Efficacy of timing-dependent infusion of nivolumab in patients with advanced gastric cancer

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Abstract. Although an association exists between the timing of immune checkpoint inhibitor (ICI) administration and therapeutic efficacy in several types of cancer, to the best of our knowledge, no reports exist regarding this relationship in gastric cancer (GC). The present study aimed to evaluate the optimal timing of ICI (nivolumab) administration in patients with advanced GC. A total of 58 consecutive patients with advanced GC who received nivolumab monotherapy after ≥2 chemotherapy regimens were retrospectively evaluated. These patients were divided into two groups according to the median time of nivolumab administration: i) Early-timing and (ii) late-timing groups, and the efficacy was assessed in both groups. The early-timing group had significantly longer overall survival (OS) than the late-timing group [median OS 8.2 months; 95% confidence interval (CI), 4.2-12.9 vs. median OS 5.4 months; 95% CI, 3.6-6.1]. Moreover, patients in the early-timing group had significantly longer progression-free survival (PFS) than those in the late-timing group (median PFS 2.6 months; 95% CI, 1.3-3.9 months vs. median PFS 1.6 months; 95% CI, 0.9-2.1 months). Furthermore, univariate analysis showed that early timing, immune-related adverse events and nonsteroidal anti-inflammatory drug administration were associated with longer OS and PFS. Cutoff Finder analysis revealed that the optimal timing of nivolumab administration for achieving better outcomes was before 12:06 p.m. Nivolumab administration in the morning, especially before 12:06 p.m., had a better clinical impact on patients with advanced GC.

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Abbreviations: ICI, immune checkpoint inhibitor; GC, gastric cancer; CI, confidence interval; PFS, progression-free survival; HER2, human epidermal growth factor receptor 2; DCs, dendritic cells; PS, performance status; irAEs, immune-related adverse events; CRP, C-reactive protein; CEA, carcinoembryonic antigen; NLR, neutrophil-to-lymphocyte ratio; NSAIDs, nonsteroidal anti-inflammatory drugs; HR, hazard ratio; ORR, overall response rate; NSCLC, non-small cell lung cancer; OS, overall survival

Key words: GC, circadian rhythm, nivolumab, immunotherapy

Introduction

The emergence of immunotherapy has marked a significant turning point in the prognosis of patients with various types of advanced cancer (1). In 2017, the results of the phase III clinical trial 'ATTRACTION-2' indicated that nivolumab, an immune checkpoint inhibitor (ICI), significantly prolonged overall survival (OS) compared to placebo, making nivolumab monotherapy the standard of care for patients with advanced gastric cancer (GC) after ≥2 chemotherapy regimens (2,3).

Advanced gastric cancer is characterized by enhanced cell proliferation through various proliferation signals, such as transforming growth factor-beta signaling (4). The human epidermal growth factor receptor 2 (*HER2*) is among the most common oncogenes involved in this process. The *HER2* gene, which has a structure similar to that of the epidermal growth factor receptor gene, is involved in cell proliferation and differentiation. ATTRACTION2 subgroup analysis showed prolonged OS with nivolumab alone compared to placebo, regardless of HER2 status or whether patients received anti-HER2 drugs (5).

Furthermore, in 2021, the CheckMate 649 and ATTRACTION-4 trials demonstrated an add-on effect of nivolumab on chemotherapy, making this combination therapy the standard first-line treatment for these patients (6,7). Although clinical trials have demonstrated the efficacy of nivolumab, a critical medical need persists to determine the patient population that would derive the most significant advantage from its use.

Recent studies have reported a direct effect of concomitant medications on ICI treatment (8-10). In particular, antibiotic administration during ICI treatment exhibits a poor prognosis

in various carcinomas (10-12). However, few studies have compared the prognostic value of ICI therapy and concurrent drugs for gastric cancer.

