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Antibiotics are associated with attenuated efficacy of anti-PD-1/PD-L1 therapies in Chinese patients with advanced non-small cell lung cancer

Sha Zhao[a,1], Guanghui Gao[a,1], Wei Li[a,1], Xuefei Li[b], Chao Zhao[b], Tao Jiang[a], Yijun Jia[a], Yayi He[a], Aiwu Li[a], Chunxia Su[a], Shengxiang Ren[a], Xiaoxia Chen[a,*], Caicun Zhou[a,*]

[a] Department of Medical Oncology, Shanghai Pulmonary Hospital &Thoracic Cancer Institute, Tongji University School of Medicine, Shanghai, 200433, PR China
[b] Department of Lung Cancer and Immunology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, 200433, PR China

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ABSTRACT

Objectives: Gut microbiome plays a dominant role in modulating therapeutic efficacy of immune checkpoint inhibitors (ICIs) targeting the programmed cell death receptor/ligand-1 (PD-1/PD-L1) pathway, suggesting that co-administration of antibiotics (Abx), which might result in dysbacteriosis, can attenuate the clinical outcomes of ICIs. The current study aimed to investigate the predictive role of Abx on ICIs treatment in patients with advanced non-small cell lung cancer (NSCLC). The impact of proton pump inhibitors (PPIs), another medication that can induce dysbacteriosis, was also investigated.

Materials and methods: We retrospectively reviewed the medical records of eligible patients who received anti-PD-1-based therapies in our hospital. Tumor responses, patients' survival, the incidence of immune-related adverse events (irAEs) and other baseline variables were examined. The application of Abx or PPIs treatment were also collected. Clinical outcomes and clinicopathologic features were compared according to the status of Abx or PPIs co-administration.

Results: A total of 109 patients were included. Of them, 20 (18.3%) patients were categorized in Abx-treated group. No major difference in baseline characteristics was observed between Abx-treated and -untreated groups. Concomitant Abx treatment was significantly associated with shorter progression-free survival (PFS) ($p < 0.0001$) and overall survival (OS) ($p = 0.0021$). And primary disease progression tended to increase in Abx-treated group ($p = 0.092$). Yet, the occurrence and grades of irAEs were comparable between two groups. In multivariable analysis, Abx treatment was markedly associated with worse PFS (HR = 0.32, 95%CI 0.18-0.59, $p < 0.0001$) and OS (HR = 0.35, 95%CI 0.16-0.77, $p = 0.009$). Regarding the use of PPIs, no significant difference was observed in clinical outcomes between the patients with or without concomitant PPIs treatment.

Conclusions: Abx treatment was significantly associated with attenuated clinical outcomes derived from anti-PD-1-based ICIs in a Chinese cohort of patients with advanced NSCLC.

1. Introduction

Immune checkpoint inhibitors (ICIs) targeting the programmed cell death-1(PD-1) and programmed cell death-ligand 1 (PD-L1) axis have dramatically shifted the therapeutic paradigm of non-small cell lung cancer (NSCLC) and now are standard treatment options in both first-line and second-line settings for patients with advanced disease [1–6]. Three monoclonal anti-PD-1/PD-L1 monoclonal antibodies (mAbs) (including nivolumab, pembrolizumab and atezolizumab) have currently been approved for the treatment of advanced NSCLC by the US Food and Drug Administration. However, despite the remarkable success of clinical applications, the efficacy of anti-PD-1/PD-L1 mAbs varies greatly across individual patients [7,8]. In unselected advanced NSCLC populations, the objective response rate [5] is only around 20% [9,10]. Moreover, although responses to PD-1/PD-L1 blockade tend to be more durable than response to chemotherapy [1–4], acquired resistance inevitably develops in most patients [11]. Therefore, the identification of reliable predictors to select those patients that who are more likely to benefit from anti-PD-1/PD-L1 mAbs is of paramount importance.

* Corresponding authors at: Department of Medical Oncology, Shanghai Pulmonary Hospital & Thoracic Cancer Institute, Tongji University School of Medicine, No. 507, Zheng Min Road, Shanghai, 200433, PR China.
E-mail addresses: cheetos_xx@126.com (X. Chen), caicunzhou_dr@163.com (C. Zhou).

[1] These authors contributed equally to this work.

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A number of biomarkers have been identified to predict the response to anti-PD-1/PD-L1 mAbs, such as intratumoral PD-L1 expression, mutational burden, lymphocytic infiltrates and neoantigens [6,10,12–15]. Interestingly, several elegant studies have recently demonstrated the crucial impact of human gut microbiome (GM) on anti-PD-1 immunotherapies [16–18]. The diversity and abundance of the GM can modulate the anti-cancer activity of ICIs in terms of both efficacy and toxicity, highlighting the possibility that concomitant antibiotics (Abx) administration, which can shift GM to long-term alternative dysbiotic states [19,20], may promote resistance to ICIs. Because of its prominent influence on microbial composition, Abx treatment can further disturb the development of systematic immune responses by affecting T cell function, altering cytokine production and interfering with dendritic cells (DCs) action etc. [21–23]. As a consequence, the use of Abx is increasingly being recognized as playing a crucial role in ICIs therapeutic outcomes. A series of previous reports have indicated a negative correlation between Abx uptake and efficacy of several anticancer therapies, such as cyclophosphamide, CpG-oligonucleotide immunotherapy, platinum chemotherapy, anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) therapy and anti-PD-1 mAbs [17,24–29]. However, the data on the predictive role of Abx usage in the clinical outcomes of ICIs are scarce, and this issue has not yet been extensively explicated, especially in lung cancer. Furthermore, the human gut microbiota variations appear to vary distinctly depending on ethnicity and geography [30]. For instance, previous literatures demonstrated that *Bacteroides* shows domination in Chinese and French population in their GM, whereas American community has higher abundance in *Firmicutes* and Japanese community has abundance in *Actinobacteria* [30–32]. This phenomenon indicates that the influence of Abx on GM may differ according to ethnicity and geography specific diversity, and further hints at the potential heterogeneous responses to ICIs induced by concomitant Abx treatment in different patient populations. Therefore, more data from diverse patient cohorts are needed to comprehensively reveal the correlation between Abx treatment and ICIs efficacy.

In the present study, we compared the clinical features and responses in a Chinese cohort of NSCLC patients who received ICIs with or without concomitant Abx treatment, and analyzed the prognostic impact of Abx on anti-PD-1-based immunotherapeutic efficacy.

