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Kunimitsu, Yoko ; Morio, Kayoko ; Hirata, Sachi ; Yamamoto, Kazuhiro ; Omura, Tomohiro ; Hara, Takuto ; Harada, Kenichi ; Fujisawa, Masato ;…

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Effects of Proton Pump Inhibitors on Survival Outcomes in Patients with Metastatic or Unresectable Urothelial Carcinoma Treated with Pembrolizumab

Yoko Kunimitsu,[a] Kayoko Morio,*[,a] Sachi Hirata,[a] Kazuhiro Yamamoto,[a] Tomohiro Omura,[a] Takuto Hara,[b] Kenichi Harada,[b] Masato Fujisawa,[b] and Ikuko Yano[a]

[a] *Department of Pharmacy, Kobe University Hospital; 7–5–2 Kusunoki-cho, Chuo-ku, Kobe 650–0017, Japan: and* [b] *Department of Urology, Kobe University Graduate School of Medicine; 7–5–2 Kusunoki-cho, Chuo-ku, Kobe 650–0017, Japan.*

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The gut microbiome influences tumor response to immune checkpoint inhibitors (ICIs). The proton pump inhibitors (PPI) significantly impair diversity of the gut microbiota and can affect the efficacy of ICIs. Therefore, the present study aimed to evaluate the influence of PPI on survival in patients with metastatic or unresectable urothelial carcinoma receiving pembrolizumab. We conducted a retrospective cohort study of patients with metastatic or unresectable urothelial carcinoma receiving pembrolizumab. The use of PPI was defined as any administration for ≥30 d within 60 d prior and/or 30 d after treatment initiation. Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan–Meier method, and Cox proportional hazards regression analysis was performed to investigate prognostic factors based on patient characteristics. Seventy-nine patients were included in the analysis, and 34 patients (43.0%) received PPI. There were no significant differences in OS and PFS between PPI users and nonusers (median OS: 8.2 months *vs.* 11.2 months, hazard ratio (HR): 1.36, 95% confidence interval (CI): 0.75–2.42, $p = 0.296$; median PFS: 3.5 months *vs.* 5.1 months, HR: 1.63, 95% CI: 0.95–2.80, $p = 0.069$). In the multivariable analysis, PPI use was not associated with OS (HR 0.80, 95% CI 0.40–1.56, $p = 0.526$) or PFS (HR 1.44, 95% CI 0.79–2.60, $p = 0.233$). In conclusion, the estimated effect size of PPI use on survival in Japanese patients with metastatic or unresectable urothelial carcinoma treated with pembrolizumab was not reproducible.

Key words pembrolizumab; urothelial carcinoma; proton pump inhibitor; survival; gut microbiome

INTRODUCTION

Pembrolizumab is an immune checkpoint inhibitor (ICI) administered to patients with metastatic or unresectable urothelial carcinoma. A randomized controlled study[1] showed that the objective response rate of pembrolizumab was 21.1% in patients with advanced urothelial carcinoma. Pembrolizumab was significantly associated with prolonged overall survival (OS) in patients with no liver metastases and those with tumor programmed death ligand-1 (PD-L1) positive score >1%.[1]

Several recent studies have demonstrated that the gut microbiome modulates antitumor immune response.[2–4] In case of melanoma, the responders treated with anti-programmed cell death 1 (PD-1) therapy showed significantly higher diversity of the gut microbiome than non-responders.[2,3] A preclinical study indicated that administration of *Bifidobacterium* to mice inoculated with bladder cancer cells delayed tumor growth.[5] Moreover, a combined treatment of *Bifidobacterium* and anti-PD-1 monoclonal antibody increased tumor-specific T cell response. Therefore, the gut microbiome plays an important role in the efficacy of ICI therapy.

Proton pump inhibitors (PPIs) change the composition of the gut microbiota.[6–8] Additionally, the diversity of the gut microbiome in PPI users is significantly lower than that in PPI nonusers. A pooled analysis reported that PPI use was associated with shorter OS and progression-free survival (PFS) than PPI nonuse in patients with non-small cell lung cancer treated with atezolizumab.[9] The analyses of individual patient data showed that PPI use was associated with shorter OS and PFS in patients with advanced urothelial carcinoma treated with atezolizumab.[10] However, effects of PPI on the therapeutic efficacy of pembrolizumab remains unknown in patients with metastatic or unresectable urothelial carcinoma. In this study, we evaluated the possibility that PPI affects survival outcomes in patients with metastatic or unresectable urothelial carcinoma who received pembrolizumab therapy.