Immunotherapy primarily exerts antitumor effects by modulating the interactions between cancer cells and immune cells, such as T lymphocytes, natural killer cells, and dendritic cells (DCs), which are responsible for recognizing and attacking cancer cells. Specifically, immunotherapy targets key signaling pathways, such as PD-1/PD-L1 and cytotoxic T-lymphocyte-associated protein 4, which regulate immune responses to cancer cells (1). Thus, the success of immunotherapy against cancer depends heavily on the activation of immune cells.

The circadian rhythm has a well-established impact on the number of circulating immune cells in the bloodstream (13). This rhythm influences the immune parameters of these cells, such as their functional activity and transport capacity, which vary throughout the day. Therefore, the effectiveness of cancer immunosurveillance may vary at different times of the day, emphasizing the significance of the timing of immunotherapy administration (14). Previous research has proposed that patients with advanced solid tumors who receive ICI infusions in the morning may experience longer OS than those who receive infusions in the afternoon (15-19). Currently, no information is available regarding the association between the timing of ICI administration and its clinical effectiveness in patients with advanced GC.

This study aimed to evaluate the optimal timing of nivolumab administration in patients with advanced GC to determine its clinical efficacy.

Materials and methods

Study design and patients. We retrospectively evaluated 58 consecutive patients with advanced GC (stage IV) who received nivolumab monotherapy after ≥2 chemotherapy regimens at Kurume University Hospital (Kurume, Japan) between October 2017 and December 2023. All patients had histologically confirmed advanced GC with a performance status (PS) of ≤2. The following data were collected from medical charts and reviewed: age, sex, PS, previous gastrectomy, liver metastasis, peritoneal dissemination, number of organs with metastases, HER2 status, occurrence of immune-related adverse events (irAEs), serum levels of C-reactive protein (CRP), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9, and neutrophil-to-lymphocyte ratio (NLR), treatment method, and outcome. We defined drug administration (e.g., proton pump inhibitors (PPIs), nonsteroidal anti-inflammatory drugs (NSAIDs), opioid analgesics, probiotics, and antibiotics) as drug use during treatment and/or within 6 months before the date of the first nivolumab treatment.

The median of all cases was used to stratify the cutoff for this administration time. For example, if only one cycle of nivolumab was administered for each case, the time was defined as the administration time. In cases where multiple cycles of nivolumab were administered, the median value was defined as the administration time. The median of all cases was analyzed as the cutoff value. Patients were divided into two groups according to the median timing of nivolumab administration: (I) those who received nivolumab earlier than the median start time (before 11:41 a.m) (early-timing group) and (II) those who received nivolumab later (after 11:41 a.m) (late-timing group).

This retrospective study was performed at the Kurume University Hospital (Kurume, Japan). This study conformed to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Kurume University (approval number 21118). An opt-out approach was employed to obtain informed consent from patients, and personal information was protected during data collection.

Evaluation of therapeutic response and definition of progression-free survival (PFS) and OS. Therapeutic response was evaluated at 8-week intervals using dynamic computed tomography or magnetic resonance imaging scans following the Response Evaluation Criteria in Solid Tumors version 1.1 (20). PFS was defined as the time from the enrollment date to disease progression with a 20% or greater increase in the diameters of target lesions or the appearance of new lesions during treatment or to death from any cause. OS was defined as the duration from enrollment to death from any cause.

Nivolumab dosing regimen and schedule. The patients were intravenously administered nivolumab at a dose of 240 mg for 30 min once every 2 weeks, following the clinical guidelines for advanced GC (21). This dose was continued until disease progression, deterioration of the patient's condition, the onset of intolerable adverse effects, or the patient's decision to discontinue treatment.

Statistical analysis. Categorical variables were compared using the χ^2 test or the Fisher's exact test, while continuous variables were compared using the Wilcoxon rank sum test. Univariate and multivariate analyses were conducted using the Cox regression model to identify risk factors associated with OS or PFS. OS and PFS were calculated using the Kaplan-Meier method and assessed using the log-rank test. To evaluate the optimal cutoff value for administered time, we employed the Cutoff Finder application (22). The optimal cutoff was defined as the point with the most significant split (log-rank test). $P<0.05$ was considered to indicate statistical significance. Data analysis was performed using JMP Pro version 16.0 and SAS 9.4 (both from the SAS Institute, Inc., NC, USA).