2. Materials and methods

2.1. Patients

We conducted a retrospective review of all the patients diagnosed with advanced NSCLC who started anti-PD-1/PD-L1based therapies between January 2016 and January 2018 in Shanghai Pulmonary Hospital, Tongji University. Patients who were evaluable for response assessment were selected for the present study. All of them had received anti-PD-1 mAbs as monotherapy alone or in combination with chemotherapy or anti-angiogenesis, regardless of treatment lines. Electronic medical records were used to collect the detailed patient information, mainly including age, sex, smoking history, histological types, Eastern Cooperative Oncology Group (ECOG) performance status (PS), mutation status, primary tumor sites, comorbidities, treatment lines, therapeutic regimens and the date of death or last follow-up. The comorbidity was diagnosed when any of the following conditions were identified: chronic obstructive lung disease (COPD), bronchiectasis, tuberculosis, interstitial lung diseases, cardiovascular diseases, hypertension, diabetes mellitus, hepatitis and renal insufficiency. The status of proton pump inhibitors (PPIs) treatment was also collected. Objective tumor responses were assessed by investigators according to the Response Evaluation Criteria In Solid Tumors (RECIST; version 1.1). Adverse events (AEs) were evaluated according to the Common Terminology Criteria for Adverse Events (version 4.0). Immunotherapy-related AEs (irAEs) were defined as AEs that having an immunological basis [33]. One pack year (PY) of smoking was equivalent to average 20 cigarettes per day for one year. Based on the smoking status, never smokers were defined as patients with a smoking history of < 5PY cigarettes within their lifetime. The cumulative smoking dosage of 30 or more PY were used as the cut-off to divide the smokers (including former and current) into moderate smokers (5–29 PY) and heavy smokers (≥30 PY) [34,35]. The status of Abx treatment was evaluated: patients who received Abx within 1 month before or after the first administration of anti-PD-1 mAbs were defined as the Abx-treated group, and the others were defined as the Abx-untreated group.

This study was approved by the Ethics Committee of Shanghai Pulmonary Hospital, Tongji University School of Medicine.

2.2. Statistical analysis

Clinicopathologic features were descriptively summarized by percentages. Fisher's exact test or the chi-square test was used to analyze relationships between clinicopathologic variables. Progression-free survival (PFS) was defined from the date of ICIs treatment initiation to the date of physician assessment of disease progression or death, whichever occurred first. Overall survival (OS) was calculated from the date of ICIs treatment start to the date of death of any cause or last follow-up in surviving participants. PFS and OS curves were estimated using the Kaplan-Meier methodology and compared by log-rank test. At data cutoff (May 2018), an event of RECIST-defined progression disease (PD) was observed in 62 (56.9%) patients for PFS analysis, and death had occurred in 35 (32.1%) patients for OS analysis. Cox proportional hazards model was used for univariate and multivariate analysis to calculate the hazard ratio (HR) and 95% confidence interval (CI). The variables with a p value < 0.1 identified in univariable analysis were selected for the multivariable analysis. Statistical analysis was performed using SPSS statistical software (version 22.0; IBM Corporation, Armonk, NY). All p values were two-sided and were considered significant at $p < 0.05$.

3. Results

3.1. Patient characteristics

A total of 142 consecutive patients with advanced NSCLC received various ICIs approaches at our institution. Among them, 109 patients were evaluable for objective response and finally included in the current study. All the eligible patients did not harbor epidermal growth factor receptor (*EGFR*) activating mutations or anaplastic lymphoma kinase (*ALK*) rearrangements. Of the included patients, 57 received anti-PD-1 mAbs (including pembrolizumab, nivolumab or SHR-1210) as monotherapy. Of the remaining patients, 33 received anti-PD-1 mAbs plus chemotherapy, and 19 were treated with anti-PD-1 mAb (SHR-1210) plus an anti-angiogenic agent (apatinib or bevacizumab). 28 patients (25.7%) received anti-PD-1-based therapies as first-line therapy, and the others (74.3%) received ICIs treatment as second- or higher-line therapy.

We firstly dichotomized the included patients according to Abx use. Overall, 20 patients (18.3%) received Abx treatment within 1 month before or after the first administration of PD-1 blockade (hereafter referred to as the Abx-treated group), and 89 (81.7%) were identified as the Abx-untreated group. The most frequently used Abx were β-lactam inhibitors and fluoroquinolones. And the most common indication for Abx treatment was pneumonitis, which occurred in 13 (65.0%) of the patients in Abx-treated group, with another 3 (15.0%) patients had urinary tract infection, 3 (15.0%) patients suffered skin or perianal abscess, and 1 (5.0%) patient experienced COPD exacerbation. The clinicopathologic variables were typical of patients with advanced/metastatic NSCLC and were well balanced across the Abx-treated and Abx-untreated groups. While additionally, the patients in Abx-treated group were more frequently diagnosed with *KRAS* mutation (25.0% versus 4.5%, $p = 0.010$), and a greater proportion of the patients in the

Table 1
Demographic data and clinical characteristics.

Variable	Total N = 109	Abx n = 20	Non-Abx n = 89	*P* Value
Age, yr, No (%)				
Median, Range	62(34-89)	57.5(34-79)	62(37-89)	
< 65	63(57.8)	13(65.0)	50(56.2)	0.470
≥65	46(42.2)	7(35.0)	39(43.8)	
Gender, No (%)				
Female	20(18.3)	5(25.0)	15(16.9)	0.395
Male	89(81.7)	15(75.0)	74(83.1)	
Smoking, No (%)				
Never	35(32.1)	5(25.0)	30(33.7)	0.333
Moderate	12(11.0)	4(20.0)	8(9.0)	
Heavy	62(56.9)	11(55.0)	51(57.3)	
ECOG PS, No (%)				
0-1	107(98.2)	20(100.0)	87(97.8)	1.000
≥2	2(1.8)	0(0.0)	2(2.2)	
Histology, No (%)				
Squamous	27(24.8)	5(25.0)	22(24.7)	0.979
Non-squamous	82(75.2)	15(75.0)	67(75.3)	
Oncogenic mutation, No (%)				
Kras	9(8.3)	5(25.0)	4(4.5)	**0.010**
None	100(91.7)	15(75.0)	85(95.5)	
Primary site, No (%)				
Superior lobe	57(52.3)	10(50.0)	47(52.8)	0.115
Middle/inferior lobe	39(35.8)	5(25.0)	34(38.2)	
both	13(11.9)	5(25.0)	8(9.0)	
Distant metastasis*, No (%)				
Yes	64(58.7)	15(75.0)	49(55.1)	0.102
No	45(41.3)	5(25.0)	45(44.9)	
Comorbidities				
Yes	52(47.7)	8(40.0)	44(49.4)	0.445
No	57(52.3)	12(60.0)	45(50.6)	
No.of treatment line, No (%)				
1	28(25.7)	4(20.0)	24(27.0)	0.718
≥2	81(74.3)	16(80.0)	65(73.0)	
Treatment regimen, No (%)				
αPD-1	57(52.3)	11(55.0)	46(51.7)	0.835
αPD-1 + Chemo	33(30.3)	5(25.0)	28(31.5)	
αPD-1 + Apatinib	19(17.4)	4(20.0)	15(16.8)	
PPIs use				
Yes	40(36.7)	4(20.0)	36(40.4)	0.145
No	69(63.3)	16(80.0)	53(59.6)	
Clinical trial, No (%)				
Yes	76(69.7)	10(50.0)	66(74.2)	**0.034**
No	33(30.3)	10(50.0)	23(25.8)	