MATERIALS AND METHODS

Study Design and Patients We conducted a retrospective cohort study in patients with metastatic or unresectable urothelial carcinoma who received pembrolizumab at the Kobe University Hospital between May 2017 and December 2020. Eligible patients were aged ≥20 years and received at least one infusion of pembrolizumab with a standard dose of 200 mg/body every three weeks or 400 mg/body every six weeks. The exclusion criteria were: concomitant autoimmune diseases, PPI use as needed, and inability to track PPI prescription history. This study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethics Committee of the Kobe University Hospital (B210135). We obtained following information from the electronic medical charts of each patient: age, sex, performance status (PS), tumor histology, PPI type, smoking status, primary urothelial cancer site, adverse drug reactions (ADRs) with pembrolizumab, presence of liver metastases, history of operation, number of prior treatment

* To whom correspondence should be addressed. e-mail: kayoko.morio0826@gmail.com

regimens, clinical laboratory test values (hemoglobin (Hb), albumin (Alb), and neutrophil-to-lymphocyte ratio (NLR)), and concomitant use of steroids. PPI use was defined as any administration for ≥30 d within 60 d prior and/or 30 d after treatment initiation.[4,7] PS was estimated according to the Eastern Cooperative Oncology Group system.

The primary endpoint was OS, which was defined as the time from the initiation of treatment to death from any cause. The secondary endpoint was PFS. PFS was defined as the time from the initiation of treatment to disease progression or death from any cause. The follow-up period was 36 months for both OS and PFS. Response to treatment was assessed as per the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1). ADRs were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Sample Size Estimation We performed log-rank test to select a sample size to determine the difference in OS according to PPI use. Based on the results from a previous study by Hopkins *et al.*[10] we set the following parameters: a study accrual period of 3 years, event rates of 0.50 in the placebo group during the 1-year follow-up period, minimum 0.4 years of follow-up, and pooled HR of 2.10. When the ratio of PPI users and nonusers was 1 : 1, 80 patients were required to detect a significant difference assuming an α of 0.05 and power of 0.8. Thus, considering that 5% of the patients were excluded, 84 patients were included in the present study.

Statistical Analysis Continuous variables are presented as medians (range), and categorical variables as frequencies and proportions. The Kaplan–Meier method was used to calculate OS and PFS, and differences in survival were evaluated using log-rank test. Cox proportional hazard regression was performed to examine the prognostic factors associated with OS or PFS, and estimating the hazard ratio (HR) and 95% confidence interval (CI). The explanatory variables examined were selected referring to predictive factors and prognostic factors associated with survival outcomes in previous studies: age, sex, PPI use, PS, smoking status, history of operation, Hb, Alb, NLR, and liver metastasis. Explanatory variables with an effect *p*-value <0.10 in the univariate analysis were included in the multivariate analysis. PPI use was included in the multivariate analysis regardless of the *p*-value in the univariate analysis. *p*-Value <0.05 (two-sided) was considered to be statistically significant. All statistical analyses were performed using R 4.0.3 software and packages "Hmisc" (version 4.5-0) for sample size calculation.

RESULTS

Patient Characteristics Between May 2017 and December 2020, 83 patients were included in the study. Of these, four patients with autoimmune diseases were excluded. There were no patients who received PPI as needed or were unable to track PPI prescription history. Therefore, 79 patients were included in the analysis. The median duration of follow-up was 7.2 months. Thirty-four patients (43.0%) received PPIs. PPIs included lansoprazole, esomeprazole, vonoprazan, and rabeprazole (Table 1).

