

ORIGINAL RESEARCH



# Antibiotic use and the efficacy of immune checkpoint inhibitors in cancer patients: a pooled analysis of 2740 cancer patients

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## ABSTRACT

The gut microbiota plays a critical role in the anti-tumor immune response. There is increasing data showing that antibiotics (ATBs) change the composition of the gut microbiota and affect the efficacy of immune checkpoint inhibitors (ICIs). However, this is the first meta-analysis to evaluate the association between ATB use and ICI efficacy in cancer patients to provide a better understanding of the strength of this association. We performed a literature search for relevant studies that evaluated the relationship between ATB use and ICI efficacy using the PubMed, Embase, and conference databases. The primary outcomes consisted of overall survival (OS) and progression-free survival (PFS) measured by hazard ratios (HR) and corresponding 95% confidence intervals (CI). Subgroup and sensitivity analyses were also performed. A total of 19 eligible studies comprising 2,740 cancer patients treated with ICIs were included in the analysis. Our results indicated that ATB use was negatively associated with OS in cancer patients (HR = 2.37; 95% CI = 2.05–2.75;  $P < .001$ ), without heterogeneity ( $I^2 = 0.0\%$ ;  $P = .851$ ). Moreover, ATB use significantly reduced PFS in patients treated with ICIs (HR = 1.84; 95% CI = 1.49–2.26;  $P < .001$ ;  $I^2 = 56.2\%$ ). Similar results were obtained in the subgroup analyses stratified by the time of ATB use and cancer type. Sensitivity analyses confirmed the stability of our results. Therefore, the findings of our meta-analysis indicated that ATB use is negatively associated with OS and PFS in cancer patients treated with ICI immunotherapy.

## ARTICLE HISTORY

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## KEYWORDS

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## Introduction

Immune checkpoint inhibitors (ICIs) that target programmed cell death-1 (PD-1), programmed cell death-ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), have altered the therapeutic landscape and have become an attractive treatment strategy in several malignancies due to their high efficacy and lower toxicity compared with traditional cytotoxic drugs.<sup>1,2</sup> However, in clinical practice, the clinical efficacy of ICIs is highly variable among cancer patients, and a considerable proportion of patients still progress with disease or relapse due to ICI resistance and limited efficacy.<sup>3,4</sup> Recently, researchers have sought to identify prognostic and predictive biomarkers associated with the response to ICI immunotherapy (e.g., PD-L1 expression, tumor mutational burden, tumor infiltrating lymphocytes, mismatch repair deficiency, and high microsatellite instability); however, the predictive precision of such potential biomarkers remains limited.<sup>5,6</sup> Thus, it is critical to explore reliable predictors to improve the clinical response to ICIs.

Evidence has shown that a balanced gut microbiota is an important regulator of the systemic immune system.<sup>7,8</sup> Furthermore, preclinical studies have reported that the gut microbiota is involved in the response to ICI immunotherapy.<sup>9,10</sup>

Moreover, it has been well-documented that antibiotics (ATBs) can alter the diversity and composition of the commensal gut microbiota.<sup>11</sup> Thus, researchers hypothesize that dysbiosis of the gut microbiota caused by ATBs may be associated with resistance to ICI immunotherapy and have a negative impact on the efficacy of ICI immunotherapy. Although several studies have evaluated the association between ATB use and ICI efficacy, inter-study heterogeneity may exist between ATBs and ICI efficacy; thus, a pooled analysis may provide a greater understanding of the strength of this relationship.

The purpose of this study was to evaluate the association between ATB use and ICI efficacy in cancer patients treated with ICIs.


