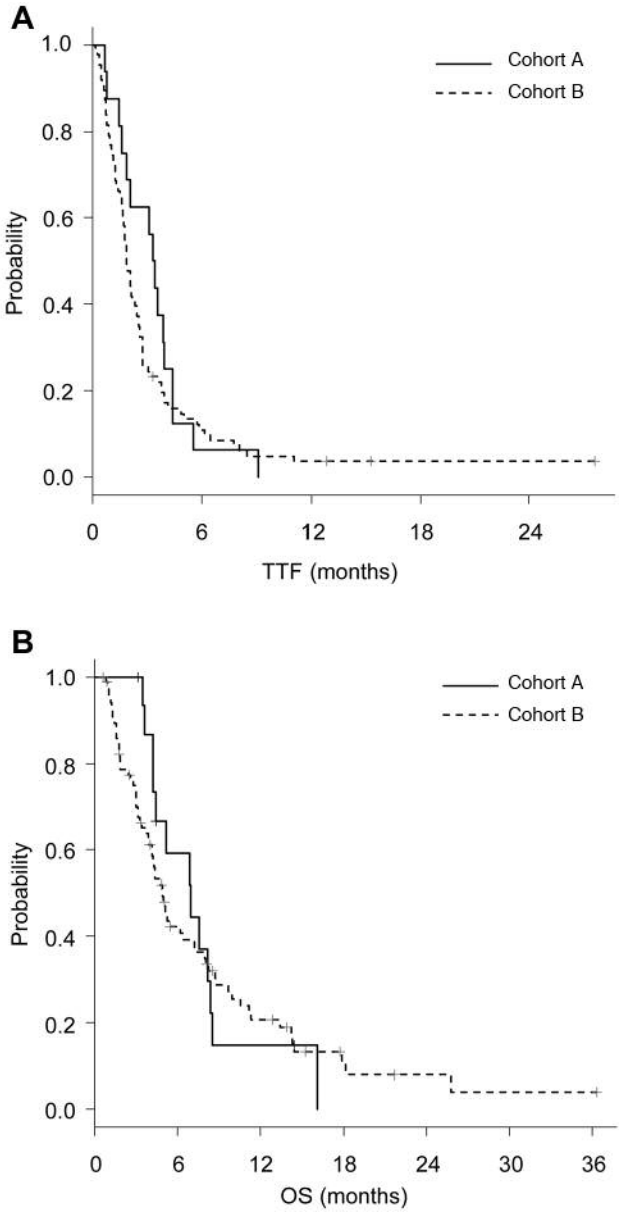

Figure 3. *Time to treatment failure (TTF) and overall survival (OS). Kaplan-Meier survival curves for TTF (A) and OS (B) according to cohorts A and B.*

immunogenic cell death and can enhance cytotoxic T-cell responses. On the other hand, lymphopenia induced by CTx can promote antigen-specific T-cell responses, thereby augmenting antitumor immunity, particularly during the recovery phase from lymphopenia. For example, it has been shown that a subset of circulating CD8+ T cells expressing the chemokine receptor CX3CR1 are able to withstand the toxicity of CTx and increase in patients with metastatic melanoma who respond to chemoimmunotherapy (17). Preclinical data have indicated that nivolumab controlled the PD-1 on the surface of lymphocytes for >2 months (18). In the KEYNOTE-062 trial, subgroup analysis showed that the progression-free survival was lower or equal in the pembrolizumab alone group in Asians compared to that recorded for the CTx group, even though the OS was better in the former. In fact, post-progression survival was longer in the pembrolizumab group compared to the CTx group. The reason for this difference is the high implementation rate of subsequent CTx in Asian countries and the enhanced effectiveness of CTx after treatment with pembrolizumab (19).

In the univariate analysis of this study, the ORR was significantly worse in patients treated with irinotecan. Moreover, the number of patients who received irinotecan was higher in cohort B compared to A. Preclinical data have shown that irinotecan in combination with the anti-PD-L1 antibody decrease the number of regulatory T cells and