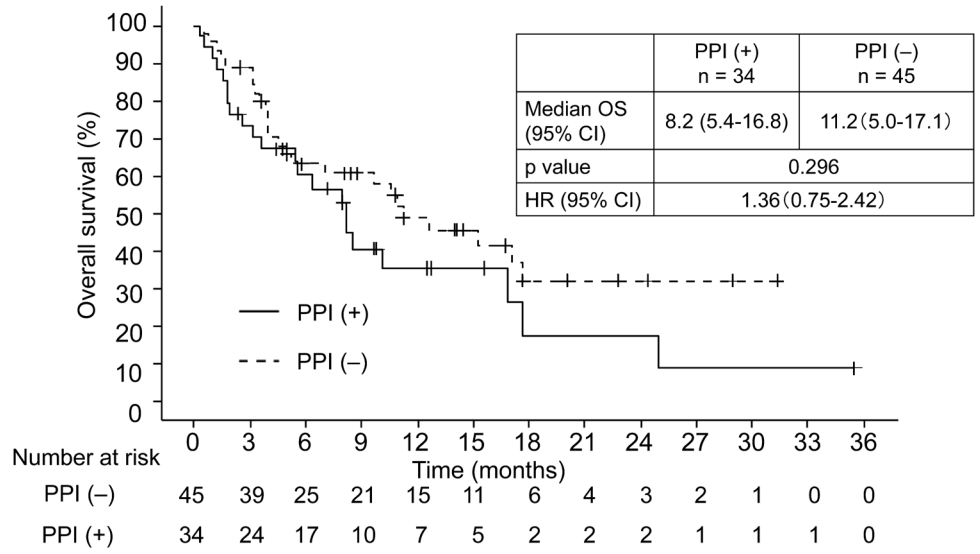
Effects of PPI on Survival Outcomes There were no significant differences in OS and PFS between PPI users and nonusers (median OS: 8.2 months *vs.* 11.2 months, HR: 1.36, 95% CI: 0.75–2.42, $p = 0.296$; median PFS: 3.5 months *vs.* 5.1 months, HR: 1.63, 95% CI: 0.95–2.80, $p = 0.069$) (Figs. 1, 2). Univariate Cox proportional hazards regression analyses for OS and PFS were performed (Tables 2, 3). Poorer PS, lower Hb, lower Alb, higher NLR, and presence of liver metastases were associated with shorter OS with an effect *p*-value <0.10. Poorer PS, lower Hb, lower Alb, higher NLR, and presence of liver metastases were associated with poorer PFS, with an effect *p*-value <0.10. These factors and PPI use were included in the multivariate analysis as covariates. In the multivariable analysis, poor PS (HR 4.83, 95% CI 1.97–11.3, $p < 0.001$), lower Alb (HR 0.49, 95% CI 0.29–0.86, $p = 0.010$), higher NLR (HR 1.10, 95% CI 1.03–1.17, $p = 0.022$), and presence of liver metastases (HR 6.08, 95% CI 2.57–13.7, $p < 0.001$) were associated with shorter OS. Poorer PS (HR 3.16, 95% CI 1.42–6.59, $p = 0.006$) and the presence of liver metastases (HR 2.66, 95% CI 1.18–5.44, $p = 0.010$) were associated with worse PFS. PPI use failed to have an effect on OS (HR 0.80, 95% CI 0.40–1.56, $p = 0.526$) and PFS (HR 1.44, 95% CI 0.79–2.60, $p = 0.233$).

Treatment-Related ADRs Treatment-related ADRs of any grade in patients with PPI use and nonuse are shown in Table 4. Grade 3 events occurred in four patients (11.8%) in the PPI use group and three patients (6.7%) in PPI nonuse group. None of the patients experienced grade 4 events. Six (17.6%) and four (8.9%) patients in the PPI users and nonuser groups, respectively, showed treatment-related discontinuation of pembrolizumab, including ADRs and progression disease. The most common ADRs of any grade between PPI users and nonusers were interstitial pneumonia (5.9 and 13.3%) and rash (5.9 and 13.3%), respectively.