Results

Baseline patient characteristics. In this study, the median timing of nivolumab administration was 11:41 a.m. Subsequently, we divided the patients into early- and late-timing groups, comprising a total of 29 patients. The early group received nivolumab between 9:40 and 11:40 a.m., whereas the late group received it between 12:20 and 3:30 p.m. The histograms depict the administration times for both groups (Fig. S1). In the early timing group, most cases were administered nivolumab from 10:00 to 11:41 a.m. In contrast, the late timing group received it any time after 11:41 a.m. Baseline patient characteristics are summarized in Table I. No substantial differences were observed in age, PS, previous gastrectomy, liver metastasis, peritoneal dissemination,

Table I. Baseline patient characteristics.

Characteristic	Early timing (n=29)	Late timing (n=29)	P-value
Median time of administration (IQR)	10:48 a.m. (9:40 a.m. -11:38 a.m., 9:33 a.m.-11:21 a.m.)	12:57 p.m. (11:45 a.m. -3:30 p.m., 12:03 p.m.-1:40 p.m.)	
Median age, years (IQR)	69 (67-73)	65 (57-74)	0.22
Sex, female/male	10%/90% (3/26)	24%/76% (7/22)	0.30
Performance status, 0/1/2	45%/45%/10% (13/13/3)	38%/52%/10% (11/15/3)	0.86
Previous gastrectomy, no/yes	72%/28% (21/8)	59%/21% (17/12)	0.27
Liver metastasis, absence/presence	52%/48% (15/14)	69%/31% (20/9)	0.18
Peritoneal dissemination, absence/presence	52%/48% (15/14)	28%/72% (8/21)	0.06
Number of organs with metastases, <2/≥2	62%/38% (18/11)	62%/38% (18/11)	1.00
HER2 status, negative/positive	72%/28% (21/8)	83%/17% (24/5)	0.34
irAEs, no/yes	73%/17% (24/5)	79%/21% (23/6)	0.73
Median C-reactive protein, mg/dl (IQR)	0.30 (0.04-9.03)	0.39 (0.04-6.04)	0.27
Median CEA, ng/dl (IQR)	3.3 (1.2-82.2)	5.7 (1.6-226)	0.14
Median CA19-9, ng/d (IQR)	43.8 (2.3-1,537)	16.5 (2-8,477)	0.94
Median neutrophil-to-lymphocyte ratio (IQR)	2.58 (0.82-6.72)	3.22 (0.77-18.3)	0.09
Medicine			
Proton pump inhibitor, no/yes	34%/66% (10/19)	28%/72% (8/21)	0.57
NSAIDs, no/yes	55%/45% (16/13)	31%/69% (9/20)	0.06
Opioid analgesics, no/yes	62%/38% (18/11)	59%/41% (17/12)	0.79
Probiotics, no/yes	83%/17% (24/5)	79%/21% (23/6)	0.74
Antibiotic, no/yes	69%/31% (20/9)	67%/33% (19/10)	0.78

Values are expressed as median (IQR) or percentage (value). IQR, interquartile range; HER2, human epidermal growth factor receptor 2; irAEs, immune-related adverse events; NSAIDs, nonsteroidal anti-inflammatory drugs; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen.