Table II. *Univariate and multivariate analyses for ORR/DCR.*

Variable	ORR				DCR			
	Univariate		Multivariate		Univariate		Multivariate	
	OR (95%CI)	*p*-Value	OR (95%CI)	*p*-Value	OR (95%CI)	*p*-Value	OR (95%CI)	*p*-Value
Age								
≥65 years vs. <65 years	1.75 (0.51-6.04)	0.38			1.60 (0.67-3.79)	0.29		
Gender								
Female *vs*. Male	0.46 (0.09-2.25)	0.33			1.58 (0.66-3.77)	0.30		
ECOG PS								
2 *vs*. 0-1	1.11 (0.21-5.77)	0.90			0.43 (0.12-1.53)	0.19		
Histology								
Diffuse *vs*. Intestinal	1.94 (0.54-7.04)	0.31			0.47 (0.19-1.11)	0.086	0.50 (0.19-1.30)	0.16
Prior gastrectomy								
Yes *vs*. No	1.96 (0.57-6.77)	0.29			1.93 (0.81-4.63)	0.14		
HER2 status								
Pos. *vs*. NE/Neg.	1.07 (0.29-3.90)	0.92			1.60 (0.64-4.02)	0.32		
No. of metastatic sites								
≥3 *vs*. 1-2	1.12 (0.32-3.89)	0.86			0.71 (0.29-1.71)	0.44		
Metastatic lesions								
P Yes *vs*. No	0.33 (0.08-1.33)	0.12	4.59 (1.03-20.5)	0.046	0.61 (0.26-1.45)	0.26		
H Yes *vs*. No	3.54 (0.97-12.9)	0.055			0.65 (0.27-1.56)	0.33		
L Yes *vs*. No	0.34 (0.04-3.17)	0.37			0.69 (0.22-2.14)	0.52		
ALP, iu/l								
≥ULN *vs*. <ULN	1.53 (0.45-5.18)	0.49	6.88 (0.94-50.2)	0.057	0.51 (0.22-1.22)	0.13	9.07 (0.95-86.4)	0.055
LDH, iu/l								
≥ULN *vs*. <ULN	1.85 (0.54-6.39)	0.33			0.55 (0.23-1.31)	0.18		
CEA, ng/ml								
≥ULN *vs*. <ULN	0.88 (0.24-3.23)	0.85			1.71 (0.67-4.40)	0.26		
Alb, g/dl								
≥4.0 *vs*. <4.0	5.67 (1.09-29.5)	0.039			7.59 (0.87-66.1)	0.067		
No. of prior regimens								
≥3 vs. 2	3.96 (1.06-14.8)	0.040			6.58 (1.71-25.3)	<0.01		
CTx regimen								
irinotecan *vs*. FU + Ox, taxane	0.20 (0.04-0.98)	0.047	0.23 (0.04-1.35)	0.11	0.81 (0.34-1.92)	0.63		
Time from the first-line CTx, months								
<6 vs. ≥6	1.55 (0.16-15.1)	0.71			0.26 (0.03-2.40)	0.23		
Cohort								
A vs. B	3.96 (1.06-14.8)	0.040	1.93 (0.41-9.02)	0.40	6.58 (1.71-25.3)	<0.01	5.96 (1.49-23.8)	0.012

Alb: Albumin; ALP: alkaline phosphatase; CEA: carcinoembryonic antigen; CI: confidence interval; CTx: chemotherapy; DCR: disease control rate; ECOG PS: Eastern Cooperative Oncology Group performance status; H: liver; L: lung; LDH: lactate dehydrogenase; NE: not evaluated; Neg.: negative; OR: odds ratio; ORR: overall response rate; P: peritoneum; Pos.: positive; ULN: upper limit of the normal.

enhance the proliferation of CD8+ cells in both tumours and lymph nodes (20). A report recently demonstrated that oxaliplatin and paclitaxel induce immunogenic cell death, increase the level of tumour antigen presented by antigen presenting cells, and prevent the suppression of immune responses *via* STAT6 (21-25). Despite the availability of preclinical data regarding the interaction of irinotecan, oxaliplatin, and paclitaxel with ICI, the differences in the effects of these regimens remain unclear.

In this study, there was no association between subsequent CTx and pre-ICI. However, the relationship between the effects of CTx and pre-ICI was reported in NSCLC. Park *et al*. have reported that the ORR of subsequent CTx was 71.4% in patients who had previously achieved partial response to PD-(L)1 inhibitors; the ORR in all other patients was 51.5% (p=0.31) (5). Activation of lymphocytes by ICI may increase the effectiveness of subsequent CTx. In a preclinical study, non-specifically activated CD4+ T cells were used as a chemosensitizer before the administration of CTx in *in vitro* and *in vivo* tumour xenograft models. The results showed a drastic cytotoxic enhancement by CTx, either as active or nonactive single agents, after exposure to CD4+ T cells (26).