Abbreviations: Abx, antibiotics; yr, years; No, number; ECOG, Eastern Cooperative Oncology Group; PS, performance status; PPIs, proton pump inhibitors; Chemo, chemotherapy.

* Include brain, bone, liver, adrenal gland, cervical/abdominal lymph nodes, subcutaneous tissue, cardiac vesicle.

Abx-untreated group were enrolled in clinical trials (74.2% versus 50.0%, $p = 0.034$). The clinicopathologic features of all included patients were summarized in Table 1.

3.2. Correlation between Abx use and outcomes

We firstly assessed the impact of Abx treatment on clinical outcomes during ICIs treatment. Of note, we found that concomitant Abx use was associated with shorter PFS (median 3.73 versus 9.63 months, $p < 0.0001$) and reduced OS (median 6.07 versus 21.87 months, $p = 0.0021$) in the entire cohort (Fig. 1A). Meanwhile, we observed a slightly higher rate pf primary PD in the Abx-treated group compared to Abx-untreated group (35.0% versus 18.0%, $p = 0.092$) (Fig. 1A), although no statistical difference was observed in the ORR (22.5% versus 15.0%, $p = 0.662$). To address whether the results were confounded by therapeutic approaches, we further evaluated the impact of Abx usage according to different therapeutic regimens. Detailed survival and response data are presented in Fig. 1B–D. Among the 57 patients treated with single-agent anti-PD-1 mAbs, both PFS (median 3.73 months versus 9.63 months, $p = 0.0006$) and OS (median 6.00 months versus 21.87 months, $p = 0.015$) were significantly shorter in the Abx-treated group than that in the Abx-untreated group. However, no increased rate of primary PD was observed in patients with Abx treatment (36.4% versus 23.9%, $p = 0.645$). As for the 19 patients who received anti-PD-1 plus antiangiogenic therapies, median PFS was 2.24 months versus 5.57 months ($p = 0.0001$), and median OS was 3.27 months versus undefined ($p = 0.0013$) in the groups with and without Abx usage, respectively. And the rate of primary PD also trended to increase in Abx-treated group (50.0% versus 6.7%, $p = 0.097$). On the contrary, among the 33 patients treated with anti-PD-1 mAbs plus chemotherapy, no statistical difference in PFS was observed (undefined versus 8.50 months, $p = 0.838$) in the Abx-treated and Abx-untreated groups, and OS could not yet be identified ($p = 0.952$). The rates of primary PD were also similar between these two groups (20.0% versus 14.3%, $p = 1.000$). In the individual subgroups of patients included, concomitant Abx use showed a consistently negative effect on the survival during ICI treatment, with the diminished PFS and OS were observed in nearly every subgroup examined (Fig. 2A and B).

We also evaluated the influence of PPIs, another medication that could alter gut microbial composition, on ICIs efficacy. Similarly, the patients who received PPIs within 1 month before or after the first dose of ICIs were defined as PPI-treated. However, no difference was observed in terms of PFS (median 9.63 versus 6.23 months, $p = 0.343$) or OS (median 11.90 versus 23.70 months, $p = 0.754$) during ICI treatment among PPI-treated and PPI-untreated patients (Supplementary Fig. 1A). Tumor responses were also similar in the PPI-treated and PPI-untreated groups (Supplementary Table 1). Furthermore, no significant difference in survival was found when comparing patients with or without PPI treatment according to diverse therapeutic regimens (Supplementary Fig. 1B–D).

3.3. Abx treatment and toxicity

The occurrence of irAEs of any grade was documented in 46 (42.2%) patients during ICIs treatment, with 8 (40.0%) patients were in the Abx-treated group and 38 (42.7%) were classified in the Abx-untreated group. The overall irAEs profile was listed in Supplementary Table 2. The most common irAEs was dermatitis, which occurred in 16 (20.8%) patients. And as depicted in Table 2, most of the patients experienced mild irAEs in grade one or two, and none of them discontinued therapy because of irAEs. Grade 3 irAEs were recorded in 4 patients, and no grade 4 or greater irAEs were observed. Overall, the development of irAEs did not differ significantly between Abx-treated and -untreated groups ($p = 0.621$). Meanwhile, the grading of irAEs was similar across the two groups ($p = 1.000$). The numbers of concurrent irAEs were also analyzed, and the rates of patients who concurrently developed multiple irAEs between the Abx-treated and -untreated groups were equivalent ($p = 0.800$). In addition, most of the patients developed irAEs within 8 weeks since ICIs treatment started, and no significant difference was observed in the time to the onset of irAEs between the two groups ($p = 1.000$).

Because no difference in tumor response and survival was observed between the patients in PPI-treated and -untreated groups, we did not further evaluate the association between irAEs and PPI utilization.

3.4. Multivariate analysis

The univariate and multivariate analysis data were presented in Tables 3A and 3B. In univariate analysis, we found that patients with concomitant Abx treatment had significantly shorter PFS (HR = 0.32, 95% CI 0.18–0.59, $p < 0.0001$) and OS (HR = 0.33, 95% CI 0.16–0.69, $p = 0.003$) than the patients without Abx. Patients with ECOG PS ≥ 2 also had worse PFS (HR = 0.20, 95% CI 0.05–0.83, $p = 0.027$) and OS (HR = 0.12, 95% CI 0.03–0.52, $p = 0.005$). Meanwhile, patients who were diagnosed with squamous histology had worse PFS (HR = 0.60,