Table 1. Patient Characteristics

Patient characteristics	PPI (−) ($n = 45$)	PPI (+) ($n = 34$)
Male, *n* (%)	35 (77.8)	24 (70.6)
Age (years), median (range)	71 (57–86)	72 (56–87)
Primary urothelial cancer site, *n* (%)		
Upper urinary tract	27 (60.0)	19 (55.9)
Lower urinary tract	17 (37.8)	15 (44.1)
Upper and lower urinary tract	1 (2.2)	0 (0)
Histology, *n* (%)		
Pure cell	40 (88.9)	28 (82.4)
Others	5 (11.1)	6 (17.6)
Prior lines of therapy		
1	29 (64.5)	27 (79.4)
2	15 (33.3)	7 (20.6)
3	1 (2.2)	0 (0)
PS, *n* (%)		
0–1	40 (88.9)	25 (73.5)
2–4	5 (11.1)	9 (26.5)
History of operation, *n* (%)	25 (55.6)	14 (41.2)
Smoking status, *n* (%)	26 (62.2)	22 (64.7)
Concomitant use of steroids, *n* (%)	1 (2.2)	4 (11.8)
Liver metastases, *n* (%)	8 (17.8)	7 (20.6)
Laboratory test values		
NLR (%), median (range)	2.7 (0.6–21.7)	4.2 (1.2–46.3)
Alb (g/dL), median (range)	3.9 (1.2–4.8)	3.6 (2.0–4.3)
Hb (g/dL), median (range)	10.7 (7.8–15.3)	10.6 (6.1–15.0)

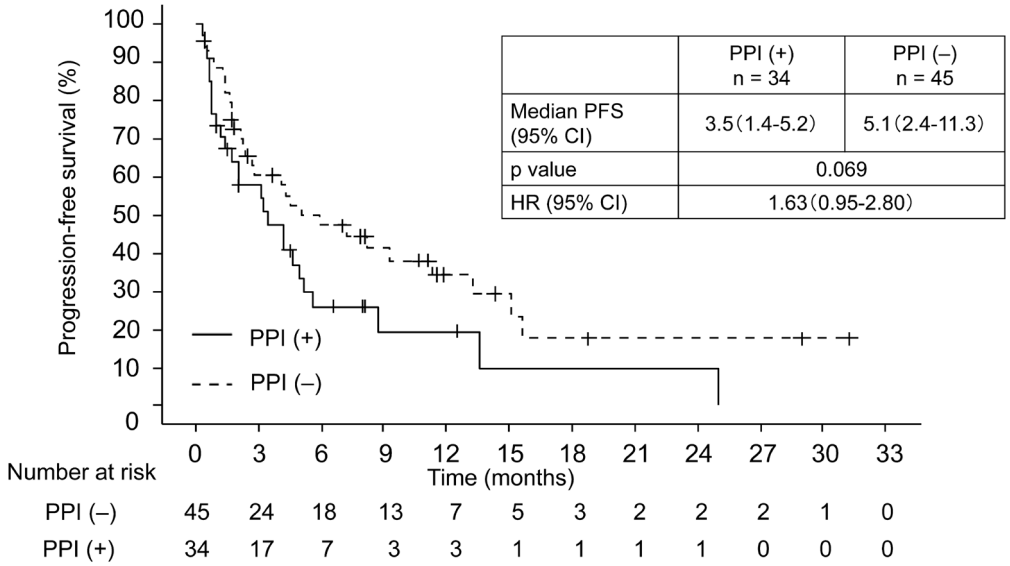
Abbreviations: Alb, albumin; Hb, hemoglobin; NLR, neutrophil-to-lymphocyte ratio; PPI, proton pump inhibitor; PS, performance status.



	PPI (+) n = 34	PPI (–) n = 45
Median OS (95% CI)	8.2 (5.4-16.8)	11.2(5.0-17.1)
p value	0.296	
HR (95% CI)	1.36(0.75-2.42)	

Fig. 1. Kaplan–Meier Curves of Overall Survival in Patients Treated with Pembrolizumab According to PPI Use or Nonuse

Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival; PPI, proton pump inhibitor. *p*-Value was evaluated using log-rank test.



	PPI (+) n = 34	PPI (–) n = 45
Median PFS (95% CI)	3.5(1.4-5.2)	5.1(2.4-11.3)
p value	0.069	
HR (95% CI)	1.63(0.95-2.80)	

Fig. 2. Kaplan–Meier Curves of Progression-Free Survival in Patients Treated with Pembrolizumab According to PPI Use or Nonuse

Abbreviations: CI, confidence interval; HR, hazard risk; PFS, progression-free survival; PPI, proton pump inhibitor. *p*-Value was evaluated using log-rank test.