Table III. *Adverse events related to chemotherapy.*

	Cohort A (N=16)		Cohort B (N=86)		*p*-Value
	Any G N (%)	G≥3 N (%)	Any G N (%)	G≥3 N (%)	
Haematological					
Neutropenia	11 (69)	9 (56)	50 (58)	26 (30)	0.082
Anaemia	16 (100)	2 (12)	84 (98)	19 (22)	0.51
Thrombocytopenia	6 (38)	0 (0)	30 (35)	3 (3)	1.0
Non-haematological					
Nausea	8 (50)	0 (0)	42 (49)	0 (0)	1.0
Diarrhoea	7 (44)	0 (0)	28 (33)	2 (2)	1.0
Constipation	6 (38)	0 (0)	21 (24)	0 (0)	1.0
Fatigue	12 (75)	1 (6)	44 (51)	2 (2)	0.40
Anorexia	10 (63)	0 (0)	61 (71)	5 (6)	1.0
Stomatitis	1 (6)	0 (0)	6 (7)	0 (0)	1.0
Peripheral sensory neuropathy	8 (50)	1 (6)	47 (55)	4 (5)	0.58
Increased AST	5 (31)	0 (0)	44 (51)	5 (6)	1.0
Increased ALT	4 (25)	0 (0)	36 (42)	3 (3)	1.0
Febrile neutropenia	0 (0)	0 (0)	5 (6)	5 (6)	1.0

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; G: grade.

Table IV. *Immune-related adverse events at the initiation and during chemotherapy.*

	Remaining irAEs (Grade)	Management		Outcomes
		At the initiation of CTx	During CTx	
1	Rash (1)	Follow-up	Follow-up	Not recovered
2	Rash (1)	Steroid ointment	Follow-up	Recovered
3	Rash (1) Pemphigoid (1)	PSL 10 mg, po Steroid ointment	PSL 5 mg, po Steroid ointment	Recovered

CTx: Chemotherapy; irAEs: immune-related adverse events; po: per os; PSL: prednisolone.

Although there was no increase in AEs in this study, worsening of AEs during TKI therapy after administration of ICI has been reported. In EGFR mutation-positive lung cancer, it has been suggested that the use of EGFR TKI increases the risk of interstitial pneumonia after administration of ICI (8). In patients with melanoma, there are reports that skin, liver, and neurological disorders occur or worsen following the use of BRAF inhibitors after treatment with ICI (27-29). Thus far, there are no reports of AEs caused by cytotoxic agents after ICI. Studies with longer follow-ups and higher numbers of cases are warranted to determine whether the occurrence of AEs (including irAEs) increases with CTx after ICI.

There were several limitations in our study. Firstly, biomarker analysis (*e.g.*, MSI-H, PD-L1 positivity, etc.) was not performed, which may be responsible for not detecting the predictive effectiveness of CTx. Secondly, this was a retrospective study performed at the single institution with a small sample size and a highly selected population. Finally, the CTx regimens included various types of agents. An ongoing prospective, observational study evaluating the efficacy and safety of CTx (*i.e.*, irinotecan, oxaliplatin combination regimen, or trifluridine/tipiracil) after therapy with nivolumab for mGC (UMIN000032182) will provide further insight into this strategy.

In conclusion, the findings of this study suggest that the use of CTx after progression on anti-PD-(L)1 antibody is favourable efficacy and feasibility, even in patients with mGC who have undergone intensive pre-treatment.

Conflicts of Interest

The Authors declare the following conflicts of interest: YN reports grants and personal fees from Ono and Bristol-Mayers Squibb, personal fees from Eli Lilly, Yakult Honsha, Daiichi Sankyo, and Taiho, outside the submitted work; SM reports personal fees from Taiho, Eli Lilly, Takeda, and Ono, outside the submitted work; TM reports grants from MSD, Daiichi Sankyo, and Ono, personal fees from Takeda, Chugai, Merck Serono, Taiho, Bayer, Eli Lilly, Yakult Honsha, and Sanofi, outside the submitted work; HT reports grants and personal fees from Takeda, grants from Daiichi Sankyo and Sysmex, personal fees from Eli Lilly, Taiho, and Chugai, outside the submitted work; SK reports grants and personal fees from Taiho, Eli Lilly, and Ono, grants from Merck Sharp and Dohme, personal fees from Bristol-Myers Squibb, Yakult Honsha, Chugai, Bayer, and Merck Serono, outside the submitted work; MA reports personal fees from Astra Zeneca, Novartis, Chugai, Kyowa Hakko Kirin, Eisai, Ono, Eli Lilly, and Taiho, outside the submitted work; MT reports personal fees from EA Pharma and Olympus Corporation, outside the submitted work; KM reports grants and personal fees from Ono and Sanofi, grants from Daiichi Sankyo, Parexel International, Shionogi Pharma, Sumitomo Dainippon, MSD, Pfizer, Mediscience Planning, Solasia Pharma, personal fees from Eli Lilly, Chugai, Takeda, Taiho, Bristol-Myers Squibb, and Bayer, outside the submitted work. No financial support or compensation was received for the study or its publication.

Authors' Contributions

KK and YN contributed equally to this work, collected the data, generated and edited the figures, and wrote the manuscript. The manuscript was reviewed by SM, KH, TM, HT, SK, TU, MA, MT, and KM. All authors read and approved the final version of the manuscript.

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