SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 17: 2946-2952, 2019

Impact of prior antibiotic use on the efficacy of nivolumab for non-small cell lung cancer

TAIKI HAKOZAKI[1], YUSUKE OKUMA[1,2], MIWAKO OMORI[3] and YUKIO HOSOMI[1]

[1]Department of Thoracic Oncology and Respiratory Medicine, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo 113-8677; [2]Division of Oncology, Research Center for Medical Sciences, Jikei University School of Medicine, Tokyo 105-8461; [3]Department of Pulmonary Medicine and Oncology, Graduate School of Medicine, Nippon Medical School, Tokyo 113-8603, Japan

Received May 6, 2018; Accepted October 18, 2018

DOI: 10.3892/ol.2019.9899

Abstract. Gut microbiota serves an important role in shaping systemic immune responses. Antibiotics cause changes in the gut microbiota that may influence the efficacy of cancer immunotherapy. In the present study, a retrospective analysis of the data from 90 patients treated with nivolumab for non-small cell lung cancer (NSCLC) was conducted. A total of 13 patients were treated with antibiotics prior to nivolumab therapy. The median progression-free survival time in patients treated with antibiotics was 1.2 months [95% confidence interval (CI), 0.5-5.8], while the time for patients who were not treated with antibiotics was 4.4 months (95% CI, 2.5-7.4). The median overall survival time in patients treated with antibiotics was 8.8 months, while it was not reached in those not treated with antibiotics, respectively. The differences between the survival curves with regard to PFS and OS were statistically significant (P=0.04 and P=0.037, respectively). However, in multivariate analysis, no statistically significant association was indicated between survival and prior antibiotic use, although a certain trend concerning the negative influence of antibiotic use was conveyed.

Introduction

Lung cancer is one of the leading causes of cancer-associated mortality globally, with a poor prognosis and a 5-year survival rate of <10% in patients with advanced-stage cancer, according to an international surveillance published in 2016 (1). Recent advancements in molecular targeted therapies for oncogenic driver mutations of advanced non-small cell lung cancer (NSCLC) have improved the prognosis in those individuals with tumors that express the appropriate molecular targets for inhibitory agents (2). However, the majority of patients with advanced NSCLC do not possess any molecular aberrations that can be targeted by any current agents. Therefore, further studies are required to identify and establish novel agents and concepts for molecular targeted therapy.

Antibody-mediated blockade of the interaction between programmed cell death-1 (PD-1) and activated cytotoxic T lymphocytes (CTLs), and between programmed cell death ligand-1 (PD-L1) and tumor cells, has exhibited significant clinical efficacy in a number of types of cancer, including NSCLC. Antibody-mediated blockade inactivates the tumoricidal activity of CTLs and therefore allows tumor cell immune evasion. Immune checkpoint inhibitors (ICIs) nivolumab, pembrolizumab and atezolizumab are currently approved for treating advanced-stage NSCLC. The CheckMate-017 (3), CheckMate-057 (4), KEYNOTE-010 (5) and OAK (6) trials demonstrated the superiority of these agents over docetaxel, which was the standard care for second-line therapy. However, the response to ICIs is only ~20%. In immunohistochemistry, despite the fact that PD-L1 has been approved as a biomarker, it is not sufficient for predicting the response to ICIs.

Efficacy of ICIs could be influenced not only by the intrinsic factors of patients, but also by extrinsic factors. Increasing focus has been placed on the role of gut microbiota in shaping systemic immune responses (7-9). Antibiotics cause changes in the gut microbiota (10-12) that may influence the efficacy of ICIs (13,14). A recent study indicated that prior use of antibiotics negatively influenced the efficacy of ICIs in the clinical settings (15). Using a prospective observational database, the present study performed a retrospective analysis to examine the influence of antibiotics on the clinical outcomes of patients treated with nivolumab for advanced NSCLC.

