ANTICANCER RESEARCH *39*: 6265-6271 (2019)
doi:10.21873/anticanres.13836

The Impact of Antibiotics on Prognosis of Metastatic Renal Cell Carcinoma in Japanese Patients Treated With Immune Checkpoint Inhibitors

KOSUKE UEDA[1,2], SATORU YONEKURA[2], NAOYUKI OGASAWARA[1], YOSHIHIRO MATSUNAGA[1], RYUJI HOSHINO[1], HIROFUMI KUROSE[1], KATSUAKI CHIKUI[1], KEIICHIRO UEMURA[1], MAKOTO NAKIRI[1], KIYOAKI NISHIHARA[1], MITSUNORI MATSUO[1], SHIGETAKA SUEKANE[1] and TSUKASA IGAWA[1]

[1]*Department of Urology, Kurume University School of Medicine, Kurume, Japan;*
[2]*Gustave Roussy Cancer Campus, Villejuif, France*

Abstract. *Background/Aim: The present study aimed to examine the influence of antibiotics (AB) on the clinical outcomes of Japanese patients treated with immune check point inhibitors (ICIs) for metastatic renal cell carcinoma (RCC) patients. Patients and Methods: A total of 31 patients with metastatic RCC treated with ICIs from November 2016 to April 2019 were retrospectively reviewed and analyzed. Results: Five patients were treated with AB prior to ICIs treatment. Median progression free survival (PFS) of patients treated with AB vs. patients not treated with AB was 2.8 months and 18.4 months, respectively. The difference between PFS was statistically significant (p=0.0004). In multivariate analyses, AB use (p=0.0377) and presence of immune related adverse events (p=0.0042) were independent prognostic factors for PFS in association with ICIs therapy. Conclusion: The use of AB before ICIs treatment was a predictor of poor ICIs response in metastatic RCC.*

Immune checkpoint inhibitors (ICIs) that target programmed cell death-1 (PD-1) protein, programmed cell death-ligand 1 protein, and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), have changed the therapeutic landscape and are currently standard treatment options in patients with advanced and metastatic renal cell carcinoma (RCC) (1, 2). Despite the remarkable success of clinical applications, the efficacy of ICIs in RCC varies greatly across individual patients. Some researchers have reported biomarkers for predicting prognosis in patients treated with ICIs, such as PD-L1 and PD-L2 positivity, tumor mutation burden, and profile of immune-related genes (3). Furthermore, the association of immune related adverse events (irAEs) with prognosis in metastatic RCC has been recently reported (4, 5). Thus, it is critical to explore reliable predictors to improve prognosis of RCC patients treated with ICIs.

Recently, several studies have demonstrated the crucial impact of human gut microbiota on ICIs therapies (6-9). It is well recognized that antibiotics (AB) alter the diversity and composition of gut microbiota and consequently shift their metabolic capacity (10). The hypothesis was that modulation of gut microbiota by AB may be associated with poor response to ICIs. However, the data on the association between AB use and clinical outcomes with ICIs are limited, especially in Japanese patients with genitourinary cancer.

In the present study, we performed a retrospective analysis to examine the influence of AB on the clinical outcomes of Japanese patients treated with ICIs therapy for metastatic RCC patients.

Patients and Methods

Study design and patients. We retrospectively examined clinical information collected from 31 RCC patients treated with ICIs at Kurume University Hospital from November 2016 to April 2019. All patients received nivolumab or the combination of nivolumab and ipilimumab. Nivolumab was intravenously administered at 3 mg/kg or 240 mg/body every 2 weeks. Nivolumab and ipilimumab were administered intravenously at a dose of 240 mg/body and 1 mg/kg, respectively, every 3 weeks in four doses (induction phase), followed by nivolumab monotherapy at a dose of 240 mg/body every 2 weeks (maintenance phase). Dose reductions were not permitted for any reason. However, the dose interval could be modified according to the patient condition.