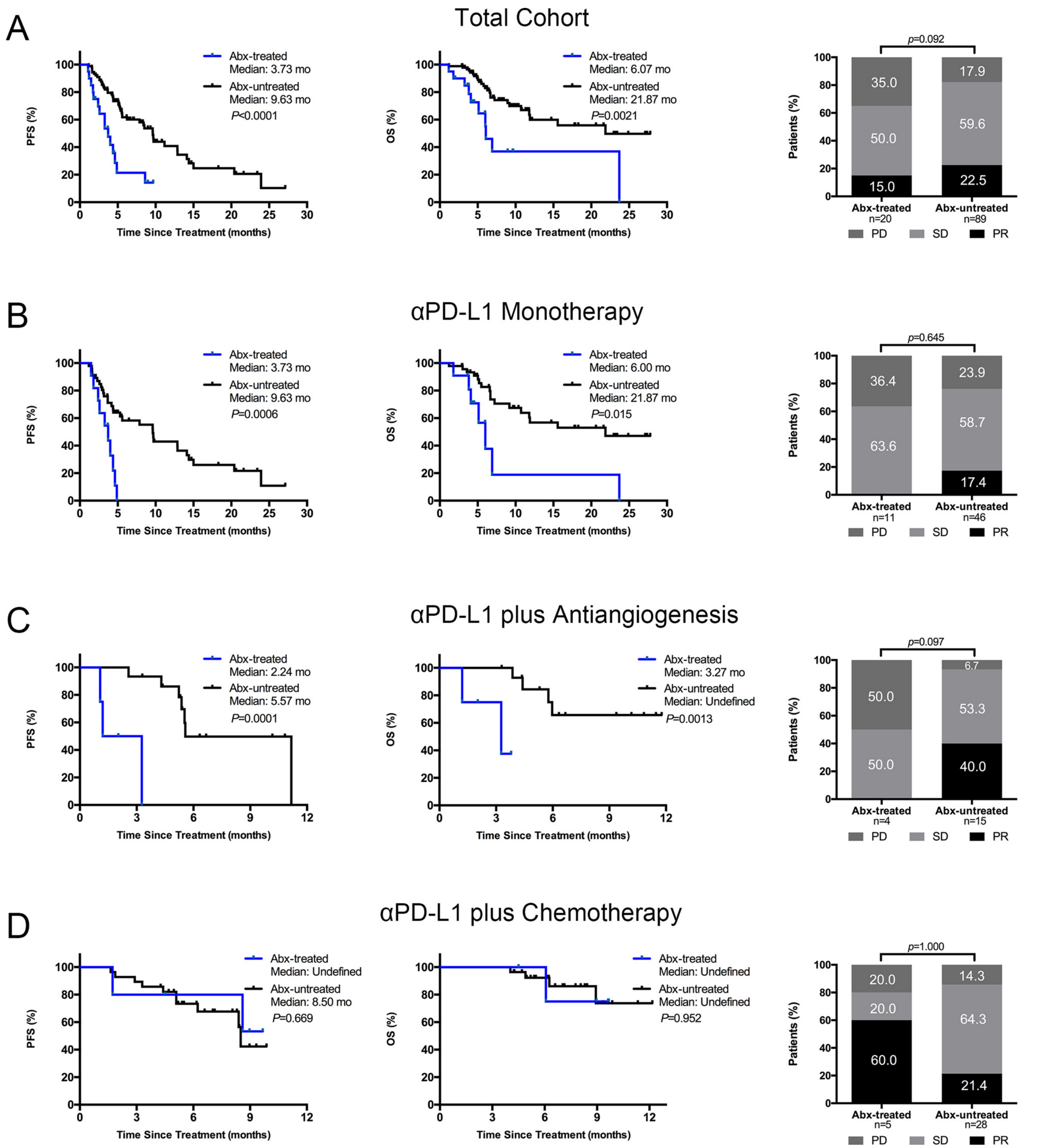

Fig. 1. The correlations of concomitant Abx treatment and clinical outcomes during ICIs therapies. (A): PFS, OS and responses in the entire cohort. (B): PFS, OS and responses in patients receiving anti-PD-1 monotherapy. (C): PFS, OS and responses in patients treated with anti-PD-1 plus anti-angiogenesis. (D): PFS, OS and responses in patients treated with anti-PD-1 plus chemotherapy. Abbreviations: Abx, antibiotics; mo, months; αPD-1, anti-PD-1 antibody; PFS, progression-free survival; OS, overall survival; PD, progression disease; SD, stable disease; PR, partial response.

95% CI 0.34–0.99, $p = 0.047$). Yet, the patients who received ICIs as first-line treatment (PFS: HR = 2.09, 95% CI 1.09–4.03, $p = 0.027$; OS: HR = 3.43, 95% CI 1.21–9.77, $p = 0.021$) or who were included in clinical trials (PFS: HR = 2.11, 95% CI 1.25–3.55, $p = 0.005$; OS: HR = 3.62, 95% CI 1.81–7.24, $p < 0.0001$) had longer PFS, as well as OS. Furthermore, the former/current smokers tended to have better PFS than never smokers (HR = 1.65, 95%CI 0.99–2.74, $p = 0.055$). These significant factors, together with the variables with $p < 0.1$ identified in univariable analysis, were further included in multivariate analysis for both PFS and OS. Significantly, concomitant Abx treatment remained an independent prognostic factor for both PFS (HR = 0.29, 95%CI 0.15-0.56, $p < 0.0001$) and OS (HR = 0.35, 95%CI 0.16-0.77, $p = 0.009$) after adjusting for other confounding factors.