Materials and methods

Database acquisition. Clinical data from 90 patients with advanced NSCLC were retrospectively analyzed. Patients were treated with nivolumab as the second or later line of chemotherapy at the Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital (Tokyo, Japan) between

Correspondence to: Dr Yusuke Okuma, Department of Thoracic Oncology and Respiratory Medicine, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, 3-18-22 Honkomagome, Bunkyo, Tokyo 113-8677, Japan
E-mail: y-okuma@cick.jp

Key words: non-small cell lung cancer, microbiota, nivolumab, prognosis

January 2016 and April 2017. The database of a prospective observational study [University hospital Medical Information Network (UMIN) registry: UMIN000021694] was used. The following clinical factors of the patients were examined: Age, sex, Eastern Cooperative Oncology Group performance status (ECOG-PS) (16), histological subtype, oncogenic driver mutation status (EGFR mutations and anaplastic lymphoma kinase gene rearrangement), Tumor-Node-Metastasis (TNM) staging (1), lines of chemotherapy, use of antibiotics, use of proton pump inhibitors (PPIs) or histamine H_2-blockers (H_2B) and use of antiflatulents.

Patients treated with antibiotics for ≥3 days within 30 days of nivolumab therapy were defined as those who were treated with antibiotics, regardless of the spectrums or the dosages of the antibiotics, the administration routes (intravenous or oral) or the purpose of antibiotic use. The same criteria were employed for defining patients who used PPIs or H_2B, and antiflatulents.

Statistical analysis. Descriptive statistics were used to summarize the baseline characteristics of the patients. Progression-free survival (PFS) time was defined as the period from the date of initial nivolumab administration to the date of clinical disease progression, mortality from any cause or the last follow-up. Overall survival (OS) time was defined as the period from the date of initial nivolumab administration to the date of mortality from any cause or the last follow-up. The Kaplan-Meier method was used to assess PFS and OS time. Data of patients who were lost to follow-up were censored at the time of last contact. The log-rank test was used for identifying prognostic indicators using univariate and multivariate analyses. The candidate variables analyzed included ECOG-PS, driver mutations, use of antibiotics, use of PPIs or H_2B, and use of antiflatulents. $P<0.05$ using the Cox proportional hazard model was considered to indicate a statistically significant difference. All statistical analyses were performed using the JMP 11.0 software (SAS Institute, Inc., Cary, NC, USA).

The study protocol was approved by the Ethics Committee of the Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital (approval no., 1469) and was conducted according to the Declaration of Helsinki. The study was registered with the UMIN Clinical Trials Registry (ID no., UMIN000021694).

Results

Baseline characteristics. A total of 90 patients with NSCLC (57 male and 33 female) were treated with nivolumab as the second or later line of chemotherapy. All patients were treated with nivolumab monotherapy at the recommended dose (2 mg/kg, day 1, every 2 weeks). The median age of the patients was 68 years (range, 36-87 years). At the time of nivolumab initiation, according to 8th Edition of TNM Classification for Lung Cancer, 12 patients (13.3%) presented with stage IVA disease, 38 (42.2%) with stage IVB disease and 40 (44.4%) with recurrent disease. Overall, 55 patients (61.1%) had adenocarcinoma and 21 (23.3%) had squamous cell carcinoma. A total of 21 patients (23.3%) exhibited oncogenic driver mutations. During the 30 days prior to nivolumab therapy, 13 patients (14.4%) were treated with antibiotics, 47 (52.2%) with PPIs or H_2B, and 11 (12.2%) with antiflatulents. Other patient characteristics are presented in Table I. The details of the patients with prior antibiotic use are summarized in Table II.

Table I. Baseline characteristics of enrolled patients divided into those treated with (n=13) and without (n=77) antibiotics.

Characteristics	Abx^+ group	Abx^- group
Median age (range), years	67 (47-78)	68 (36-87)
Sex, n (%)		
Male	9 (69.2)	48 (62.3)
Female	4 (30.8)	29 (37.7)
ECOG-PS, n (%)		
0/1	4 (30.8)	60 (77.9)
2	3 (23.1)	10 (13.0)
3	6 (46.2)	7 (9.1)
Histological subtypes, n (%)		
Adenocarcinoma	11 (84.6)	44 (57.1)
SQC	2 (15.4)	19 (24.7)
NSCLC, NOS	0 (0.0)	9 (11.7)
ADSQC	0 (0.0)	2 (2.6)
LCNEC	0 (0.0)	2 (2.6)
NEC	0 (0.0)	1 (1.3)
Driver mutations, n (%)		
None	12 (92.3)	57 (74.0)
EGFR exon19 del	0 (0.0)	6 (7.8)
EGFR exon20	0 (0.0)	1 (1.3)
EGFR exon21 L861Q	0 (0.0)	1 (1.3)
EGFR exon21 L858R	1 (7.7)	10 (13.0)
KRAS	0 (0.0)	1 (1.3)
ROS-1	0 (0.0)	1 (1.3)
Staging, n (%)		
IVA	3 (23.1)	9 (11.7)
IVB	6 (46.2)	32 (41.6)
Recurrent	4 (30.8)	36 (46.8)
Median number of chemotherapy lines (range)	2 (2-5)	2 (2-5)
Use of PPIs or H_2Bs, n (%)		
Yes	12 (92.3)	35 (45.5)
No	1 (7.7)	42 (54.5)
Use of antiflatulents, n (%)		
Yes	4 (30.8)	7 (9.1)
No	9 (69.2)	70 (90.9)