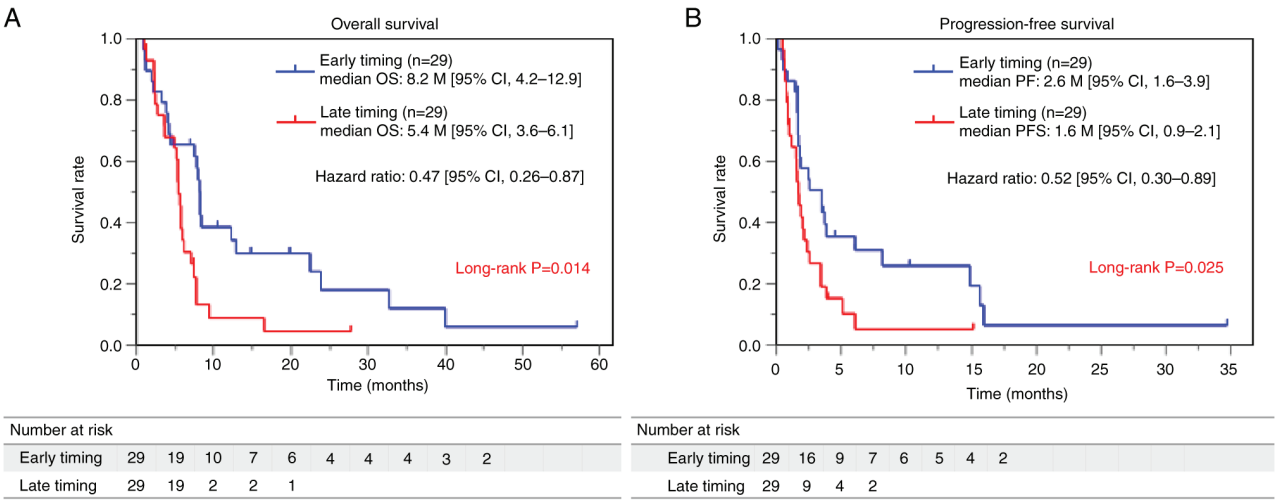

Figure 1. Kaplan-Meier curves for OS and PFS between the early- and late-timing groups. (A) OS and (B) PFS following nivolumab treatment in the early timing (n=29) and late timing (n=29) groups. The blue and red lines represent the early-timing and late-timing groups, respectively. OS, overall survival; PFS, progression-free survival; CI, confidence interval.

number of organs with metastases, HER2 status, irAEs, NLR, administration of proton pump inhibitors, opioid analgesics, NSAIDs, probiotics, antibiotics, serum CRP levels, or CEA levels between the two groups. However, the prevalence of male sex was significantly higher in the early-timing group than in the late-timing group (Table I).

Patients in the early-timing group had a long PFS and OS with a better therapeutic response. To assess the effect of the timing of drug administration on PFS and OS, we plotted Kaplan-Meier curves for the two groups (Fig. 1A and B). The early-timing group demonstrated a significantly longer OS compared to the late-timing group (median OS 8.2 months [95% confidence

Table II. Univariate analysis of OS and PFS using the Cox regression model (n=58).

		OS			PFS		
Characteristic	Cut-off	HR	95% CI	P-value	HR	95% CI	P-value
Age	>65 years	1.31	0.74-2.33	0.36	1.18	0.69-2.01	0.53
Sex	Female	1.26	0.59-2.70	0.55	1.01	0.51-2.01	0.97
Performance status	>1	1.25	0.70-2.24	0.46	1.07	0.63-1.83	0.80
Nivolumab administration timing	Early	0.47	0.26-0.87	0.02	0.52	0.30-0.89	0.02
Number of metastatic sites	≥2	0.92	0.51-1.65	0.78	0.73	0.42-1.25	0.25
Liver metastasis	Yes	0.93	0.52-1.66	0.80	0.88	0.51-1.50	0.63
Peritoneal dissemination	Yes	1.65	0.90-3.04	0.11	1.42	0.82-2.44	0.20
Previous gastrectomy	Yes	1.24	0.67-2.30	0.50	1.51	0.86-2.63	0.16
IrAE	Yes	0.27	0.12-0.62	<0.01	0.31	0.15-0.64	<0.01
C-reactive protein	>0.4 mg/dl	1.11	0.63-1.99	0.71	1.13	0.63-2.00	0.69
CEA	>5.0 ng/dl	0.85	0.48-1.52	0.59	1.13	0.67-1.91	0.65
CA19-9	>37 ng/dl	0.87	0.49-1.55	0.64	1.06	0.63-1.78	0.83
Neutrophil-to-lymphocyte ratio	>3.0	1.10	0.62-1.95	0.75	0.93	0.55-1.57	0.92
Medicine							
Proton pump inhibitor	Yes	0.83	0.45-1.54	0.56	0.70	0.37-1.30	0.26
NSAIDs	Yes	2.66	1.42-4.97	<0.01	2.50	1.40-4.60	<0.01
Opioid analgesics	Yes	1.31	0.73-2.36	0.36	1.33	0.75-2.38	0.33
Probiotics	Yes	1.45	0.71-2.95	0.30	1.56	0.77-3.20	0.22
Antibiotic	Yes	1.46	0.81-2.63	0.21	1.55	0.86-2.79	0.14