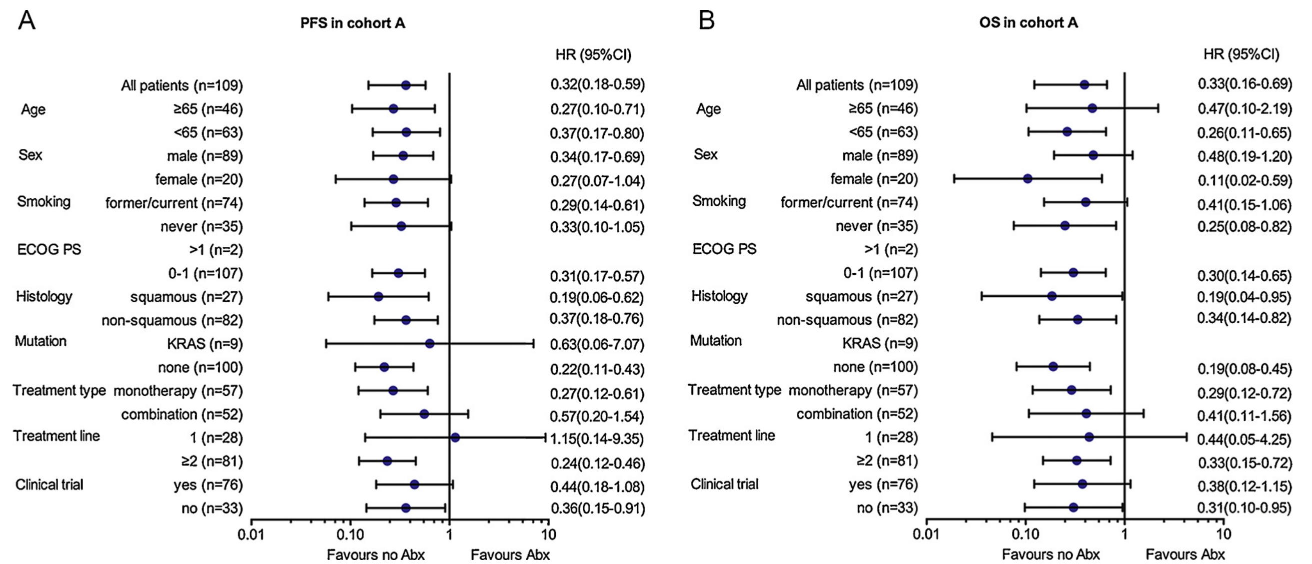

Fig. 2. Forest plot of subgroup analysis by baseline characteristics for PFS (A) and OS (B) in all the included patients. PFS, progression-free survival; OS, overall survival; Abx, antibiotics; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

Table 2
The incidence of irAEs.

	Abx-treated	Abx-untreated	*P* Value
Any irAEs[a], No (%)			
Yes	8(40.0)	38(42.7)	0.621
No	10(50.0)	47(52.8)	
Unknown	2(10.0)	4(4.5)	
Highest grade of irAEs, No (%)			
1-2	8(100.0)	34(89.5)	1.000
≥3	0(0.0)	4(10.5)	
Concurrent irAEs[b], No (%)			
1	5(62.5)	19(50.0)	0.800
≥2	3(37.5)	19(50.0)	
Time from IO treat to onset of irAE			
Within 8 weeks	8(100.0)	36(94.7)	1.000
Exceed 8 weeks	0(0.0)	2(5.3)	

Abbreviations: irAEs, immune-related adverse events; Abx, antibiotics; No, number; IO: immunotherapy.
[a] Indicate the patients experienced at least one irAE of any grade.
[b] Indicate the patients experienced more than one irAE.

4. Discussion

Clinically, patients with lung cancer are usually prone to necessitate Abx treatment, which might be attributed to the immunosuppressive nature of malignancies and anti-cancer therapy induced lymphodepletion. As the therapeutic paradigm for lung cancer has radically evolved with the incorporation of ICIs, the influence of Abx use on ICIs is undeniably an emerging area requiring major focus. Accumulating evidence suggests that loss of diversity and shift in composition of GM can attenuate the therapeutic efficacy of ICIs [16–18]. Since Abx treatment can reduce the diversity of human GM transiently [19], the dysbiosis induced by Abx has been addressed as a detrimental predictor to ICIs therapy, whereas the data is limited from only a few reports [17,28,29]. Furthermore, owing to the ethnicity and geography-specific divergences in gut microbial composition [30], the impact of Abx treatment in promoting ICIs resistance is still needed to be confirmed in different patient cohorts. To our knowledge, the current study is the first to compare the clinical outcomes of patients who received anti-PD-1-based therapies with or without concomitant Abx use in a cohort of NSCLC patients mostly from Shanghai, China, whose GM composition is totally different from the cohorts in previous investigations.

As mentioned earlier, several groups have indicated associations between Abx treatment and worse clinical outcomes of PD-1 mAbs in cancer treatment, including lung cancer [17,28,29]. Routy et al. [17] made an initial observation of the deleterious impact of Abx on PFS and OS during PD-1 blockade in patients with advanced NSCLC form American and French populations. Derosa et al. [28] subsequently expanded the observation to a larger patient cohort and observed that

Table 3A
Univariate and Multivariate Analyses of Clinical Parameters on PFS.

	Univariate Analysis		Multivariate Analysis	
Factor	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age, year (< 65 vs. ≥65)	0.95(0.57–1.59)	0.838	—	—
Gender (female vs. male)	1.65(0.90–3.00)	0.104	—	—
Smoking (never vs. smoker)	1.65(0.99–2.74)	**0.055**	1.89(1.11–3.23)	**0.020**
ECOG PS (0-1 vs. ≥ 2)	0.20(0.05–0.83)	0.027	0.36(0.08–1.72)	0.200
Histology (non-squamous vs. squamous)	0.60(0.34–0.99)	0.047	0.57(0.32–1.03)	0.061
Mutation (none vs. Kras)	1.22(0.44–3.39)	0.702	—	—
Treatment regimen (combination vs. monotherapy)	0.75(0.44–1.30)	0.307	—	—
Treatment line (≥2 vs. 1)	2.09(1.09–4.03)	0.027	1.92(0.96–3.83)	0.064
Clinical trial (no vs. yes)	2.11(1.25–3.55)	0.005	1.33(0.72–2.49)	0.364
Abx treatment (no vs. yes)	0.32(0.18–0.59)	< **0.0001**	0.29(0.15–0.56)	< **0.0001**
PPIs treatment (no vs. yes)	1.10(0.65–1.85)	0.725	—	—

Abbreviations: PFS, progression-free survival; HR, hazard rate; ECOG, Eastern Cooperative Oncology Group; PS, performance status; irAEs, immune-related adverse events; Abx, antibiotics; PPIs, proton pump inhibitors.

Table 3B
Univariate and Multivariate Analyses of Clinical Parameters on OS.

Factor	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age, year (< 65 vs. ≥ 65)	0.80(0.41–1.58)	0.526	—	—
Gender (female vs. male)	0.95(0.41–2.18)	0.906	—	—
Smoking (never vs. smoker)	0.803(0.41–1.58)	0.526	—	—
ECOG PS (0-1 vs. ≥ 2)	0.12(0.03–0.52)	**0.005**	0.17(0.03–0.80)	**0.025**
Histology (non-squamous vs. squamous)	0.55(0.27–1.12)	0.098	0.67(0.32–1.41)	0.288
Mutation (none vs. Kras)	1.73(0.41–7.21)	0.455	—	—
Treatment regimen (combination vs. monotherapy)	0.68(0.32–1.43)	0.305	—	—
Treatment line (≥2 vs. 1)	3.43(1.21–9.77)	0.021	2.58(0.87–7.61)	0.087
Clinical trial (no vs. yes)	3.62(1.81–7.24)	< 0.0001	2.10(0.95–4.64)	0.067
Abx treatment (no vs. yes)	0.33(0.16–0.69)	**0.003**	0.35(0.16–0.77)	**0.009**
PPIs treatment (no vs. yes)	1.47(0.70–3.06)	0.309	—	—