Abx, antibiotics; ECOG-PS, Eastern Cooperative Oncology Group-performance status; SQC, squamous cell carcinoma; NSCLC, non-small cell lung cancer; NOS, not other specified; ADSQC, adeno-squamous cell carcinoma; LCNEC, large cell neuroendocrine carcinoma; NEC, neuroendocrine carcinoma; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma; ROS-1, ROS, proto-oncogene 1, receptor tyrosine kinase; PPIs, proton pump inhibitors; H_2Bs, histamine H_2-blockers.

Clinical outcomes of nivolumab therapy. The median PFS time of all patients treated with nivolumab was 3.9 months

Table II. Cases of antibiotic use prior to nivolumab therapy (n=13).

Patient no.	Reasons for Abx use	Duration, days	Types of Abx	Administration routes
1	Prophylaxis (steroid use)	8	TMP/SMX	Oral
2	Prophylaxis (steroid use)	22	TMP/SMX	Oral
3	Prophylaxis (steroid use)	31	TMX/SMX	Oral
4	Prophylaxis (steroid use)	35	TMX/SMX	Oral
5	Lung infection	11	AMPC/CVA	Oral
6	Lung infection	13	CTRX, MEPM	Intravenous
7	Lung infection	14	AMPC/CVA	Oral
8	Lung infection	18	PIPC/TAZ	Intravenous
9	Obstructive pneumonia	10	ABPC/SBT	Intravenous
10	Obstructive pneumonia	60	AMPC/CVA	Oral
11	Pyelonephritis	21	CEZ, TMP/SMX	Oral
12	Fever	5	LVFX	Oral
13	Fever	10	AMPC/CVA	Oral

Abx, antibiotics; TMP/SMX, trimethoprim/sulfamethoxazole; AMPC/CVA, amoxicillin/clavulanate; CTRX, ceftriaxone; MEPM, meropenem; PIPC/TAZ, piperacillin/tazobactam; ABPC/SBT, ampicillin/sulbactam; CEZ, cefazolin; LVFX, levofloxacin.