NSAIDs, nonsteroidal anti-inflammatory drugs; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen; HR, hazard ratio; CI, confidence interval.

interval (CI), 4.2-12.9] vs. 5.4 months; 95% CI, 3.6-6.1: hazard ratio (HR) 0.47; 95% CI, 0.26-0.87) (Fig. 1A). Moreover, patients in the early-timing group demonstrated a substantially longer PFS than those in the late-timing group (median PFS 2.6 months, with a 95% CI of 1.3-3.9 months vs. median PFS 1.6 months, with a 95% CI of 0.9-2.1 months). The HR for the early-timing group was 0.52, with a 95% CI of 0.30-0.89 (Fig. 1B).

The overall response rate (ORR) was greater in the early-timing group, with three patients achieving a complete or partial response (17.2%), than in the late-timing group (3.4%) (Fig. 2). The ORR was higher in the early-timing group than in the late-timing group (17.2% vs. 3.4%) (Fig. 2). The disease control rate was higher in the early-timing group than in the late-timing group (48.3% vs. 31.0%) (Fig. 2).

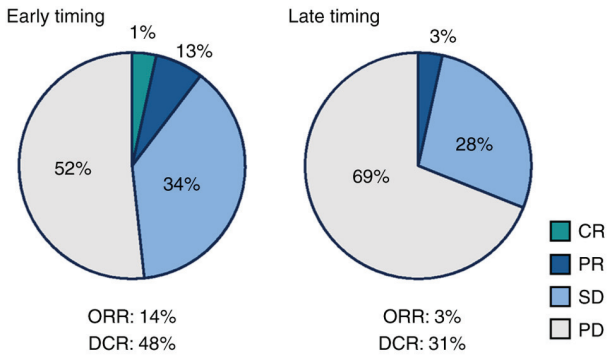

Figure 2. Best overall response between the early- and late-timing groups. Pie graphs indicate the percentages of patients who achieved CR (green), PR (blue), SD (light blue), or PD (gray). The ORR and DCR were determined as the proportions of patients who achieved CR, PR, CR, PR, and SD, respectively. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, overall response rate; DCR, disease control rate.

Evaluation of concomitant medications and prognosis in treatment with nivolumab. We used Kaplan-Meier survival curves to assess the effect of concomitant medications with nivolumab on OS (Fig. S2). The group not administered NSAIDs had a significantly better prognosis than the NSAID-administered group (median OS 8.2 months [95% CI, 5.4-32.7] vs. 5.4 months [95% CI, 3.3-6.1]). However, the use of concomitant medications, such as PPIs, opioid analgesics, probiotics, and antibiotics, did not affect prognosis.

Univariate Cox model analyses for PFS and OS. A univariate analysis was performed to identify factors associated with OS and PFS. In the analysis, the early-timing group, irAEs, and NSAID administration were associated with a longer OS. Additionally, univariate analysis showed that early timing, irAEs, and NSAID administration were associated with longer PFS (Table II).

Multivariate analysis and optimizing the correlation with OS. We performed multivariable Cox regression analysis to adjust for baseline patient background in the early- and late-timing

Table III. Multivariate Cox regression analysis to adjust for baseline patient background.