prior Abx use was associated with shorter PFS and OS in patients with NSCLC receiving anti-PD-1 mAb alone or plus anti-CTLA-4 mAb. More recently, Huemer et al. [29] demonstrated that the survival benefit derived from ICIs was attenuated by concomitant Abx use in a small cohort of patients with advanced non-squamous NSCLC. The results of the current study echo previous reports. In more detail, we observed that Abx treatment was significantly associated with reduced PFS and OS in the entire patient cohort. We further investigated the impact of Abx according to different immunotherapeutic regimens. As expected, our data demonstrated a negative correlation of Abx treatment with PFS and OS in patients receiving anti-PD-1 mAbs monotherapy. Besides, our study firstly demonstrated the detrimental impact of Abx on prognosis in patients who received ICIs plus antiangiogenesis. Yet, regarding the cohort received anti-PD-1 mAbs plus chemotherapy, no significant difference in PFS and OS was observed between patients with or without Abx use. We could find that on the basis of previous studies [19,26,27], an intact gut microbiota was not only necessary for immunotherapy, but also a mechanical prerequisite for systemic chemotherapy, and Abx were proved to impair the efficacy of multiple chemotherapeutic agents. Therefore, it was somewhat unexpected about the data in the cohort of ICIs plus chemotherapy. However, it should be noted that the numbers of patients included in subgroup analysis was relatively small and heterozygous. Among the 33 patients in this cohort, only 5 patients were identified in the Abx-treated group. Yet, a higher rate of *KRAS* mutant tumors, which might display favorable response to ICIs [37–39], were observed in Abx-treated group (3/5) than that in Abx-untreated group (3/28). Hence, we speculated that the small sample size and the potential imbalance of patient selections might confound the survival analysis. Due to the limited data on the prognostic impact of Abx on the combination of ICIs and chemotherapy in lung cancer, more data from larger patient cohorts are undoubtedly needed.

Our findings also have significant clinical implications when using ICIs to treat patients with NSCLC. It should be noted that since many infections can only be cured by Abx, total avoidance of Abx treatment is not feasible in clinical practice. It is therefore of great importance to find suitable approaches to counteract the negative impact of Abx on ICIs efficacy. In addition to diversity, distinct compositional species in GM in response to ICIs are also investigated. Both mouse models and human studies have suggested that the bacterial species favorable for ICI efficacy vary according to therapeutic strategies [16–18,40]. Meanwhile, it is well understood that Abx are usually not restricted to their limited anti-microbial spectrum because of the mutual co-dependence of gut microbiota [20]. For these reasons, characterizing the microbial species favorable for different ICI regimens, as well as elucidating detailed underlying mechanism by which Abx affects microbiota and ICIs efficacy in lung cancer, is necessary in future work.

As with therapeutic efficacy, the dysbiosis of GM is also involved in the development of immune-mediated toxicity in the context of ICIs treatment [41]. Therefore, we explored the correlation between Abx treatment and irAEs. We reviewed the incidence of irAEs and found that patients in the Abx-treated and Abx-untreated groups had a similar risk of experiencing irAEs. Meanwhile, no statistical difference was observed in the grades of irAEs between the two groups. This, however, was potentially attributed to the small number of patients included in our analysis. Our data are consistent with the previous report from Huemer et al [29], in which the authors reported that the occurrence and grades of irAEs were similar regardless of Abx usage during ICIs treatment in non-squamous NSCLC patients. However, their study is also concluded from a small patient cohort. Since clinical data on the prognostic impact of Abx use on irAEs are still limited, more studies in larger cohorts of patients and long-term investigations are needed.

Except for Abx, the use of several additional medications, such as PPIs, also has a potential impact on the microbial composition [42,43]. As is known, PPIs are also widely used in patients with cancer to prevent oversecretion of gastric acid and indigestion, which are often induced by anti-cancer therapies. It is becoming increasingly clear that PPI treatment can alter the composition of the human gut microbiome and reduce microbial diversity [43,44]. However, concomitant PPI treatment failed to affect survival benefit in our analysis, and this finding was consistent with the results from Routy et al. [17]. Referring to previous reports, we find that PPIs can affect the pharmacokinetics and attenuate the efficacy of various anti-cancer agents, such as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) and anaplastic lymphoma kinase [36] TKIs for the treatment of NSCLC [45,46]. Several case reports also demonstrate the association between PPIs and increased rates of adverse events during ICI treatment in patients with NSCLC [47,48]. Collectively, the role of concomitant PPI administration on ICI therapeutic efficacy still remains controversial, and more data from large patient cohorts will help to address this issue.

The limitations in present study must be acknowledged. Firstly, this was a retrospective study from a single-center institution and selection bias was inevitable. Several discrepancies in clinicalpathologic characteristics were observed, including a higher rate of *KRAS* mutation in Abx-treated group and more patients were included in clinical trials in Abx-untreated group, which might confound our analysis. To compensate for these discrepancies, we evaluated the impact of Abx treatment across individual subgroups and further performed multivariable analysis to adjust for multiple prognostic factors. Another important issue is that the number of patients in present study was relatively small and they were heterogeneous according to treatment lines, so, the findings presented need to be validated in additional larger cohort with well-balanced patients. We must also mention that since most of the patients were enrolled as part of clinical trials, they were less representative of the patients treated in routine clinical practice. Moreover, owing to the detection of tumor PD-L1 expression was not mandatory for the patients before they received ICIs, we failed to address their PD-L1 status characterization, which might also skew the clinical responses and survival benefits.

In conclusion, our study provided additional evidence for the notion that concomitant Abx use could limit the clinical prognosis in patients with advanced NSCLC received anti-PD-1/PD-L1-based therapies. The shift in GM composition induced by Abx treatment might generate the negative impact on ICIs treatment. These findings should be viewed with caution in clinical practice, suggesting that the use of Abx should be limited to strict indications in patients receiving ICIs. In addition, it should be emphasized that our data, together with previous studies, facilitate the potential to promote anti-tumor efficacy of ICIs by modulating microbiome diversity. Interestingly, investigators have demonstrated the feasibility of modifying GM to a more "favorable" phenotype for enhancing ICIs efficacy in *in vivo* preclinical models. The explorations in clinical setting are urgent and promising.

Conflict of interest

The authors declare no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.01.017.

References

[1] J. Brahmer, K.L. Reckamp, P. Baas, L. Crino, W.E. Eberhardt, E. Poddubskaya, S. Antonia, A. Pluzanski, E.E. Vokes, E. Holgado, et al., Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer, N. Engl. J. Med. 373 (2) (2015) 123–135.