The irAEs included cutaneous, gastrointerstinal, endocrine, pulmonary, hepatobiliary, pancreatitis (elevated pancreatic enzymes), and ocular toxicity according to previous studies (5). The severity of

Correspondence to: Kosuke Ueda, MD, Ph.D., Department of Urology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. Tel: +81 942317572, Fax: +81 942317866, e-mail: ueda_kousuke@med.kurume-u.ac.jp

Key Words: Renal cell carcinoma, immune checkpoint inhibitor, antibiotics, gut microbiota, progression-free survival.

Table I. *Baseline characteristics of patients.*

Feature	Total (n=31)	AB (n=5)	No AB (n=26)	*p*-Value
Age, years, range	67 (44-80)	67 (46-68)	68 (44-80)	0.4043
Gender, n (%)				
Male	24 (77.4)	4 (80.0)	20 (76.9)	0.8788
Female	7 (22.6)	1 (20.0)	6 (23.1)	
BMI (kg/m^2), median	20.8	20.9	19.3	0.6868
Prior nephrectomy, n (%)				
Yes	27 (87.1)	3 (60.0)	24 (92.3)	0.0828
No	4 (12.9)	2 (40.0)	2 (7.7)	
Performance status, n (%)				
0, 1	28 (90.3)	4 (80.0)	24 (92.3)	0.4362
≥2	3 (9.7)	1 (20.0)	2 (7.7)	
Histological subtype, n (%)				
Clear cell type	27 (87.1)	3 (60.0)	24 (92.3)	0.2221
Papillary type	2 (6.5)	1 (20.0)	1 (3.8)	
unknown	2 (6.5)	1 (20.0)	1 (3.8)	
IMDC risk classification, n (%)				
Favorable	3 (9.7)	0 (0.0)	3 (11.5)	0.5098
Intermediate	20 (64.5)	4 (80.0)	16 (61.5)	
Poor	8 (25.8)	1 (20.0)	7 (26.9)	
Number of prior regimens, n (%)				
0	3 (9.7)	1 (20.0)	2 (7.7)	0.3362
1	13 (41.9)	3 (60.0)	10 (38.5)	
≥2	15 (48.4)	1 (20.0)	14 (53.8)	
Treatment, n (%)				
Nivolumab	28 (90.3)	4 (80.0)	24 (92.3)	0.4362
Ipilimumab + Nivolumab	3 (9.7)	1 (20.0)	2 (7.7)	
irAE, n (%)				
Present	11 (35.5)	0 (0.0)	11 (42.3)	0.0269
Absent	20 (64.5)	5 (100.0)	15 (57.7)	

AB: Antibiotics; BMI: body mass index; IMDC: International Metastatic Renal Cell Carcinoma Database Consortium; irAE: immune related adverse events.

irAEs was graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (11). Radiological evaluations were performed for all patients by computed tomography (CT). Tumor response was evaluated as best response according to the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1 (12).

We defined patients who received any oral or intravenous AB within 30 days before ICIs treatment as the AB group as previously reported (8). PFS was measured from the time of ICI initiation to clinical or radiographic progression or death from any cause. OS was measured from the time of ICIs initiation to death from any cause.

Statistical analysis. Kaplan–Meier curves were generated for progression free survival (PFS) and overall survival (OS). Comparison of PFS and OS among groups was achieved *via* log-rank test. The relationships between groups were compared using Chi-squared test, Fisher's exact test or Student's *t*-test. Cox proportional hazard model was used for univariate and multivariate analyses to calculate the hazard ratio (HR) and 95% confidence interval (CI). The variables with *p*-values <0.1 identified in univariate analyses were selected for multivariate analyses. All statistical analyses were performed using JMP version 13 (SAS Institute, Inc., Cary, NC, USA). All *p*-values were two-sided, statistical significance was set at $p<0.05$.

Ethical approval. This study was conducted in full accordance with the World Medical Association Declaration of Helsinki and was independently reviewed and approved by the Ethics Review Committee at Kurume University School of Medicine. Patients were not solicited for informed consent, given the retrospective nature of our study. All patient data were processed in anonymity and de-identified prior to analysis.