		PFS			OS		
Model	Adjusted value	HR	95% CI	P-value	HR	95% CI	P-value
0	Unadjusted	0.52	0.30-0.89	0.017	0.47	0.26-0.87	0.016
1	Age, sex, PS	0.50	0.29-0.86	0.013	0.45	0.24-0.85	0.013
2	IrAEs, NSAIDs	0.42	0.24-0.76	0.004	0.39	0.19-0.77	0.001
3	Age, sex, PS, irAEs, NSAIDs	0.36	0.20-0.67	0.001	0.34	0.17-0.69	0.003

PS, performance status; irAEs, immune-related adverse events; NSAIDs, nonsteroidal anti-inflammatory drugs; HR, hazard ratio; CI, confidence interval.

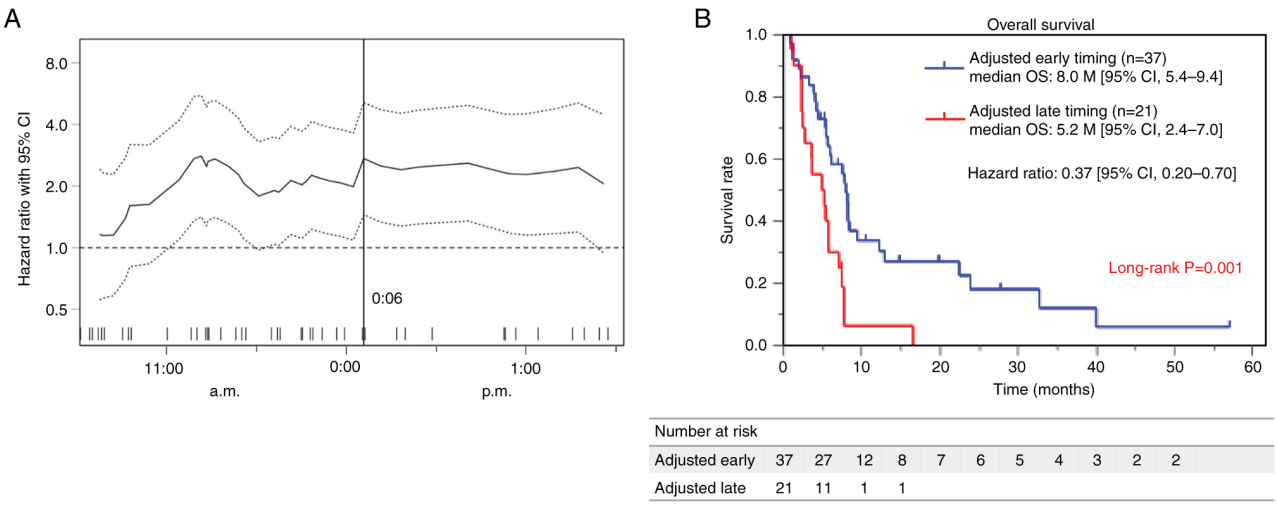

Figure 3. Plot of the differences in hazard ratio of OS for each cutoff value of the timing of administration. (A) Significance (P<0.05) test: Thirty of the 38 patients (78.9%). (B) Kaplan-Meier curve using cutoff value of the timing of administration. OS, overall survival; CI, confidence interval.

groups (Table III). The early-timing group had significantly better PFS than the late-timing group in model 1, adjusted for age, sex, and PS (HR, 0.50; 95% CI, 0.29-0.86). In addition, the early-timing group had significantly better OS than the late-timing group in model 1, adjusted for age, sex, and PS (HR, 0.45; 95% CI, 0.24-0.85). Next, in model 2, adjusted for irAEs and NSAIDs, which were identified as prognostic factors in this study, PFS/OS was significantly longer in the early-timing group than in the late-timing group (HR 0.42; 95% CI, 0.24-0.76; P=0.004/HR 0.39; 95% CI, 0.19-0.77; P=0.001), respectively. Finally, in model 3, adjusted for age, sex, PS, irAEs, and NSAIDs, the early-timing group had significantly longer PFS than the late-timing group (HR 0.36; 95% CI, 0.20-0.67). In addition, in model 3, adjusted for age, sex, PS, irAE, and NSAIDs, the early-timing group had significantly better OS than the late-timing group (HR 0.34; 95% CI, 0.17-0.69) (Table III).