## Results

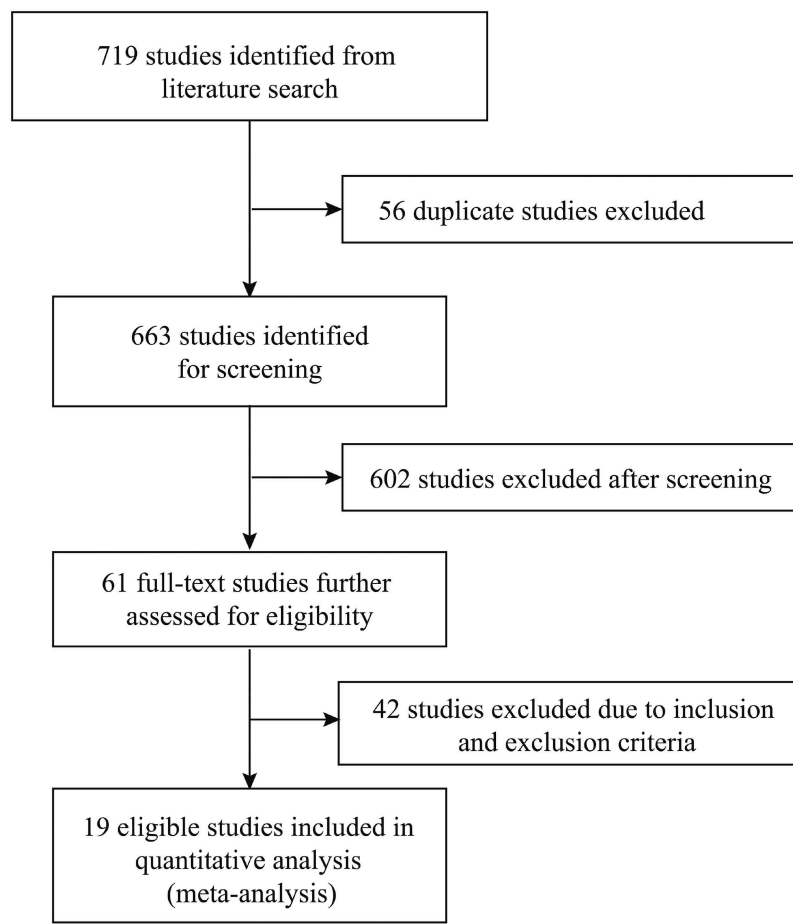
### Study selection and associated characteristics

A total of 719 studies were identified from our literature search, of which 658 studies were excluded after reviewing the titles and abstracts. The remaining 61 studies were further reviewed, and 42 studies were excluded based on the eligibility criteria. Finally, 19 studies were included in our quantitative analysis (Figure 1).<sup>10,12-29</sup>

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 Supplemental data for this article can be accessed on the [publisher's website](#).



**Figure 1.** Literature search and study selection.

A total of 2,740 patients [mean: 144; median with range: 109 (30–360)] were included in our analysis. The included studies were published between 2017 and 2019 (17/19 studies, 89%, were published in 2018 and 2019) from the United States, the United Kingdom, China, Japan, Canada, Switzerland, Greece, Korea, Austria, and France. With regards to the time of ATB use, 11 studies provided results on the association between pre-therapy ATB use and ICI efficacy,<sup>13,15–19,21,23,24,26,28</sup> nine studies provided data on the association between pre-therapy or post-therapy ATB use and ICI efficacy,<sup>10,12,14,20,22,24,25,27,29</sup> and only one study provided results on the association between post-therapy ATB use and ICI efficacy.<sup>24</sup> A total of 14 studies used anti-PD-1/PD-L1 inhibitors,<sup>10,12,13,15,16,18,20,22–25,27–29</sup> three studies used anti-PD-1/PD-L1 inhibitors and/or anti-CTLA-4 inhibitors,<sup>17,21,26</sup> and two studies did not report the type of ICI drug.<sup>14,19</sup> Of the eligible studies, eight studies were complete cohort studies<sup>10,12,16,17,24,26,27,29</sup> and 11 studies provided only an abstract.<sup>13–15,18–23,25,28</sup> The main characteristics of the included studies are listed in Table 1.

### **ATB use and overall survival (OS)**

Our results indicated that ATB use was negatively associated with OS in cancer patients treated with ICIs (hazard ratios [HR] = 2.37, 95% confidence intervals [CI] = 2.05–2.75,  $P < .001$ ; Figure 2), without obvious

heterogeneity among the analyzed studies ( $I^2 = 0.0\%$ ,  $P = .851$ ). Sensitivity analyses using the leave-one-out approach confirmed the stability of our results, and no single study substantially dominated the results (Figure 3). Regarding the time of ATB use, pre-therapy ATB use had an unfavorable impact on OS without heterogeneity (HR = 2.29, 95% CI = 1.92–2.73,  $P < .001$ ,  $I^2 = 0.0\%$ ; Figure 4), and similar results were observed for patients who received pre-therapy or post-therapy ATBs without heterogeneity (HR = 2.56, 95% CI = 1.96–3.36,  $P < .001$ ;  $I^2 = 0.0\%$ ). Further analysis of pre-therapy ATB use indicated that the HR for ATB use within one month prior to ICI (HR = 2.23) was larger than that within two months before ICI (HR = 1.97), although the difference was small. Results for non-small-cell lung cancer (NSCLC), renal cell carcinoma (RCC), and urothelial carcinoma (UC) were provided in 10, 2, and 2 studies, respectively. The pooled results also showed that patients treated with ATBs had poor OS without heterogeneity (NSCLC: HR = 2.68, 95% CI = 2.19–3.28,  $P < .001$ ,  $I^2 = 0.0\%$ ; RCC: HR = 1.68, 95% CI = 1.00–2.83,  $P = .052$ ,  $I^2 = 0.0\%$ ; UC: HR = 2.01, 95% CI = 1.23–3.29,  $P = .005$ ,  $I^2 = 0.0\%$ ). Moreover, these results were confirmed by a subgroup analysis based on the type of ICI drug (PD-1 inhibitors: HR = 2.45, 95% CI = 2.04–2.97,  $P < .001$ ,  $I^2 = 0.0\%$ ; PD-1/CTLA-4/PD-1+CTLA-4 inhibitors: HR = 2.23, 95% CI =