Results

Patients characteristics. The clinicopathological characteristics of the 31 study participants are summarized in Table I. All patients had metastatic RCC and received ICIs therapy. Nearly all patients (28/31, 90.3%) received nivolumab monotherapy after 1 or more molecular targeted therapies. Three patients (9.7%) received the combination of nivolumab and ipilimumab as first line therapy. Of the 31 patients, 5 patients (16.1%) received AB within 30 days of initiating ICIs therapy. AB were prescribed for infection in the upper respiratory tract, urinary tract, and skin or acute cholangitis. All of them were prescribed β-Lactums inhibitors. Clinicopathological characteristics

Table II. *Profile of immune related adverse events (irAEs).*

irAE categories	n=31	
	All Grades (n=11) (%)	Grade ≥3 (n=3) (%)
Rash/pruritus	7 (63.6)	0 (0.0)
Elevated hepatic enzymes	3 (27.2)	1 (33.3)
Interstitial pneumonia	2 (18.2)	0 (0.0)
Colitis	1 (9.1)	0 (0.0)
Adrenal insufficiency	1 (9.1)	1 (33.3)
Elevated pancreatic enzymes	1 (9.1)	1 (33.3)
Uveitis	1 (9.1)	0 (0.0)

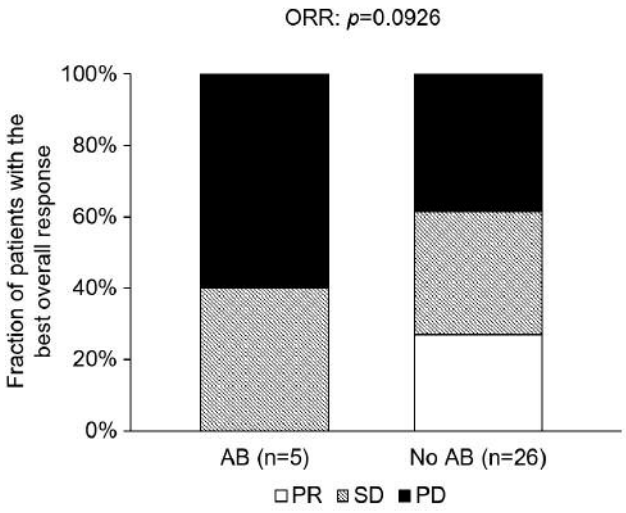

Figure 1. *Best overall response to immune checkpoint inhibitors therapy. The objective response rate tended to be higher in patients who did not receive antibiotics (AB) than in those who received AB.*

between AB group and no AB group were well balanced, except that no patients in the AB group had experienced irAEs upon ICIs therapy (p=0.0269). However, there was no significant difference in heamatological and biochemical analysis between AB and no AB group.

irAEs profile of ICIs therapy. Of the 31 patients, 11 patients (35.5%) experienced irAEs. Most irAEs were grade 1 or 2 (Table II). Cutaneous irAEs such as rash and pruritus were the most frequent (7/11, 63.6%), followed by elevated hepatic enzymes and interstitial pneumonia. One patient in each group experienced Grade 3 or higher elevated hepatic enzymes, adrenal insufficiency and elevated pancreatic enzymes. However, there was no case of treatment-related death in this study.

Best overall response according to AB use. Figure 1 shows patients' ratio regarding the best overall response during ICIs therapy. Complete response was not obtained as best response to ICIs therapy in our study. The objective response rate (ORR) tended to be higher in patients without AB than in those with AB. However, there was no statistically significant difference between these two groups (p=0.0926).

Clinical course according to AB use. Figure 2 shows the estimated curves of PFS and OS according to AB use. Median PFS in patients treated with AB vs. patients not treated with AB were 2.8 months [95% confidence interval (CI)=1.6-5.6] and 18.4 months (95%CI=6.5-not reached), respectively (p=0.0004, log-rank test) (Figure 2A). Median OS was not significantly different between those with AB and those without AB (not reached for both groups). Nivolumab monotherapy after second line treatment was also examined, and the PFS of patients with AB was significantly inferior compared to that without AB (p=0.0023, data no shown).