The optimal cutoff value for survival, determined using the Cutoff Finder application, was 12:06 p.m. (Fig. 3A). We compared the OS between the two groups, which were divided by this cutoff, using the Kaplan-Meier curve. Patients in the early-timing group demonstrated a substantially longer OS than those in the late-timing group (median OS 8.0 months, with a 95% CI of 5.4-9.4 months vs. median of 5.2 months, with a 95% CI of 2.4-7.0 months, P=0.001) (Fig. 3B).

Discussion

To the best of our knowledge, this is the first report on patients with advanced GC stratified according to the timing of nivolumab infusion. The results of this study indicate that individuals who received nivolumab infusion earlier in the day, specifically between 9:40 and 11:40 a.m., experienced significantly better outcomes in terms of ORR, PFS, and OS than those who received the infusion later in the day, between 12:20 and 3:30 p.m. These results remained consistent after multivariable Cox regression analysis. Moreover, Cutoff Finder analysis revealed that the optimal timing of nivolumab administration for achieving better outcomes was before 12:06 p.m. The findings of these studies collectively provide evidence for the enhanced efficacy of nivolumab administration in the morning.

The relationship between circadian rhythms and the adaptive immune system is an area of growing research interest, with potential implications for ICI treatment. The influence of specific time-of-day patterns of ICI injections on therapeutic efficacy is highly debated. Several studies have

been conducted on various solid tumors, including melanoma, non-small cell lung cancer (NSCLC), and colorectal, head and neck, breast, urinary, renal, and pancreatic cancer. A recent study reported that pembrolizumab monotherapy for advanced esophageal cancer resulted in a better prognosis when treatment was initiated in the morning than in the afternoon (19). These studies suggest that administering ICIs early in the morning rather than in the evening improves clinical outcomes (15-19). Thus, our findings align with a growing body of evidence that adaptive immune responses are more robust when initially stimulated in the morning than in the evening. The circadian oscillation of lymphocytes is believed to be an underlying mechanism. According to research conducted by Wang *et al*, the capacity of immune cells to identify and eradicate cancer cells depends on their biological clock (23). According to various experiments and human studies, $CD8^+$ T lymphocytes promote blood circulation at night and distinguish cancer antigens in the early morning to eradicate target cells based on the serum level of cortisol (24,25). In cellular experiments on melanoma and colorectal cancer, treatment with anti-PD-1 antibody therapy was more effective in the morning than in the evening (26). This variation in efficacy based on timing was critically dependent on CD8+ T cells, and anti-PD-1 therapy was ineffective in the evening because CD8+ T cells were depleted (26). According to recent studies, DCs and $CD8^+$ T cells are crucial in regulating tumor volume through a circadian antitumor function. Additionally, the rhythmic migration of DCs to lymph nodes draining from the tumor appears to be a key factor in the circadian response of tumor antigen-specific $CD8^+$ T lymphocytes, which is dependent on the circadian expression of the costimulatory molecule CD80 (23). Another study found that T lymphocytes did not migrate from the blood to the lymph nodes in mice during the latter part of the nocturnal active period (27). This corresponds to the afternoon and evening in humans, which may explain the reason for the observed differences in nivolumab efficacy. In a phase I study of nivolumab, pharmacokinetic parameters of nivolumab administration at 10 mg/kg, with ≥1 dose administered, showed a Tmax of 3 h (1.0-9.0 h) (28). Therefore, nivolumab therapy in the morning, when CD8+ T cells are activated, is likely to reach an effective blood concentration and reflect the therapeutic effect.