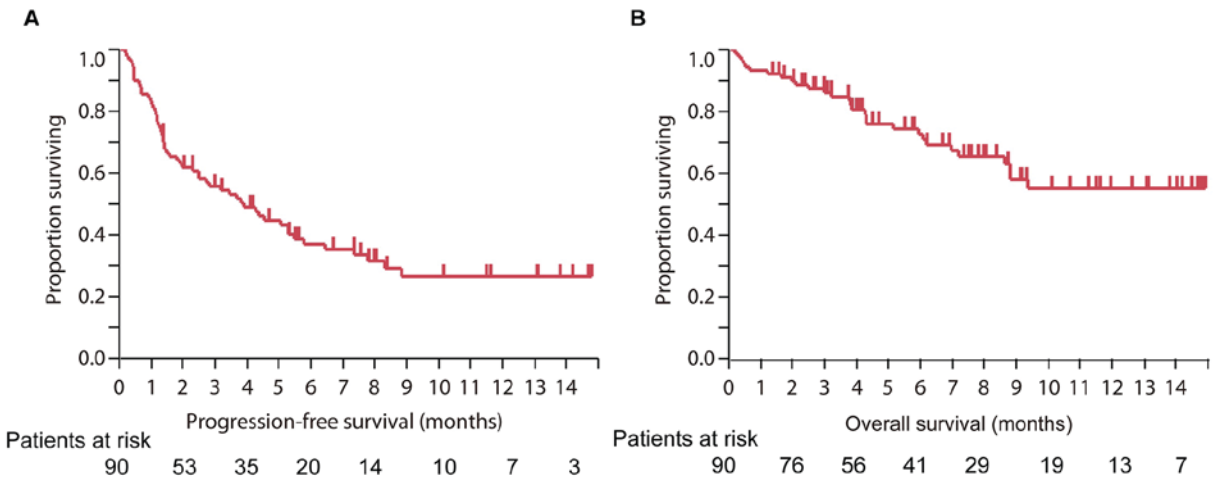

Figure 1. Survival analysis of patients treated with nivolumab. The estimated Kaplan-Meier survival curves for the (A) progression-free survival and (B) overall survival of patients with non-small cell lung cancer (n=90).

[95% confidence interval (CI), 2.3-5.5], and the median OS time was not reached (Fig. 1).

Clinical outcomes of nivolumab therapy in the subgroups previously treated or not treated with antibiotics, H_2B or PPIs, and antiflatulents. The median PFS time of patients treated with antibiotics was 1.2 months (95% CI, 0.5-5.8) and the median PFS time of patients not treated with antibiotics was 4.4 months (95% CI, 2.5-7.4). The median OS of patients treated and those not treated with antibiotics was 8.8 months and not reached, respectively (Fig. 2). The differences between the survival curves with regard to PFS and OS were statistically significant (P=0.04 and P=0.037, respectively).

Univariate and multivariate analyses. Univariate analysis revealed that ECOG-PS, oncogenic driver mutations, use of antibiotics, and use of PPIs or H_2B were significantly associated with OS (Table III). Multivariate analysis indicated that driver mutations were significantly associated with patient survival, whereas significant associations were not observed between OS and use of antibiotics, PPIs or H_2Bs (Table IV).

Discussion

In recent years, clinical responses to ICIs have been observed to be more favorable in patients with an indicative active endogenous T-cell response in the tumor microenvironment (16-19). However, the underlying mechanisms that govern the presence or absence of this phenotype remain unclear. In the present study, a retrospective analysis of 90 patients treated with nivolumab for NSCLC was performed. A statistically significant association between survival and prior antibiotic use was not indicated, although

Table III. Univariate analysis of survival in patients treated with nivolumab.

Variants	n	MST (95% CI), months	P-value
Age, years			
<70	56	NR (7.0-NR)	0.64
≥70	34	NR (7.2-NR)	
Sex			
Male	57	NR (8.8-NR)	0.19
Female	33	9.4 (4.3-NR)	
ECOG-PS			
<2	64	NR (8.8-NR)	0.01[a]
≥2	26	7.0 (3.8-NR)	
Histology			
Adenocarcinoma	55	8.8 (5.9-NR)	0.06
Squamous cell carcinoma	21	NR (NR-NR)	
Other	14	NR (7.0-NR)	
Driver mutations			
Yes	21	4.3 (2.1-NR)	<0.001[a]
No	69	NR (8.8-NR)	
Lines of chemotherapy			
2	54	NR (8.8-NR)	0.14
≥3	36	8.6 (5.2-NR)	
Use of antibiotics			
Yes	13	8.8 (0.7-NR)	0.04[a]
No	77	NR (8.6-NR)	
Use of PPIs or H_2Bs			
Yes	47	8.8 (5.9-NR)	0.04[a]
No	43	NR (NR-NR)	
Use of antiflatulents	11	NR (2.5-NR)	0.64
Yes	11	NR (2.5-NR)	0.64
No	79	NR (8.8-NR)	

[a]P≤0.05; ECOG-PS, Eastern Cooperative Oncology Group-performance status; PPIs, proton pump inhibitors; H_2Bs, histamine H_2-blockers; MST, median survival time; CI, confidence interval; NR, not reached.

Table IV. Multivariate analysis of survival in patients treated with nivolumab.

Variants	HR	95% CI	P-value
ECOG-PS (poor vs. good)	2.17	0.89-5.25	0.09
Driver mutations (yes vs. no)	4.82	2.05-11.3	<0.001[a]
Use of antibiotics (yes vs. no)	2.02	0.70-5.83	0.19
Use of PPIs or H_2Bs (yes vs. no)	1.90	0.80-4.51	0.15

[a]P≤0.05; ECOG-PS, Eastern Cooperative Oncology Group-performance status; PPIs, proton pump inhibitors; H_2Bs, histamine H_2-blockers; HR, hazard ratio; CI, confidence interval.

a certain trend toward the negative influence of antibiotic use was suggested.