**Table 1.** The baseline characteristics of included studies.

Author	Year	Country	Cancer type	Definition of antibiotics use	Treatment	Sample (Y/N)	Outcome
Zhao	2019	China	NSCLC	Within 1 month before or after initiation of anti-PD-1 therapy	PD-1 inhibitors alone or in combination with chemotherapy	109(20/89)	OS, PFS
Hakozaki	2019	Japan	NSCLC	For $\geq 3$ days within 30 days of nivolumab	Nivolumab monotherapy	90(13/77)	OS, PFS
Elkrief	2019	Canada	Melanoma	Within 30 days prior to ICI initiation	PD-1 inhibitors or CTLA-4 inhibitors alone or in combination with chemotherapy	74(10/64)	OS, PFS
Agarwal	2019	US	Urothelial carcinoma	Within 1 month before starting to during anti-PD1/PDL1 therapy	PD1/PD-L1 inhibitors	101(26/75)	OS
Schett	2019	Switzerland	NSCLC	Within 2 months prior to start of therapy	PD-(L)1 inhibitors	218(44/174)	OS, PFS
Rounis	2019	Greece	NSCLC	Within 30 days pre- or during therapy	ICI	44(NR)	OS, PFS
Pinato	2019	United Kingdom	NSCLC and melanoma	Within 1 month prior to ICI or until ICI cessation	PD-1/PD-L1 inhibitors	196(97/99)	OS
Routy	2018	France	NSCLC and urothelial carcinoma	Within 2 month before or 1 month after starting anti-PD-1 therapy	Nivolumab or durvalumab	182 (132/50)	OS, PFS
Tinsley	2018	United Kingdom	Melanoma, RCC and NSCLC	Within 2 weeks of ICI initiation or 6 weeks after	ICI	303(94/209)	OS, PFS
Swami	2018	United Kingdom	Melanoma	Within 2 months before or after starting anti-PD-1 therapy	PD-1 inhibitors	199(NR)	PFS
Sen	2018	United States	RCC, NSCLC, melanoma, sarcoma and gastrointestinal stromal tumors	during ICI use; within 30 days of ICI; 30–60 days prior to ICI	CTLA-4 or PD-1 inhibitors	172(NR)	OS, PFS
Lalani	2018	United States	RCC	between 8-weeks pre- and 4-weeks post initiation of therapy	PD-1/PD-L1 inhibitors	146(31/115)	OS, PFS
Kim	2018	Korea	Advanced cancer	Within 30 days of ICI initiation	Nivolumab, pembrolizumab or atezolizumab	199(57/142)	OS
Huemer	2018	Austria	Non-squamous NSCLC	Within 1 month before or 1 month after ICI initiation	Nivolumab or pembrolizumab	30(11/19)	OS, PFS
Do	2018	United States	Lung cancer	Within 30 days before nivolumab initiation to 30 days after the last dose of nivolumab	Nivolumab	109(87/22)	OS
Derosa	2018	Canada	RCC and NSCLC	Within the 30 or 60 days before the start of anti-PD-(L)1 therapy	PD-(L)1 inhibitors alone or in combination with CTLA-4 inhibitors or bevacizumab	360(90/270)	OS, PFS
Ahmed	2018	United States	Advanced cancer	Within 2 weeks prior to and after therapy initiation and within 10 weeks prior to disease progression	Nivolumab, pembrolizumab or atezolizumab	60(17/43)	OS, PFS
Thompson	2017	United States	NSCLC	Within 6 weeks of initiating anti-PD-1 therapy	PD-1 inhibitors	74(18/56)	OS, PFS
Kaderbhai	2017	France	NSCLC	Within 3 months before nivolumab initiation or during therapy	Nivolumab in monotherapy	74(15/59)	PFS

NOTE, ICI: Immune Checkpoint Inhibitors; NR: Not Reported; NSCLC: Non-Small-Cell Lung Cancer; OS: Overall Survival; PFS: Progression-Free Survival; RCC: Renal Cell Carcinoma; Y/N: antibiotics use/no antibiotics use

1.68–2.97,  $P < .001$ ,  $I^2 = 0.0\%$ ), sample size, publication country, and study type, which indicated that ATB use was associated with a decreased OS (Table 2).