Table 2. Univariate and Multivariate Cox Regression Analysis for Overall Survival

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Sex (female)	1.57	0.79–2.93	0.185	—	—	—
Age (years)	1.01	0.97–1.05	0.503	—	—	—
PPI use	1.36	0.75–2.42	0.303	0.80	0.40–1.56	0.526
PS (2–3)	3.72	1.83–7.07	<0.001	4.83	1.97–11.3	<0.001
Smoking status (current smoker)	0.70	0.39–1.31	0.259	—	—	—
History of operation (yes)	0.97	0.54–1.74	0.919	—	—	—
Hb (g/dL)	0.80	0.67–0.95	0.013	1.09	0.85–1.40	0.501
Alb (g/dL)	0.53	0.38–0.76	0.001	0.49	0.29–0.86	0.010
NLR	1.12	1.07–1.19	<0.001	1.10	1.03–1.17	0.022
Liver metastases (yes)	5.86	2.83–11.6	<0.001	6.08	2.57–13.7	<0.001

Abbreviations: Alb, albumin; CI, confidence interval; Hb, hemoglobin; HR, hazard ratio; NLR, neutrophil-to-lymphocyte ratio; PPI, proton pump inhibitors; PS, performance status.

Table 3. Univariate and Multivariate Cox Regression Analysis for Progression-Free Survival

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Sex (female)	1.56	0.84–2.75	0.154	—	—	—
Age (years)	1.01	0.97–1.05	0.707	—	—	—
PPI use	1.63	0.95–2.79	0.076	1.44	0.79–2.60	0.233
PS (2–3)	3.67	1.95–6.80	<0.001	3.16	1.42–6.59	0.006
Smoking status (current smoker)	0.82	0.47–1.46	0.492	—	—	—
History of operation (yes)	1.24	0.72–2.15	0.429	—	—	—
Hb (g/dL)	0.84	0.71–0.99	0.035	0.98	0.78–1.22	0.855
Alb (g/dL)	0.63	0.46–0.91	0.015	0.70	0.44–1.19	0.181
NLR	1.08	1.03–1.12	0.002	1.03	0.98–1.08	0.179
Liver metastases (yes)	3.16	1.56–5.94	0.002	2.66	1.18–5.44	0.010

Abbreviations: Alb, albumin; CI, confidence interval; Hb, hemoglobin; HR, hazard ratio; NLR, neutrophil-to-lymphocyte ratio; PPI, proton pump inhibitors; PS, performance status.

Table 4. Treatment-Related Adverse Drug Reactions

Adverse drug reactions	PPI (+) $n=34$				PPI (−) $n=45$			
	Any grade	Grade1	Grade 2	Grade 3	Any grade	Grade 1	Grade 2	Grade 3
Interstitial pneumonitis	2 (5.9)	1 (2.9)	1 (2.9)	0 (0)	6 (13.3)	3 (6.7)	2 (4.4)	1 (2.2)
Rash	2 (5.9)	0 (0)	2 (5.9)	0 (0)	6 (13.3)	1 (2.2)	4 (8.9)	1 (2.2)
Liver failure	2 (5.9)	0 (0)	0 (0)	2 (5.9)	1 (2.2)	0 (0)	1 (2.2)	0 (0)
Colitis	2 (5.9)	0 (0)	1 (2.9)	1 (2.9)	1 (2.2)	0 (0)	0 (0)	1 (2.2)
Hyperthyroidism	0 (0)	0 (0)	0 (0)	0 (0)	4 (8.9)	2 (4.4)	2 (4.4)	0 (0)
Pancreatitis	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.2)	1 (2.2)	0 (0)	0 (0)
Hypothyroidism	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.2)	0 (0)	1 (2.2)	0 (0)
Adrenal insufficiency	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.2)	0 (0)	1 (2.2)	0 (0)
Hypopituitarism	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.2)	0 (0)	1 (2.2)	0 (0)
Myasthenia gravis	1 (2.9)	0 (0)	0 (0)	1 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)

All data indicated as *n* (%).