The gut microbiota serves an important role in shaping systemic immune responses (7-9). A number of studies have indicated that certain types of bacteria or bacterial products can modulate systemic inflammation and antitumor immunity. Numerous families of bacteria and metabolites from the bacterial breakdown of indigestible dietary components have been indicated to interact with specific immune components that influence the synthesis of regulatory cytokines (20).

The associations between the gut microbiota and the responsiveness to anticancer therapy have been extensively investigated. Previous studies have mainly focused on patients with colorectal cancer and have demonstrated the role of gut microbiota in carcinogenesis and the response to cytotoxic chemotherapy (21-32). However, it remains unclear whether commensal microbiota influence spontaneous immune responses against tumors, affecting the therapeutic activity of ICIs regardless of the type of cancer.

Preclinical and clinical data support the hypothesis that the gut microbiota shapes the innate and adaptive immune system, influencing the CTL-associated protein 4 (CTLA-4) and PD-1/PD-L1 axis, thereby affecting the efficacy of ICIs (13,14). The abundance of *Bifidobacterium* species

Table V. Comparison of studies examining the association of antibiotics and the efficacy of immune check point inhibitors in patients with non-small cell lung cancer.

Variables	Derosa *et al* (n=239)[a]	Kaderbhai *et al* (n=74)[b]	Present study (n=90)
Abx use, n (%)	48 (20.1)	15 (20.3)	13 (14.4)
Time of Abx treatment prior to ICI use, days	30	90	30
Reasons for Abx, n (%)			
Prophylaxis	15 (31.2)	0 (0.0)	4 (30.8)
Therapy	33 (68.8)	15 (100.0)	9 (69.2)
Duration of Abx treatment, n (%)			
≤7 days	35 (72.9)	7 (46.7)	2 (15.3)
>7 days	13 (27.1)	8 (53.3)	11 (84.6)
Administration routes, n (%)			
Oral	42 (87.5)	11 (73.3)	10 (76.9)
Intravenous/muscular	5 (10.4)	4 (26.7)	3 (23.1)
Not reported	1 (2.1)	0 (0.0)	0 (0.0)
Median PFS (Abx^+ vs. Abx^-), months	1.9 vs. 3.8	NA	1.2 vs. 4.4
Median OS (Abx^+ vs. Abx^-), months	7.9 vs. 24.6	NA	8.8 vs. NR

[a](42); [b](36). Abx, antibiotics; ICIs, immune checkpoint inhibitors; PFS, progression-free survival; OS, overall survival; CI, confidence interval; NA, not available; NR, not reached.