### ATB use and progression-free survival (PFS)

Our results indicated that ATB use significantly reduced the PFS of patients treated with ICIs (HR = 1.84, 95% CI = 1.49–2.26,  $P < .001$ ; Figure 2), with heterogeneity among the studies ( $I^2 = 56.2\%$ ,  $P = .002$ ). Sensitivity analyses reported that the results were not dominated by any single study (Figure 3). Furthermore, a subgroup analysis based on the time of ATB use also revealed unfavorable levels of PFS in the group that received ATBs pre-therapy without heterogeneity (HR = 1.70, 95% CI = 1.43–2.02,  $P < .001$ ,  $I^2 = 30.1\%$ ; Figure 4), as well as those receiving pre-therapy or post-therapy ATBs (HR = 1.91, 95% CI = 1.31–2.78,  $P = .001$ ,  $I^2 = 68.6\%$ ). The subgroup analyses based on cancer type (NSCLC: HR = 1.79, 95% CI = 1.29–2.49,  $P < .001$ ,  $I^2 = 69.3\%$ ; RCC: HR = 2.12, 95% CI = 1.51–2.96,  $P < .001$ ,  $I^2 = 0.0\%$ ), type of ICI drug (PD-1 inhibitors: HR = 1.92, 95% CI = 1.43–2.58,  $P < .001$ ,  $I^2 = 61.8\%$ ; PD-1/CTLA-4/PD-1+CTLA-4

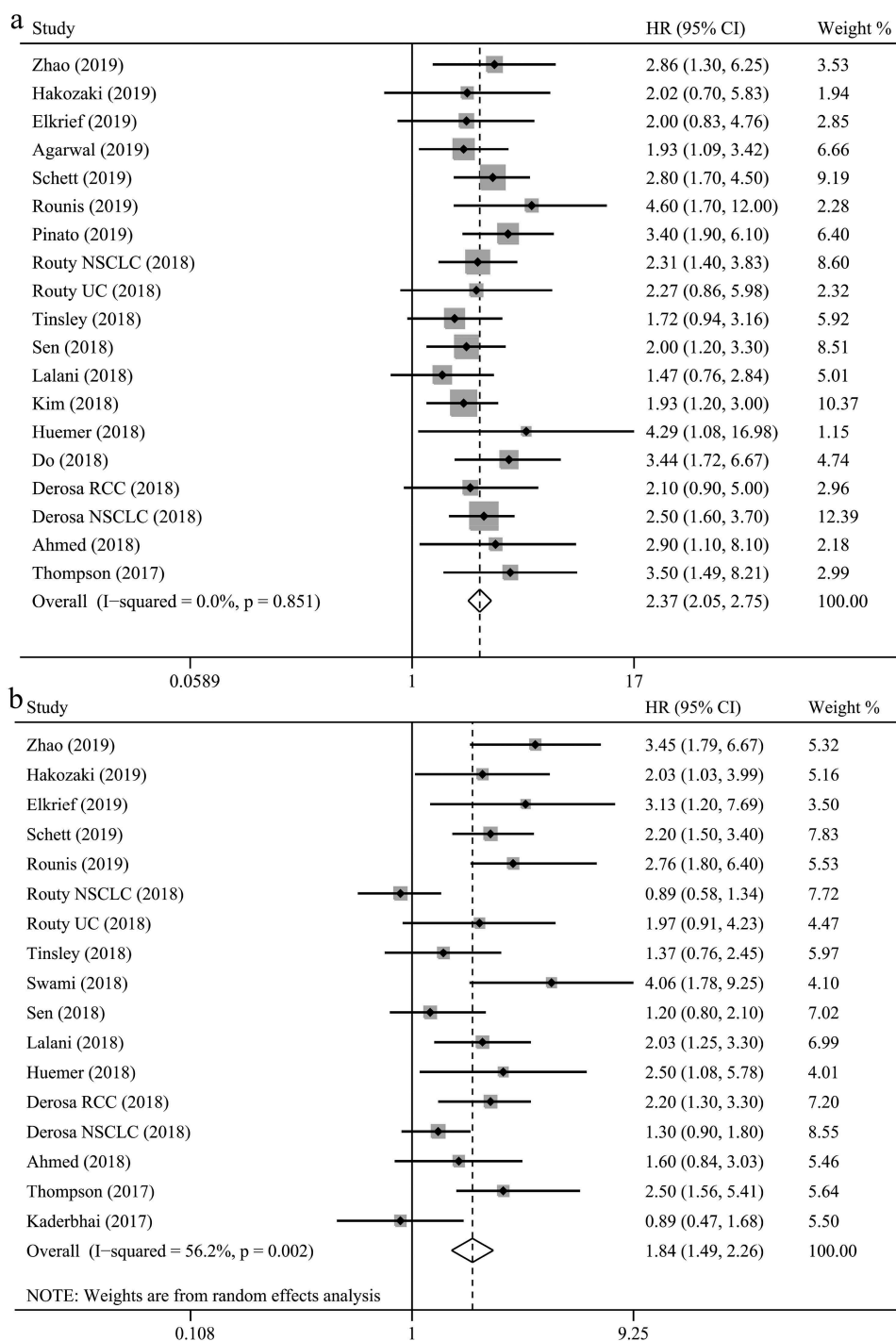
inhibitors: HR = 1.63, 95% CI = 1.13–2.36,  $P < .001$ ,  $I^2 = 53.4\%$ ), sample size, publication country, and study type obtained similar results, which indicated that ATB use was associated with a poor PFS (Table 2).