To identify the prognostic factors for ICIs treatment associated with PFS, univariate and multivariate analyses were performed using the Cox proportional hazards model (Table III). Univariate analyses showed that AB use [hazard ratio (HR)=6.518, 95%CI=1.857-21.416, p=0.0048] and the presence of irAEs (HR=0.122, 95%CI=0.019-0.443 p=0.0006) were significant factors affecting PFS. Furthermore, multivariate analyses also identified AB use (HR=3.830, 95%CI=1.086-12.717; p=0.0377) and presence of irAEs (HR=0.149, 95%CI=0.023-0.574, p=0.0042) as independent prognostic factors for PFS in association with ICIs therapy (Table III).

Discussion

This retrospective study showed that PFS after initiation of ICIs therapy was significantly shorter in patients who received AB within 30 days before initiating ICIs treatment than in those who did not receive AB. No significant association between AB use and OS was indicated, although a trend toward the negative influence of AB use on OS was observed. To our knowledge, our study is the first to examine the association of AB use with poor response to ICIs in Japanese metastatic RCC patients.

The anticancer response of ICIs therapy is enhanced by inhibiting PD-1 or CTLA-4 pathways and then re-activating host immune function (13, 14). The gut microbiota is known to play a crucial role in the anticancer response to ICIs treatment. Recently, the number of studies examining the relationship between AB use and ICIs efficacy has increased (6-9, 15). Most of them have shown a negative association between AB and clinical outcome. Dysbiosis induced by AB modifies gut microbiota composition, leading to loss of diversity (16, 17). Matson *et al*. (18) and Gopalakrishnan *et*

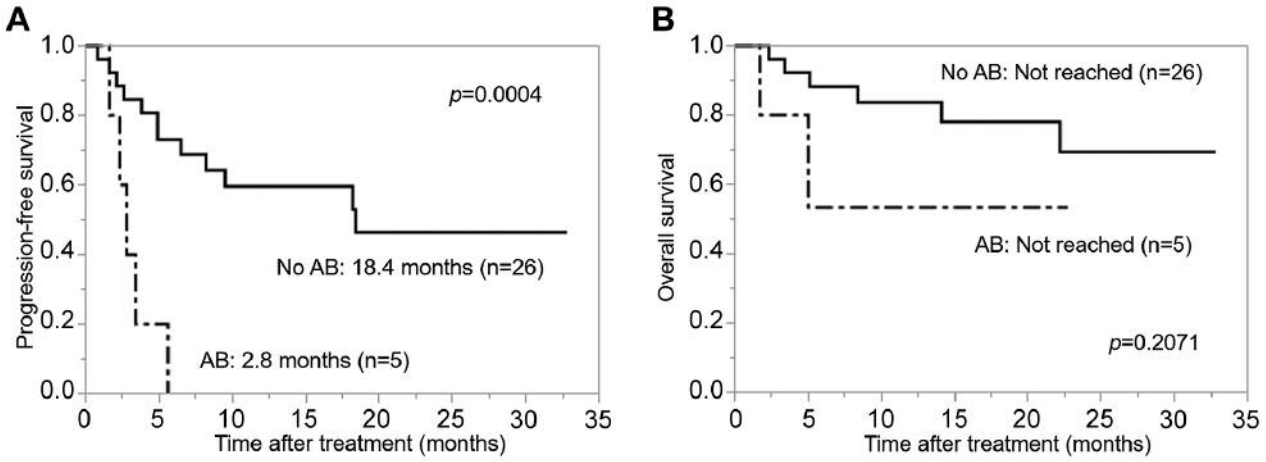

Figure 2. *Progression-free survival (A) and overall survival (B) in patients with renal cell carcinoma treated with immune checkpoint inhibitors therapy according to antibiotics use.*

al. (19) have demonstrated that bacterial species more abundant in responders to anti-PD-1 therapy included *Ruminococcaceae* family and *Bifidobacterium* spp. and reconstitution of germ-free mice with fecal transplantation from responders could lead to improved tumor control, augmented T cell responses, and greater efficacy of ICIs therapy in metastatic melanoma. Routy *et al*. (6) have also found that the abundance of *Akkermansia muciniphilia* was significantly associated with favorable outcomes. Derosa *et al*. (8) have shown that prior AB use was associated with increased rate of primary progressive disease and worse PFS in patients with RCC treated with ICIs. It has been reported that AB-induced dysbiosis can take 1-3 months to normalize (20). Thereby, they examined the influence of AB use on ICIs therapy at two time points. The results indicated that the effect of AB use within 60 days before initiating ICIs treatment was less than that of AB use within 30 days. We also examined the effect of AB within 60 days of initiating ICIs therapy. However, there were no significant differences between AB use and PFS or OS (data no shown).