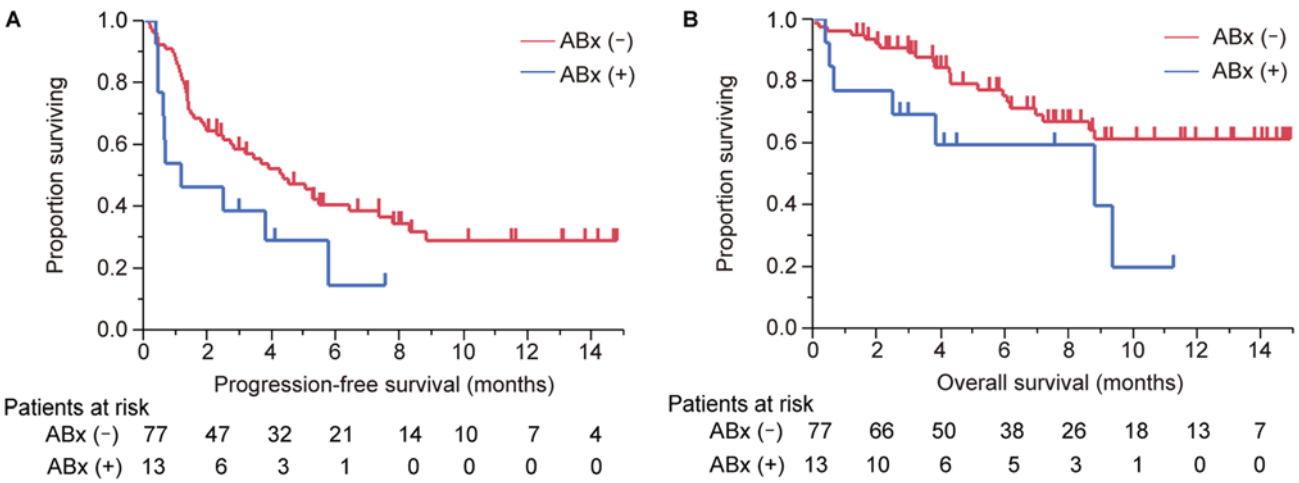

Figure 2. Survival analysis of untreated patients and those treated with antibiotics. The estimated Kaplan-Meier survival curves for (A) progression-free survival and (B) overall survival comparing the groups with (n=13) and without (n=77) antibiotics. Abx, antibiotics.

in the intestine has been indicated to improve anti-PD-L1 therapy in a tumor-bearing mouse model. In patients with metastatic melanoma, analysis of fecal samples indicated that bacterial diversity and relative abundance of bacteria of the *Ruminococcaceae* family were fecal microbial predictors of an anti-PD-1 therapy response. Metagenomic studies revealed functional differences in responders, including enrichment of anabolic pathways (33). An improved response to anti-PD-L1 therapy was observed in germ-free mice receiving fecal microbiota transplantation from responsive patients compared with that in the mice colonized with feces from non-responsive patients (34,35). The aforementioned observations suggest that a comprehensive analysis of the gut microbiota may prove valuable for detecting novel biomarkers or therapeutic targets for cancer patients treated with ICIs.

The effect of antibiotics on the efficacy of ICIs has also been investigated due to their impact on the gut microbiota, however the causal relationship is still unclear in a clinical setting (Table V). Anti-CTLA-4 antibody loses its therapeutic efficacy in mice that are reared under germ-free conditions or are treated with broad-spectrum antibiotics. In a clinical setting, a retrospective study indicated that prior antibiotic use negatively influenced the survival of patients treated with ICIs for metastatic renal cell carcinoma and NSCLC (15). This result may implicate the disruption of gut microbiota to interfere with the efficacy of ICIs. However, another study indicated that the administration of antibiotics did not influence the outcomes in

patients with NSCLC (36). In the present analysis, no statistically significant association was observed between survival and prior antibiotic use, but a certain trend toward the negative influence of antibiotic use was conveyed. The fact that certain medical conditions require the use of antibiotics should be taken into consideration, as they themselves could affect patient survival.

Considering the differences between the aforementioned studies, the timing of antibiotic use prior to the start of nivolumab therapy may serve an important role, since the composition of the microbiota changes with the passage of time following the discontinuation of antibiotics. Previous studies have mainly focused on eradication treatment for *Helicobacter pylori* and have indicated that the microbiota returns to its baseline within 1 week to 3 months after the discontinuation of antibiotics, whereas the effect of antibiotics for a number of other bacteria may remain for years. It may be difficult to set the optimal cutoff point for the 'prior antibiotics use' considering its effect on the efficacy of following ICIs. However, studies such as that by Derosa *et al* (15) may be of assistance. In this study, the associations of antibiotic use (within 30 or 60 days) and the efficacy of ICIs were examined. The impact of antibiotics prior to 60 days was not as potent as that within the first 30 days prior to ICIs. In another study, in which the prior use of antibiotics was defined as antibiotics administered in the last 3 months prior to nivolumab (36), no association between antibiotic use and the efficacy of ICIs was observed. Further interpretation of these results is required. Furthermore, future studies focusing on how the antibiotic spectrum, the administration routes and the co-administration of corticosteroids may affect the efficacy of ICIs are also required.

Recently, non-antibiotic drugs, including antacids, corticosteroids, non-steroidal anti-inflammatory drugs and antipsychotics, have been associated with changes in the gut microbiota (37-40). Regarding antacids, a previous study demonstrated the effect of PPIs on the gut microbiota (41), but the association between antacid use and the efficacy of ICIs requires further investigation. In the present retrospective analysis, the prior use of PPIs or H_2B exhibited a trend towards being negatively influential on ICI efficacy in the same way as antibiotics. The influence of antiflatulents on the efficacy of ICIs was also examined, due to potential benefits of probiotics or prebiotics suggested in previous studies (42). In the present analysis, however, no association between survival and prior antibiotic use was observed.