### Assessment of publication bias

The results of Begg's and Egger's tests indicated that there was no significant publication bias, except in the overall analysis of PFS ( $P_{Begg's} = 0.091$ ,  $P_{Egger's} = 0.035$ ; Figure 5). Furthermore, the trim-and-fill analysis indicated that publication bias did not affect the PFS results (HR = 1.54, 95% CI = 1.36–1.74) and other subgroup analyses with low  $p$  values for Begg's or Egger's tests.

### Discussion

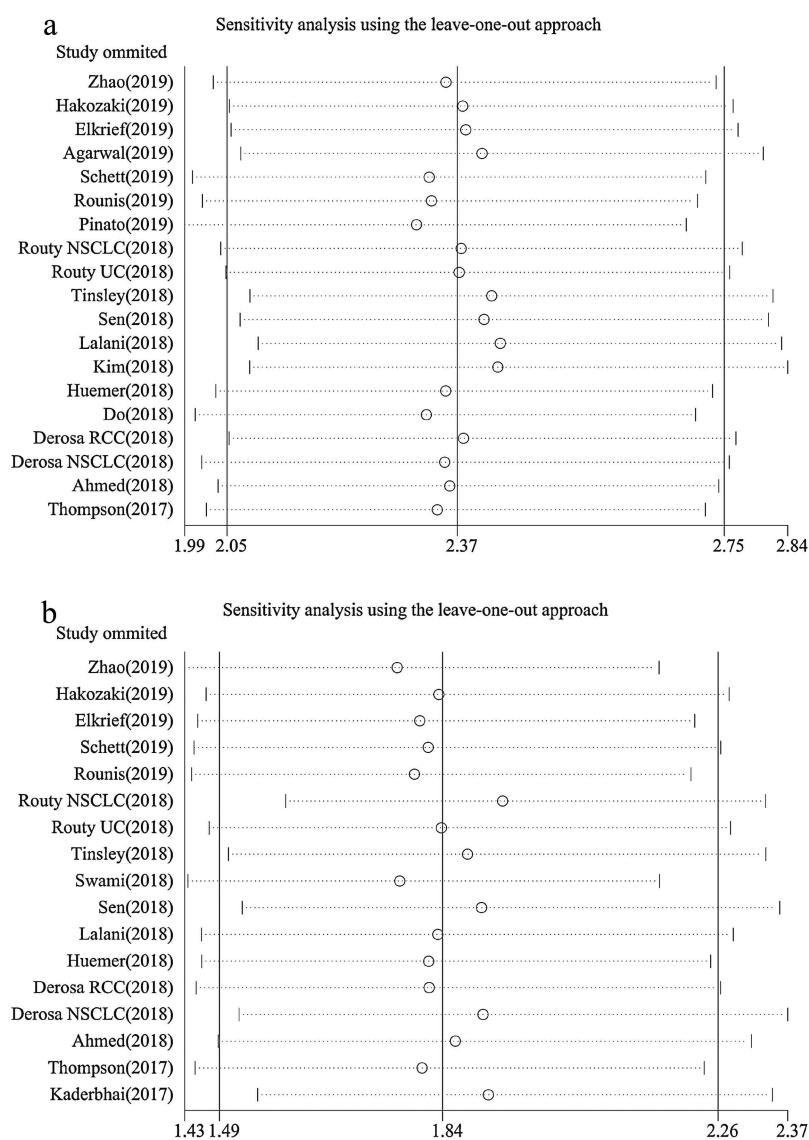
ICI immunotherapy has revolutionized cancer therapy for several solid tumors, and the anti-tumor response to ICIs is enhanced by inhibiting the PD-1 or CTLA-4 pathways and subsequently re-activating the host's immune function.<sup>30–32</sup> Although the



**Figure 2.** The associations between antibiotic use and overall survival (a) and progression-free survival (b) in cancer patients treated with immune checkpoint inhibitors.

resistance and efficacy associated with ICI immunotherapy are greatly affected by the interaction between host and tumor factors, tumor factors alone cannot completely explain the differences in ICI efficacy. As a host factor, the gut microbiota plays a critical role in the anti-tumor response of ICI immunotherapy.<sup>9,10</sup> Theoretically, ATBs, which can change the composition of the gut microbiota and lead to dysbiosis, may also affect the efficacy of ICI immunotherapy; however, the clinical data regarding the association between ATB use and ICI efficacy is limited.