Consistent with our previous report using decision tree analysis, which identified irAE development as the first divergence variable and as a prognostic factor in patients with advanced GC (29), we also found that irAE development was a good prognostic factor. The extended observation period in this study was considered to clarify whether irAE development was correlated with the prolongation of PFS in addition to OS. IrAE development has been reported to be closely linked to favorable outcomes in patients with GC (30). Although the exact reason for this association remains unclear, recent studies have proposed potential mechanisms, including ICI-activated CD8+ T lymphocytes that target common antigens in tumors and healthy tissues, leading to irAEs and antitumor efficacy (31-33). Consequently, the more potent immune-mediated anti-tumor effects of ICIs imply a similar potential for the development of irAEs. However, the current study found no significant differences in the frequency of irAEs, regardless of whether the timing of nivolumab administration was early or late.

In our study results, PPIs, opioid analgesics, probiotics, and antibiotics did not affect prognosis, whereas concomitant use of NSAIDs exhibited poor prognosis. When administering ICI treatment, considering the influence of concurrent medications on prognosis is essential. Several studies have indicated a dismal outlook on administering antibiotics before administering ICIs for cancer treatment (10-12). This may be because antibiotics affect cytokine release and immune responses by altering the composition of the gut microbiota (34). The impact of the timing of antibiotic exposure on the gut microbiota and response to ICIs has been found to be significant, with negative consequences reported when exposure occurs within a year before ICI administration (12). Our findings, which are inconsistent with those of previous research (20), suggest that antibiotic exposure has no impact on OS when ICIs are administered. However, our study only evaluated antibiotic exposure within 6 months prior to treatment, and the sample size was limited. No adjustments were made for potential confounding factors. Inconsistent with reports on patients with NSCLC and renal cell carcinoma treated with ICIs (9,35), the use of NSAIDs was identified as a poor prognostic factor in patients with advanced GC treated with ICIs. The underlying mechanisms are thought to be that NSAIDs increase the intratumoral accumulation of $CD8^+$ T lymphocytes and alter the tumor inflammatory environment to favor T cell activation by inhibiting the cyclooxygenase-2 pathway (36). However, a recent meta-analysis showed that the use of NSAIDs did not affect the prognosis (37). Therefore, the relationship between NSAID use and the prognosis of ICI-treated patients remains controversial. These results should be interpreted with caution and may change with the inclusion of additional cases in future studies. This study had several limitations. First, the sample size was relatively small, and the study was conducted retrospectively at a single institution in Japan. Additionally, the role of circadian rhythms in CD8+ T cells and the impact of circadian rhythms on other immune functions remain unclear. Furthermore, only the results of nivolumab-only treatment were available in this study. Finally, in this study, the median start time of treatment for all patients was employed to determine whether administration time was earlier or later than the median. The median was adopted because few patients received treatment extremely late in the afternoon or early in the morning; however, future studies should investigate randomizing administration between the two groups, early in the morning and late in the afternoon. To address these limitations, future studies should include large-scale clinical trials and detailed immunological evaluations.

In conclusion, we demonstrated that nivolumab administration in the morning, particularly before 12:06 p.m., was a favorable factor associated with longer OS and PFS in patients with advanced GC. These outcomes contribute to the expanding body of research on the early administration of ICIs. Furthermore, since the combination of chemotherapy and ICIs is now the mainstream therapy, we believe that it would be worthwhile to examine whether the treatment effect of chemotherapy plus ICI therapy also varies with administration time.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

TT and HS conceived the study and wrote the manuscript. SY, YS and SN collected the clinical data, and made substantial contributions to the conception and design of this study. KMu, FF, KMi and TK were involved in raw data analysis. All authors discussed the results and contributed to the final manuscript. TT and KMu confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki, and reviewed and approved by the Ethics Committee of Kurume University (approval no. 21118; Kurume, Japan). An opt-out approach was employed to obtain informed consent from patients, and personal information was protected during data collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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