The present study has a number of limitations. First, the serial changes in the gut microbiota to confirm the influence of antibiotics and antacids was not assessed. Considering the retrospective nature of the study, it was reasonable to use clinical outcomes, including PFS or OS, as surrogate indicators of these influences. Second, the influence of the use of antibiotics and antacids during nivolumab therapy was not investigated. Third, this was a retrospective, nonrandomized study that was performed at a single institution, with a relatively small number of patients who used antibiotics for a number of conditions. To the best of our knowledge, the present study is the first to examine the association among non-antibiotics drugs, antacids and antiflatulents and the efficacy of ICIs. Therefore, future focus on the experimental measures to control confounding factors with regard to the complex medications used by patients is required, and further studies are warranted to confirm the findings of the present study. Additional research is being conducted to investigate changes in the gut microbiome by obtaining stool samples to determine changes in the microbiome, or the types of microbiome that may predict responses to ICIs.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

TH and MO acquired the clinical data. TH, YO, MO and YH were responsible for the interpretation of the data. TH and YO drafted the manuscript. All authors have read and approved the current version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Committee of Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital (Tokyo, Japan) (approval number: 1952). Due to the retrospective nature of the study, written informed consent was not required.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Detterbeck FC, Boffa DJ and Tanoue LT: The new lung cancer staging system. Chest 136: 260-271, 2009.
2. Gonzalvez F, Schug ZT, Houtkooper RH, MacKenzie ED, Brooks DG, Wanders RJ, Petit PX, Vaz FM and Gottlieb E: Cardiolipin provides an essential activating platform for caspase-8 on mitochondria. J Cell Biol 183: 681-696, 2008.
3. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, *et al*: Nivolumab versus Docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 373: 123-135, 2015.
4. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, *et al*: Nivolumab versus Docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 373: 1627-1639, 2015.
5. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, *et al*: Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised controlled trial. Lancet 387: 1540-1550, 2016.

6. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, Gadgeel SM, Hida T, Kowalski DM, Dols MC, *et al*: Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): A phase 3, open-label, multicentre randomised controlled trial. Lancet 389: 255-265, 2017.
7. Hooper LV, Littman DR and Macpherson AJ: Interactions between the microbiota and the immune system. Science 336: 1268-1273, 2012.
8. Ivanov II and Honda K: Intestinal commensal microbes as immune modulators. Cell Host Microbe 12: 496-508, 2012.
9. McAleer JP and Kolls JK: Maintaining poise: Commensal microbiota calibrate interferon responses. Immunity 37: 10-12, 2012.
10. Jernberg C, Löfmark S, Edlund C and Jansson JK: Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J 1: 56-66, 2007.
11. Korpela K, Salonen A, Virta LJ, Kekkonen RA, Forslund K, Bork P and de Vos WM: Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. Nat Commun 7: 10410, 2016.
12. Becattini S, Taur Y and Pamer EG: Antibiotic-induced changes in the intestinal microbiota and disease. Trends Mol Med 22: 458-478, 2016.
13. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabri B, Alegre ML, *et al*: Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 350: 1084-1089, 2015.
14. Vétizou M, Pitt JM, Daillére R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, *et al*: Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 350: 1079-1084, 2015.
15. Derosa L, Routy B, Enot D, Baciarello G, Massard C, Loriot Y, Fizazi K, Escudier BJ, Zitvogel L and Albiges L: Impact of antibiotics on outcome in patients with metastatic renal cell carcinoma treated with immune checkpoint inhibitors. J Clin Oncol 35: 462-462, 2017.
16. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, *et al*: PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515: 568-571, 2014.
17. Spranger S, Spaapen RM, Zha Y, Williams J, Meng Y, Ha TT and Gajewski TF: Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med 5: 200ra116, 2013.
18. Ji RR, Chasalow SD, Wang L, Hamid O, Schmidt H, Cogswell J, Alaparthy S, Berman D, Jure-Kunkel M, Siemers NO, *et al*: An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother 61: 1019-1031, 2012.
19. Gajewski TF, Louahed J and Brichard VG: Gene signature in melanoma associated with clinical activity: A potential clue to unlock cancer immunotherapy. Cancer J 16: 399-403, 2010.
20. Belkaid Y and Hand TW: Role of the microbiota in immunity and inflammation. Cell 157: 121-141, 2014.
21. Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, Ojesina AI, Jung J, Bass AJ, Tabernero J, *et al*: Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. Genome Res 22: 292-298, 2012.
22. Rowland IR: The role of the gastrointestinal microbiota in colorectal cancer. Curr Pharm Des 15: 1524-1527, 2009.
23. Zhu Q, Gao R, Wu W and Qin H: The role of gut microbiota in the pathogenesis of colorectal cancer. Tumour Biol 34: 1285-1300, 2013.
24. Chen HM, Yu YN, Wang JL, Lin YW, Kong X, Yang CQ, Yang L, Liu ZJ, Yuan YZ, Liu F, *et al*: Decreased dietary fiber intake and structural alteration of gut microbiota in patients with advanced colorectal adenoma. Am J Clin Nutr 97: 1044-1052, 2013.
25. Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X, Jia W, Cai S and Zhao L: Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. ISME J 6: 320-329, 2012.
26. Peek RM Jr and Blaser MJ: Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat Rev Cancer 2: 28-37, 2002.
27. Yang Y, Wang X, Huycke T, Moore DR, Lightfoot SA and Huycke MM: Colon macrophages polarized by commensal bacteria cause colitis and cancer through the bystander effect. Transl Oncol 6: 596-606, 2013.
28. Castellarin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, Barnes R, Watson P, Allen-Vercoe E, Moore RA and Holt RA: Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome Res 22: 299-306, 2012.
29. McIntosh GH, Royle PJ and Playne MJ: A probiotic strain of L. acidophilus reduces DMH-induced large intestinal tumors in male Sprague-Dawley rats. Nutr Cancer 35: 153-159, 1999.
30. Sonnenberg GF and Artis D: Innate lymphoid cell interactions with microbiota: Implications for intestinal health and disease. Immunity 37: 601-610, 2012.
31. Wu S, Shin J, Zhang G, Cohen M, Franco A and Sears CL: The Bacteroides fragilis toxin binds to a specific intestinal epithelial cell receptor. Infect Immun 74: 5382-5390, 2006.
32. Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, Huso DL, Brancati FL, Wick E, McAllister F, *et al*: A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med 15: 1016-1022, 2009.
33. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, Luke JJ and Gajewski TF: The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 359: 104-108, 2018.
34. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC, *et al*: Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 359: 97-103, 2018.
35. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, *et al*: Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 359: 91-97, 2018.
36. Kaderbhai C, Richard C, Fumet JD, Aarnink A, Foucher P, Coudert B, Favier L, Lagrange A, Limagne E, Boidot R and Ghiringhelli F: Antibiotic use does not appear to influence response to nivolumab. Anticancer Res 37: 3195-3200, 2017.
37. Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, Poole AC, Sutter JL, Welter D, Ley RE and Bell JT: Proton pump inhibitors alter the composition of the gut microbiota. Gut 65: 749-756, 2016.
38. Huang EY, Inoue T, Leone VA, Dalal S, Touw K, Wang Y, Musch MW, Theriault B, Higuchi K, Donovan S, *et al*: Using corticosteroids to reshape the gut microbiome: Implications for inflammatory bowel diseases. Inflamm Bowel Dis 21: 963-972, 2015.
39. Rogers MAM and Aronoff DM: The influence of non-steroidal anti-inflammatory drugs on the gut microbiome. Clin Microbiol Infect 22: 178 e171-178 e179, 2016.
40. Flowers SA, Evans SJ, Ward KM, McInnis MG and Ellingrod VL: Interaction between atypical antipsychotics and the gut microbiome in a bipolar disease cohort. Pharmacotherapy 37: 261-267, 2017.
41. Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, Tigchelaar EF, Jankipersadsing SA, Cenit MC, Harmsen HJ, *et al*: Proton pump inhibitors affect the gut microbiome. Gut 65: 740-748, 2016.
42. Derosa L, Hellmann MD, Spaziano M, Halpenny D, Fidelle M, Rizvi H, Long N, Plodkowski AJ, Arbour KC, Chaft JE, *et al*: Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Ann Oncol 29: 1437-1444, 2018.