DISCUSSION

The present study showed that PPI use had no significant influence on survival outcomes in patients with metastatic or unresectable urothelial carcinoma receiving pembrolizumab. Moreover, PPI use was not a prognostic factor in Cox proportional hazards regression analysis. Several studies[9–15] have reported the impact of PPI use on the clinical outcomes of ICI therapy. A previous retrospective study[15] showed PPI use to be associated with worse OS and PFS in patients with locally advanced or metastatic urothelial carcinoma treated with ICIs. However, 70% of the patients in the previous study were received anti-PD-L1 atezolizumab and durvalumab. Therefore, it might be inappropriate to compare the results of the present study with those of a previous study.[11] A pooled analysis[10] reported that PPI use was associated with significantly worse OS and PFS in patients with advanced urothelial carcinoma treated with atezolizumab; whereas pembrolizumab was used in the present study. Furthermore, the influence of the gut microbiota on treatment efficacy differs between anti-PD-1 and anti-PD-L1 monotherapy.[16] A preclinical study[2] demonstrated that responders to anti-PD-L1 therapy had an abundance of *Faecalibacterium* spp. and *Ruminococcaceae* family than non-responders. In contrast, an association between responders to anti-PD-1 therapy and the abundance of gut microbiota, such as *Akkermansia muciniphila*, *Alistipes indistinctus*, and *Enterococcus hirae* was observed.[4] Furthermore, PPI use was associated with an increase in abundance of *Enterobacteriaceae*, *Enterococcaceae*, and *Lactobacillaceae*, and a decrease in abundance of *Bifidobacteriaceae* and *Ruminococcaceae*, a bacterial group related to response to PD-L1 therapy.[6,17] We hypothesized that the microbial composition related to response to pembrolizumab therapy remains unchanged by concomitant use of PPI. In addition, a meta-analysis showed that the survival outcome of concomitant PPI and ICI treatment differed by cancer types.[18] The results of our study may support their perception. However, the mechanisms by which microbial diversity and composition are altered by adding PPI to anti-PD-1 or anti-PD-L1 therapy is unclear. A preclinical study showed that the composition of the gut microbiome differed between Japanese and individuals of other races, indicating that differences in the hydrogen metabolism pathway could affect the microbial composition.[19] In the present study, no significant difference was observed in survival outcomes possibly because the composition of the gut microbiomes in the patients was peculiar to that of Japanese population. Additionally, we could not investigate the microbial composition of the patients; therefore, further studies are needed.

Univariate and multivariate Cox regression analyses suggested that PPI use was not a prognostic factor in patients with metastatic or unresectable urothelial carcinoma treated with pembrolizumab. Further, poor PS, lower Alb, higher

NLR, and presence of liver metastases were independent factors influencing OS; also, poor PS and the presence of liver metastases were independent factors influencing PFS. Previous studies[20–24] have demonstrated that poor PS, the presence of liver metastasis, lower Alb, and higher NLR were significant prognostic factors in patients with advanced urothelial carcinoma, consistent with results of the present study. These factors, rather than PPI use, may strongly contribute to survival.

A few grade 3 ADRs occurred in the PPI use and nonuse groups in patients receiving pembrolizumab, but none of the patients experienced grade 4 events. Thus, the addition of PPI to pembrolizumab is generally manageable.

In our study, we hypothesized a HR in sample size estimation based on the report which indicated the effect of PPI on survival in patients with urothelial cancer treated with atezolizumab.[10] Hopkins *et al.*[10] showed 1-year HR of 2.1 from the Kaplan–Meier curve for the participants whose PD-L1 expression was ≥5% of tumor-infiltrating immune cells despite the overall HR of 1.95. The effect size by PPI use was expected to be larger than the overall HR from the reported effect size, which evaluated the association between OS of Japanese patients treated with ICI and gut microbiota affected by PPI.[6,25] Therefore, we estimated the HR of 2.1 according to PPI use in patients with urothelial cancer treated with pembrolizumab.

This study has some limitations. First, we could not examine PD-L1 expression due to the retrospective study design. A meta-analysis showed that higher expression of PD-L1 associates with objective response rate, while PD-L1 expression cannot predict OS in patients with urothelial carcinoma.[26] The predictive value of PD-L1 expression remains unclear. Therefore, further studies should evaluate whether expression of PD-L1 influences the efficacy of concomitant use of pembrolizumab with PPI. Second, the concomitant medications that are well known to affect microbiota composition, such as antibiotics, steroids, and metformin may be associated with survival[8]; however, we could not perform statistical analysis because only a few patients received these drugs. Third, this was a small-scale, retrospective, observational study, and the number of patients recruited was insufficient to achieve the sample size estimated in the study protocol. However, we considered that this slight insufficiency of the sample size may not impact the primary endpoint.