To our knowledge, this is the first meta-analysis to systematically evaluate the association between ATB use and the clinical efficacy of ICIs. To assess the impact of ATB use on the clinical efficacy of ICI immunotherapy, this study included 19 eligible studies comprising 2740 cancer patients treated with ICIs. Our results indicated that ATB use is negatively associated with OS (HR = 2.37, 95% CI = 2.05–2.75,  $P < .001$ ) and PFS (HR = 1.84, 95% CI = 1.49–2.26,  $P < .001$ ) in cancer patients treated with ICIs. Similar results were obtained in the subgroup analysis of



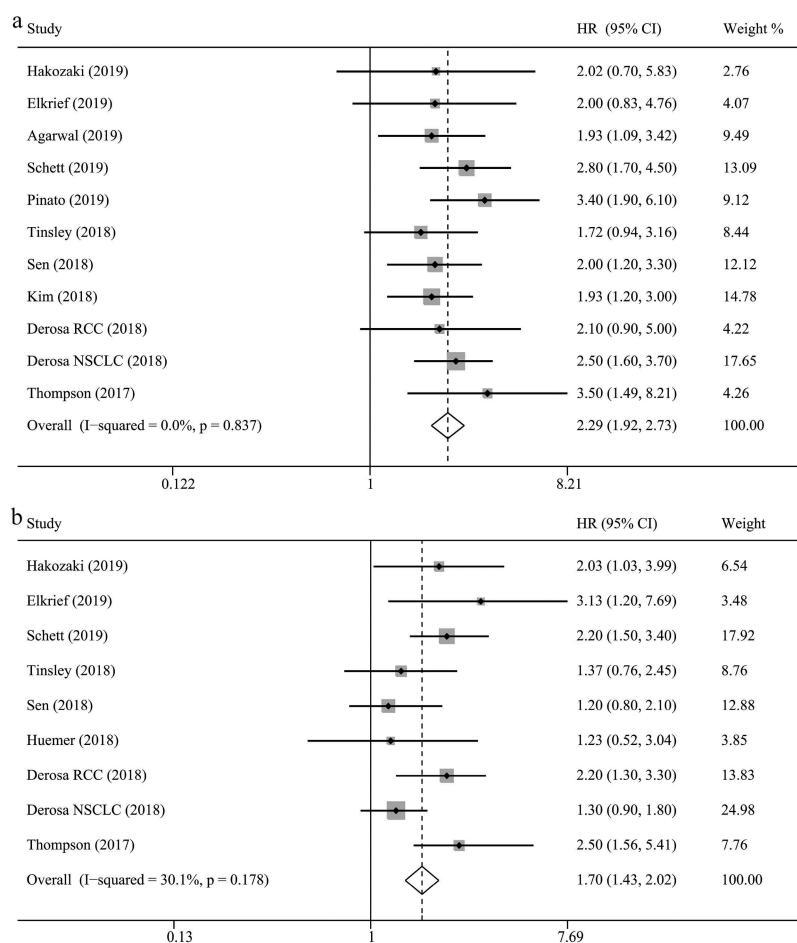
**Figure 3.** Sensitivity analysis of overall survival (a) and progression-free survival (b) based on leave-one-out approach.

pre-therapy ATB use, NSCLC, and RCC. Furthermore, subgroup analyses based on sample size, publication country, and study type confirmed these results, indicating that ATB use was associated with unfavorable OS and PFS outcomes. In addition, sensitivity analyses revealed that no single study substantially dominated the results. Moreover, no significant publication bias was found, except in the overall analysis of PFS.