In the present study, multivariate analyses identified that AB use and the presence of irAEs are independent prognostic factor for PFS in patients treated with ICIs therapy. The irAEs presumably result from activation of T cells that recognize self-proteins or commensal microorganisms (21) though neither the mechanisms driving these toxicities nor the immunological targets are fully understood so far. Some reports have shown that the presence of irAEs is a potential surrogate and predictive marker of survival in ICIs therapies (4, 22-24). Similar findings have been reported in RCC (5, 25). Interestingly, there were no patients with irAEs following ICI therapy in the AB group in our study. Chaput *et al*. (26) have demonstrated that baseline gut microbiota enriched with *Faecalibacterium* and other *Firmicutes* is associated with both clinical response to ipilimumab and increased occurrence of ipilimumab-induced colitis. Their results have suggested that certain species of bacteria might be associated with the immune activation that could cause irAEs. In our study, AB-induced dysbiosis might change gut microbiota, leading to suppressed activation of immunity and reduced irAEs.

The present study has several acknowledged limitations. First, this is a retrospective study from a single institution and selection bias may exist. In addition, the number of patients in this study was extremely small and the treatment lines in the included patients were heterogenous. Moreover, previous reported biomarkers such as the expression of PD-L1 were not examined in this study. Further prospective studies with a larger cohort will be needed to investigate whether AB use prior to ICIs treatment has a negative impact on PFS and OS in RCC.

In conclusion, our study indicates that use of AB before ICIs treatment is a predictor of poor ICIs response in metastatic RCC.

Conflicts of Interest

The Authors declare that there is no conflict of interest regarding this study.

Authors' Contributions

KU drafted the manuscript. SY contributed to the manuscript. NO, YM, RH and KUemura have contributed to data collection. HK, KC, MN, KN and MM have performed the clinical follow-up. KU, SS and TI were responsible for the conception and design of this study, interpretation of the data, and critical revision of the manuscript. All Authors read and approved the final manuscript.

Table III. *Univariate and multivariate analysis of progression-free survival.*

Parameter	Univariate		Multivariate	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age				
<67	1			
≥67	1.210 (0.463-3.342)	0.6981		
Gender				
Male	1			
Female	1.941 (0.666-5.122)	0.2106		
T stage				
T1/2	1			
T3/4	1.680 (0.603-4.444)	0.3083		
Grade (Fuhrman)				
G1/2	1			
G3/4	1.308 (0.430-3.767)	0.6222		
LN metastasis				
Absent	1			
Present	1.231 (0.390-3.332)	0.7018		
Bone metastasis				
Absent	1			
Present	0.565 (0.129-1.762)	0.3487		
Liver metastasis				
Absent	1			
Present	0.842 (0.194-2.584)	0.7830		
Number of metastatic sites				
One organ	1			
Two or more organs	1.402 (0.519-4.411)	0.5175		
IMDC risk classification				
Favorable/Intermediate	1			
Poor	0.945 (0.251-3.013)	0.9265		
C-reactive protein				
<1.0 mg/dl	1			
≥1.0 mg/dl	1.410 (0.537-3.772)	0.4811		
irAEs				
Absent	1		1	
Present	0.122 (0.019-0.443)	0.0006	0.149 (0.023-0.574)	0.0042
AB				
Absent	1		1	
Present	6.518 (1.857-21.416)	0.0048	3.830 (1.086-12.717)	0.0377

LN: Lymph node; IMDC: International Metastatic Renal Cell Carcinoma Database Consortium; irAE; immune related adverse events; AB: antibiotics.

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Received October 4, 2019
Revised October 14, 2019
Accepted October 15, 2019