In conclusion, our study showed that the PPI-caused effect size estimated from the relevant study on survival may not be reproduced for Japanese patients with metastatic or unresectable urothelial carcinoma received pembrolizumab. Furthermore, PPI use failed to qualify as a prognostic factor for OS or PFS, in addition to the previous known prognostic factors such as PS, Alb, NLR, and liver metastases. Thus, further research is needed to identify the composition of the gut microbiota related to clinical responses to concomitant administration of pembrolizumab and PPI. Additionally, it remains unclear whether concomitant medications that modulate the gut microbiome have an impact on the response to pembrolizumab. We believe that substantiating the influence of concomitant medications on clinical responses will help in optimizing the immune checkpoint therapy.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, Vogelzang NJ, Climent MA, Petrylak DP, Choueiri TK, Necchi A, Gerritsen W, Gurney H, Quinn DI, Culine S, Sternberg CN, Mai Y, Poehlein CH, Perini RF, Bajorin DF. KEYNOTE-045 Investigators. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. *N. Engl. J. Med.*, **376**, 1015–1026 (2017).
2) Gopalakrishnan V, Spencer CN, Nezi L, *et al.* Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science*, **359**, 97–103 (2018).
3) Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, Luke JJ, Gajewski TF. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science*, **359**, 104–108 (2018).
4) Routy B, Le Chatelier E, Derosa L, *et al.* Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*, **359**, 91–97 (2018).
5) Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabri B, Alegre ML, Chang EB, Gajewski TF. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science*, **350**, 1084–1089 (2015).
6) Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, Tigchelaar EF, Jankipersadsing SA, Cenit MC, Harmsen HJ, Dijkstra G, Franke L, Xavier RJ, Jonkers D, Wijmenga C, Weersma RK, Zhernakova A. Proton pump inhibitors affect the gut microbiome. *Gut*, **65**, 740–748 (2016).
7) Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, Poole AC, Sutter JL, Welter D, Ley RE, Bell JT, Spector TD, Steves CJ. Proton pump inhibitors alter the composition of the gut microbiota. *Gut*, **65**, 749–756 (2016).
8) Vich Vila A, Collij V, Sanna S, Sinha T, Imhann F, Bourgonje AR, Mujagic Z, Jonkers D, Masclee AAM, Fu J, Kurilshikov A, Wijmenga C, Zhernakova A, Weersma RK. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat. Commun.*, **11**, 362 (2020).
9) Chalabi M, Cardona A, Nagarkar DR, Dhawahir Scala A, Gandara DR, Rittmeyer A, Albert ML, Powles T, Kok M, Herrera FG. Efficacy of chemotherapy and atezolizumab in patients with non-small-cell lung cancer receiving antibiotics and proton pump inhibitors: pooled *post hoc* analyses of the OAK and POPLAR trials. *Ann. Oncol.*, **31**, 525–531 (2020).
10) Hopkins AM, Kichenadasse G, Karapetis CS, Rowland A, Sorich MJ. Concomitant proton pump inhibitor use and survival in urothelial carcinoma treated with atezolizumab. *Clin. Cancer Res.*, **26**, 5487–5493 (2020).
11) Buti S, Bersanelli M, Perrone F, *et al.* Effect of concomitant medications with immune-modulatory properties on the outcomes of patients with advanced cancer treated with immune checkpoint inhibitors: development and validation of a novel prognostic index. *Eur. J. Cancer*, **142**, 18–28 (2021).
12) Cortellini A, Tucci M, Adamo V, *et al.* Integrated analysis of concomitant medications and oncological outcomes from PD-1/PD-L1 checkpoint inhibitors in clinical practice. *J. Immunother. Cancer.*, **8**, e001361 (2020).
13) Li C, Xia Z, Li A, Meng J. The effect of proton pump inhibitor uses on outcomes for cancer patients treated with immune checkpoint inhibitors: a meta-analysis. *Ann. Transl. Med.*, **8**, 1655 (2020).
14) Qin BD, Jiao XD, Zhou XC, Shi B, Wang J, Liu K, Wu Y, Ling Y, Zang YS. Effects of concomitant proton pump inhibitor use on immune checkpoint inhibitor efficacy among patients with advanced cancer. *OncoImmunology*, **10**, 1929727 (2021).
15) Ruiz-Bañobre J, Molina-Díaz A, Fernández-Calvo O, Fernández-Núñez N, Medina-Colmenero A, Santomé L, Lázaro-Quintela M, Mateos-González M, García-Cid N, López-López R, Vázquez S,