[2] R.S. Herbst, P. Baas, D.W. Kim, E. Felip, J.L. Perez-Gracia, J.Y. Han, J. Molina, J.H. Kim, C.D. Arvis, M.J. Ahn, 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 (10027) (2016) 1540–1550.

[3] H. Borghaei, L. Paz-Ares, L. Horn, D.R. Spigel, M. Steins, N.E. Ready, L.Q. Chow, E.E. Vokes, E. Felip, E. Holgado, et al., Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer, N. Engl. J. Med. 373 (17) (2015) 1627–1639.

[4] M. Reck, D. Rodriguez-Abreu, A.G. Robinson, R. Hui, T. Csoszi, A. Fulop, M. Gottfried, N. Peled, A. Tafreshi, S. Cuffe, et al., Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer, N. Engl. J. Med. 375 (19) (2016) 1823–1833.

[5] D.P. Carbone, M. Reck, L. Paz-Ares, B. Creelan, L. Horn, M. Steins, E. Felip, M.M. van den Heuvel, T.E. Ciuleanu, F. Badin, et al., First-line nivolumab in stage IV or recurrent non-small-cell lung cancer, N. Engl. J. Med. 376 (25) (2017) 2415–2426.

[6] A. Rittmeyer, F. Barlesi, D. Waterkamp, K. Park, F. Ciardiello, J. von Pawel, S.M. Gadgeel, T. Hida, D.M. Kowalski, M.C. Dols, 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 (10066) (2017) 255–265.

[7] L. Carbognin, S. Pilotto, M. Milella, V. Vaccaro, M. Brunelli, A. Calio, F. Cuppone, I. Sperduti, D. Giannarelli, M. Chilosi, et al., Differential activity of nivolumab, pembrolizumab and MPDL3280A according to the tumor expression of programmed Death-Ligand-1 (PD-L1): sensitivity analysis of trials in melanoma, lung and genitourinary cancers, PLoS One 10 (6) (2015) e0130142.

[8] P. Sharma, S. Hu-Lieskovan, J.A. Wargo, A. Ribas, Primary, adaptive, and acquired resistance to cancer immunotherapy, Cell 168 (4) (2017) 707–723.

[9] S.L. Topalian, F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D.F. McDermott, J.D. Powderly, R.D. Carvajal, J.A. Sosman, M.B. Atkins, et al., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, N. Engl. J. Med. 366 (26) (2012) 2443–2454.

[10] T. Shukuya, D.P. Carbone, Predictive markers for the efficacy of anti-PD-1/PD-L1 antibodies in lung cancer, J. Thorac. Oncol. 11 (7) (2016) 976–988.

[11] T.K. Kim, R.S. Herbst, L. Chen, Defining and understanding adaptive resistance in cancer immunotherapy, Trends Immunol. 39 (8) (2018) 624–631.

[12] N.A. Rizvi, M.D. Hellmann, A. Snyder, P. Kvistborg, V. Makarov, J.J. Havel, W. Lee, J. Yuan, P. Wong, T.S. Ho, et al., Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer, Science 348 (6230) (2015) 124–128.

[13] H. Tang, Y. Wang, L.K. Chlewicki, Y. Zhang, J. Guo, W. Liang, J. Wang, X. Wang, Y.X. Fu, Facilitating T cell infiltration in tumor microenvironment overcomes resistance to PD-L1 blockade, Cancer Cell 30 (3) (2016) 500.

[14] L. Fehrenbacher, A. Spira, M. Ballinger, M. Kowanetz, J. Vansteenkiste, J. Mazieres, K. Park, D. Smith, A. Artal-Cortes, C. Lewanski, et al., Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial, Lancet 387 (10030) (2016) 1837–1846.

[15] M. Nishino, N.H. Ramaiya, H. Hatabu, F.S. Hodi, Monitoring immune-checkpoint blockade: response evaluation and biomarker development, Nat. Rev. Clin. Oncol. 14 (11) (2017) 655–668.

[16] V. Matson, J. Fessler, R. Bao, T. Chongsuwat, Y. Zha, M.L. Alegre, J.J. Luke, T.F. Gajewski, The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients, Science 359 (6371) (2018) 104–108.

[17] B. Routy, E. Le Chatelier, L. Derosa, C.P.M. Duong, M.T. Alou, R. Daillere, A. Fluckiger, M. Messaoudene, C. Rauber, M.P. Roberti, et al., Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors, Science 359 (6371) (2018) 91–97.

[18] V. Gopalakrishnan, C.N. Spencer, L. Nezi, A. Reuben, M.C. Andrews, T.V. Karpinets, P.A. Prieto, D. Vicente, K. Hoffman, S.C. Wei, et al., Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients, Science 359 (6371) (2018) 97–103.

[19] C. Ubeda, E.G. Pamer, Antibiotics, microbiota, and immune defense, Trends Immunol. 33 (9) (2012) 459–466.

[20] K. Lange, M. Buerger, A. Stallmach, T. Bruns, Effects of Antibiotics on Gut Microbiota, Dig. Dis. 34 (3) (2016) 260–268.

[21] A.S. Ismail, K.M. Severson, S. Vaishnava, C.L. Behrendt, X. Yu, J.L. Benjamin, K.A. Ruhn, B. Hou, A.L. DeFranco, F. Yarovinsky, et al., Gammadelta intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface, Proc. Natl. Acad. Sci. U. S. A. 108 (21) (2011) 8743–8748.

[22] D.A. Hill, C. Hoffmann, M.C. Abt, Y. Du, D. Kobuley, T.J. Kirn, F.D. Bushman, D. Artis, Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis, Mucosal Immunol. 3 (2) (2010) 148–158.

[23] K. Atarashi, T. Tanoue, T. Shima, A. Imaoka, T. Kuwahara, Y. Momose, G. Cheng, S. Yamasaki, T. Saito, Y. Ohba, et al., Induction of colonic regulatory T cells by indigenous Clostridium species, Science 331 (6015) (2011) 337–341.

[24] M. Vetizou, J.M. Pitt, R. Daillere, P. Lepage, N. Waldschmitt, C. Flament, S. Rusakiewicz, B. Routy, M.P. Roberti, C.P. Duong, et al., Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota, Science 350 (6264) (2015) 1079–1084.

[25] M.P. Kuczma, Z.C. Ding, T. Li, T. Habtetsion, T. Chen, Z. Hao, L. Bryan, N. Singh, J.N. Kochenderfer, G. Zhou, The impact of antibiotic usage on the efficacy of chemoimmunotherapy is contingent on the source of tumor-reactive T cells, Oncotarget 8 (67) (2017) 111931–111942.