The negative association between ATB use and the clinical efficacy of ICI immunotherapy verified the results of previous studies reporting that the diversity and composition of the gut microbiota play a critical role in the immune response.<sup>7,10</sup> Indeed, Matson *et al.* explored the association between the fecal microbial composition and clinical response in melanoma patients treated with anti-PD-1 or anti-CTLA-4, and found a greater abundance of bacterial species, including *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*, in the responders.<sup>9</sup> Gopalakrishnan *et al.* and Routy *et al.* also found significant differences in gut microbiota diversity and composition between responders and non-

responders (i.e., *Ruminococcaceae* family, *Faecalibacterium*, and *Akkermansia muciniphila*).<sup>10,33</sup> In addition, several studies have reported that the anti-tumor effects of ICIs were improved in germ-free or ATB-treated mice receiving a fecal microbiota transplantation from responders rather than non-responders, and a favorable gut microbiota could enhance antigen presentation and T cell function associated with the systemic and anti-tumor immune response.<sup>9,10</sup> Thus, ATB use may reduce the efficacy of ICI immunotherapy by altering the diversity and composition of the gut microbiota. Although perturbation of the gut microbiota is a highly plausible explanation for the detrimental effects of ATB exposure, this hypothesis has not been mechanistically and prospectively tested in cancer populations treated with ICI immunotherapy. Future large-scale, prospective studies are required to elucidate the mechanisms underlying the relationship between the perturbation of the gut microbiota caused by ATB and poor efficacy of ICI. Whether there are additional mechanisms for the detrimental effects that occur following ATB exposure should also be explored.





**Figure 4.** The associations between pre-therapy antibiotic use and overall survival (a) and progression-free survival (b) in cancer patients treated with immune checkpoint inhibitors.

The time of ATB use was important for the assessment of ICI efficacy because the diversity and composition of the gut microbiota was temporally altered by ATBs, after which it recovered to baseline within a certain time period following the discontinuation of ATBs. However, in the clinical practice of ICI immunotherapy, no consistent and detailed definition was found for the time of ATB use. Various definitions were used among the included studies according authors' preference. We found that most studies used a definition of 1 month before and/or after the initiation of ICI immunotherapy. This definition may be imprecise because the recovery time can differ greatly depending on the duration, route, and type of ATB used.<sup>34,35</sup> Among the included studies, Derosa *et al.* explored the impact of time of ATB use (30 versus 60 days before therapy) on ICI efficacy in patients with NSCLC and RCC, and the results demonstrated that the impact of ATB use 60 days before therapy on efficacy was lower than that of ATB use 30 days before therapy. Indeed, our subgroup analyses also indicated that the HR for ATB use within 1 month before ICI was greater than that within 2 months before ICI. The reason for this finding may be that the gut microbiota partially recovered over a longer duration following ATB use.<sup>26</sup> In addition, Kaderbhai *et al.* found that ATB use 3 months before nivolumab immunotherapy did not affect the clinical efficacy of ICI.<sup>29</sup> It should be noted that

the favorable results of ATB use 60 days prior to therapy could also be due to recall bias over the unavailability of ATB treatment data in retrospective studies. Thus, future large-scale, prospective studies are required to investigate the impact of the use time, duration, route, and drug type of ATBs on the clinical efficacy of ICI immunotherapy. Such findings may then lead to strategies that can help reduce this impact and improve ICI efficacy.

There were some limitations associated with this study: 1) this meta-analysis relied on published data from the included studies and we could not obtain detailed individual data on the tumor and host characteristics (i.e., PD-L1 expression, tumor mutational burden, tumor-infiltrating lymphocytes, mismatch repair deficiency, TNM stage, comorbidities, immune status, treatment strategies, and steroid use) that may influence the efficacy of ICIs; 2) our meta-analysis was not registered online. However, to prevent potential bias, the literature search strategy, inclusion and exclusion criteria, data extraction, and statistical analysis were defined prospectively prior to the initiation of this study; 3) our study could not assess the association between ATB use and immune-related adverse events due to the lack of eligible data. Thus, future studies are required to investigate this potential association; 4) PFS is a weaker endpoint in studies reporting outcomes from patients treated outside of trials given that the restaging interval is not standardized. Indeed,