Anido-Herranz U. Rethinking prognostic factors in locally advanced or metastatic urothelial carcinoma in the immune checkpoint blockade era: a multicenter retrospective study. *ESMO Open*., **6**, 100090 (2021).
16) Gong J, Chehrazi-Raffle A, Placencio-Hickok V, Guan M, Hendifar A, Salgia R. The gut microbiome and response to immune checkpoint inhibitors: preclinical and clinical strategies. *Clin. Transl. Med*., **8**, 9 (2019).
17) Weersma RK, Zhernakova A, Fu J. Interaction between drugs and the gut microbiome. *Gut*, **69**, 1510–1519 (2020).
18) Li M, Zeng C, Yao J, Ge Y, An G. The association between proton pump inhibitors use and clinical outcome of patients receiving immune checkpoint inhibitors therapy. *Int. Immunopharmacol*., **88**, 106972 (2020).
19) Nishijima S, Suda W, Oshima K, Kim SW, Hirose Y, Morita H, Hattori M. The gut microbiome of healthy Japanese and its microbial and functional uniqueness. *DNA Res*., **23**, 125–133 (2016).
20) Bellmunt J, Choueiri TK, Fougeray R, Schutz FA, Salhi Y, Winquist E, Culine S, von der Maase H, Vaughn DJ, Rosenberg JE. Prognostic factors in patients with advanced transitional cell carcinoma of the urothelial tract experiencing treatment failure with platinum-containing regimens. *J. Clin. Oncol*., **28**, 1850–1855 (2010).
21) Khaki AR, Li A, Diamantopoulos LN, *et al.* Impact of performance status on treatment outcomes: a real-world study of advanced urothelial cancer treated with immune checkpoint inhibitors. *Cancer*, **126**, 1208–1216 (2020).
22) Khaki AR, Li A, Diamantopoulos LN, *et al.* A new prognostic model in patients with advanced urothelial carcinoma treated with first-line immune checkpoint inhibitors. *Eur. Urol. Oncol*., **4**, 464–472 (2021).
23) Nassar AH, Mouw KW, Jegede O, Shinagare AB, Kim J, Liu CJ, Pomerantz M, Harshman LC, Van Allen EM, Wei XX, McGregor B, Choudhury AD, Preston MA, Dong F, Signoretti S, Lindeman NI, Bellmunt J, Choueiri TK, Sonpavde G, Kwiatkowski DJ. A model combining clinical and genomic factors to predict response to PD-1/PD-L1 blockade in advanced urothelial carcinoma. *Br. J. Cancer*, **122**, 555–563 (2020).
24) Sonpavde G, Manitz J, Gao C, *et al.* Five-factor prognostic model for survival of post-platinum patients with metastatic urothelial carcinoma receiving PD-L1 inhibitors. *J. Urol*., **204**, 1173–1179 (2020).
25) Hakozaki T, Richard C, Elkrief A, Hosomi Y, Benlaifaoui M, Mimpen I, Terrisse S, Derosa L, Zitvogel L, Routy B, Okuma Y. The gut microbiome associates with immune checkpoint inhibition outcomes in patients with advanced non-small cell lung cancer. *Cancer Immunol. Res*, **8**, 1243–1250 (2020).
26) Rui X, Gu TT, Pan HF, Zhang HZ. Evaluation of PD-L1 biomarker for immune checkpoint inhibitor (PD-1/PD-L1 inhibitors) treatments for urothelial carcinoma patients: a meta-analysis. *Int. Immunopharmacol*., **67**, 378–385 (2019).