[26] N. Iida, A. Dzutsev, C.A. Stewart, L. Smith, N. Bouladoux, R.A. Weingarten, D.A. Molina, R. Salcedo, T. Back, S. Cramer, et al., Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment, Science 342 (6161) (2013) 967–970.

[27] S. Viaud, F. Saccheri, G. Mignot, T. Yamazaki, R. Daillere, D. Hannani, D.P. Enot, C. Pfirschke, C. Engblom, M.J. Pittet, et al., The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide, Science 342 (6161) (2013) 971–976.

[28] L. Derosa, M.D. Hellmann, M. Spaziano, D. Halpenny, M. Fidelle, H. Rizvi, N. Long, A.J. Plodkowski, K.C. Arbour, J.E. Chaft, 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 (6) (2018) 1437–1444.

[29] F. Huemer, G. Rinnerthaler, T. Westphal, H. Hackl, G. Hutarew, S.P. Gampenrieder, L. Weiss, R. Greil, Impact of antibiotic treatment on immune-checkpoint blockade efficacy in advanced non-squamous non-small cell lung cancer, Oncotarget 9 (23) (2018) 16512–16520.

[30] V.K. Gupta, S. Paul, C. Dutta, Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity, Front. Microbiol. 8 (2017) 1162.

[31] M. Deschasaux, K.E. Bouter, A. Prodan, E. Levin, A.K. Groen, H. Herrema, V. Tremaroli, G.J. Bakker, I. Attaye, S.J. Pinto-Sietsma, et al., Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography, Nat. Med. 24 (10) (2018) 1526–1531.

[32] C.A. Gaulke, T.J. Sharpton, The influence of ethnicity and geography on human gut microbiome composition, Nat. Med. 24 (10) (2018) 1495–1496.

[33] J.M. Michot, C. Bigenwald, S. Champiat, M. Collins, F. Carbonnel, S. Postel-Vinay, A. Berdelou, A. Varga, R. Bahleda, A. Hollebecque, et al., Immune-related adverse events with immune checkpoint blockade: a comprehensive review, Eur. J. Cancer 54 (2016) 139–148.

[34] M.H. Kim, H.R. Kim, B.C. Cho, M.K. Bae, E.Y. Kim, C.Y. Lee, J.S. Lee, D.R. Kang, J.H. Kim, Impact of cigarette smoking on response to epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors in lung adenocarcinoma with activating EGFR mutations, Lung Cancer 84 (2) (2014) 196–202.

[35] H.R. Kim, D.J. Kim, D.R. Kang, J.G. Lee, S.M. Lim, C.Y. Lee, S.Y. Rha, M.K. Bae, Y.J. Lee, S.H. Kim, et al., Fibroblast growth factor receptor 1 gene amplification is associated with poor survival and cigarette smoking dosage in patients with resected squamous cell lung cancer, J. Clin. Oncol. 31 (6) (2013) 731–737.

[36] R. Brody, Y. Zhang, M. Ballas, M.K. Siddiqui, P. Gupta, C. Barker, A. Midha, J. Walker, PD-L1 expression in advanced NSCLC: Insights into risk stratification and

treatment selection from a systematic literature review, Lung Cancer 112 (2017) 200–215.
[37] A. Ribas, Adaptive immune resistance: how cancer protects from immune attack, Cancer Discov. 5 (9) (2015) 915–919.
[38] Z.Y. Dong, W.Z. Zhong, X.C. Zhang, J. Su, Z. Xie, S.Y. Liu, H.Y. Tu, H.J. Chen, Y.L. Sun, Q. Zhou, et al., Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma, Clin. Cancer Res. 23 (12) (2017) 3012–3024.
[39] I. Ferrer, J. Zugazagoitia, S. Herbertz, W. John, L. Paz-Ares, G. Schmid-Bindert, KRAS-mutant non-small cell lung cancer: from biology to therapy, Lung Cancer 124 (2018) 53–64.
[40] M. Yi, S. Yu, S. Qin, Q. Liu, H. Xu, W. Zhao, Q. Chu, K. Wu, Gut microbiome modulates efficacy of immune checkpoint inhibitors, J. Hematol. Oncol. 11 (1) (2018) 47.
[41] K. Dubin, M.K. Callahan, B.Y. Ren, R. Khanin, A. Viale, L.L. Ling, D. No, A. Gobourne, E. Littmann, C. Huttenhower, et al., Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis, Nat. Commun. (2016) 7.
[42] F. Imhann, A. Vich Vila, M.J. Bonder, A.G. Lopez Manosalva, D.P.Y. Koonen, J. Fu, C. Wijmenga, A. Zhernakova, R.K. Weersma, The influence of proton pump inhibitors and other commonly used medication on the gut microbiota, Gut Microbes 8 (4) (2017) 351–358.
[43] M.A. Jackson, J.K. Goodrich, M.E. Maxan, D.E. Freedberg, J.A. Abrams, A.C. Poole, J.L. Sutter, D. Welter, R.E. Ley, J.T. Bell, et al., Proton pump inhibitors alter the composition of the gut microbiota, Gut 65 (5) (2016) 749–756.
[44] A. Singh, G.A. Cresci, D.F. Kirby, Proton pump inhibitors: risks and rewards and emerging consequences to the gut microbiome, Nutr. Clin. Pract. (2018).
[45] M. Ohgami, T. Kaburagi, A. Kurosawa, K. Doki, T. Shiozawa, N. Hizawa, M. Homma, Effects of proton pump inhibitor co-administration on the plasma concentration of erlotinib in patients with non-small cell lung cancer, Ther. Drug Monit. (2018).
[46] Y.Y. Lau, W. Gu, T. Lin, K. Viraswami-Appanna, C. Cai, J.W. Scott, M. Shi, Assessment of drug-drug interaction potential between ceritinib and proton pump inhibitors in healthy subjects and in patients with ALK-positive non-small cell lung cancer, Cancer Chemother. Pharmacol. 79 (6) (2017) 1119–1128.
[47] R. Koda, H. Watanabe, M. Tsuchida, N. Iino, K. Suzuki, G. Hasegawa, N. Imai, I. Narita, Immune checkpoint inhibitor (nivolumab)-associated kidney injury and the importance of recognizing concomitant medications known to cause acute tubulointerstitial nephritis: a case report, BMC Nephrol. 19 (1) (2018) 48.
[48] A.C. Shirali, M.A. Perazella, S. Gettinger, Association of acute interstitial nephritis with programmed cell death 1 inhibitor therapy in lung cancer patients, Am. J. Kidney Dis. 68 (2) (2016) 287–291.