**Table 2.** The results for the association of antibiotics use and efficacy of immune checkpoint inhibitors.

	N	Hazard ratio	P for hazard ratio	Heterogeneity ( <i>P</i> , <i>I</i> <sup>2</sup> )	Publication bias
<b>Overall survival</b>					
<b>Overall</b>	17	2.37(2.05–2.75)	< 0.001	0.851, 0.0%	Begg's Test = 0.401; Egger's test = 0.235
<b>Time of antibiotics use</b>					
Before ICI initiation	10	2.29(1.92–2.73)	< 0.001	0.837, 0.0%	Begg's Test = 1.000; Egger's test = 0.975
One month before ICI initiation	7	2.23(1.82–2.74)	< 0.001	0.865, 0.0%	Begg's Test = 0.711; Egger's test = 0.778
Two month before ICI initiation	2	1.97(1.49–2.59)	< 0.001	0.199, 38.0%	Begg's Test = 1.000; Egger's test = /
Before or after ICI initiation	7	2.56(1.96–3.36)	< 0.001	0.572, 0.0%	Begg's Test = 0.266; Egger's test = 0.207
<b>Cancer type</b>					
Non-small-cell lung cancer	10	2.68(2.19–3.28)	< 0.001	0.911, 0.0%	Begg's Test = 0.283; Egger's test = 0.105
Urothelial carcinoma	2	2.01(1.23–3.29)	0.005	0.778, 0.0%	Begg's Test = 1.000; Egger's test = /
Renal cell carcinoma	2	1.68(1.00–2.83)	0.052	0.518, 0.0%	Begg's Test = 1.000; Egger's test = /
<b>ICI drug</b>					
Anti-PD-1	12	2.45(2.04–2.97)	< 0.001	0.765, 0.0%	Begg's Test = 0.669; Egger's test = 0.316
Anti-PD-1/CTLA-4/PD-1+ CTLA-4	3	2.23(1.68–2.97)	< 0.001	0.910, 0.0%	Begg's Test = 0.734; Egger's test = 0.398
<b>Treatment type</b>					
ICI monotherapy	5	2.90(1.80–4.68)	< 0.001	0.861, 0.0%	Begg's Test = 0.806; Egger's test = 0.494
<b>Sample size</b>					
<100	6	2.97(1.98–4.44)	< 0.001	0.779, 0.0%	Begg's Test = 1.000; Egger's test = 0.617
≥100	11	2.29(1.95–2.68)	< 0.001	0.780, 0.0%	Begg's Test = 0.855; Egger's test = 0.983
<b>Country</b>					
Asia	3	2.12(1.46–3.07)	< 0.001	0.695, 0.0%	Begg's Test = 1.000; Egger's test = 0.647
No-Asia	14	2.41(2.06–2.84)	< 0.001	0.768, 0.0%	Begg's Test = 0.499; Egger's test = 0.323
<b>Progression-free survival</b>					
<b>Overall</b>	15	1.84(1.49–2.26)	< 0.001	0.002, 56.2%	Begg's Test = 0.091; Egger's test = 0.035
<b>Time of antibiotics use</b>					
Before ICI initiation	8	1.70(1.43–2.02)	< 0.001	0.178, 30.1%	Begg's Test = 0.466; Egger's test = 0.421
Before or after ICI initiation	8	1.91(1.31–2.78)	0.001	0.001, 68.8%	Begg's Test = 0.175; Egger's test = 0.066
<b>Cancer type</b>					
Non-small-cell lung cancer	9	1.79(1.29–2.49)	< 0.001	0.001, 69.3%	Begg's Test = 0.602; Egger's test = 0.176
Renal cell carcinoma	2	2.12(1.51–2.96)	< 0.001	0.815, 0.0%	Begg's Test = 1.000; Egger's test = /
<b>ICI drug</b>					
Anti-PD-1	10	1.92(1.43–2.58)	< 0.001	0.003, 61.8%	Begg's Test = 0.436; Egger's test = 0.190
Anti-PD-1/CTLA-4/PD-1+ CTLA-4	3	1.63(1.13–2.36)	0.009	0.092, 53.4%	Begg's Test = 0.734; Egger's test = 0.317
<b>Treatment type</b>					
ICI monotherapy	5	1.94(1.20–3.13)	0.007	0.076, 52.7%	Begg's Test = 0.221; Egger's test = 0.081
<b>Sample size</b>					
<100	7	1.96(1.51–2.54)	< 0.001	0.157, 35.5%	Begg's Test = 0.764; Egger's test = 0.391
≥100	8	1.76(1.34–2.32)	< 0.001	0.002, 65.3%	Begg's Test = 0.210; Egger's test = 0.098
<b>Country</b>					
Asia	2	2.67(1.66–4.27)	< 0.001	0.271, 17.5%	Begg's Test = 1.000; Egger's test = /
No-Asia	13	1.75(1.41–2.18)	< 0.001	0.004, 55.9%	Begg's Test = 0.138; Egger's test = 0.070

NOTE, CTLA-4: Cytotoxic T-Lymphocyte-associated Antigen 4; *I*<sup>2</sup>: Degree of Heterogeneity; ICI: Immune Checkpoint Inhibitors; PD-1: Programmed cell death Drotein-1; PD-L1: Programmed cell Death-Ligand 1; "/>: Not applicable because Egger's test cannot be performed when the number of studies was 2

heterogeneity was observed among the included studies regarding PFS analysis. Moreover, the degree of heterogeneity could not be definitively eliminated in the subgroup analysis stratified by the time of ATB use, cancer type, sample size, type of ICI drug, publication country, and study type. This unexplained heterogeneity may result from differences in tumor and host characteristics, as well as other confounding factors; and 5) the limited number of subgroup analyses may affect the statistical power of the results.

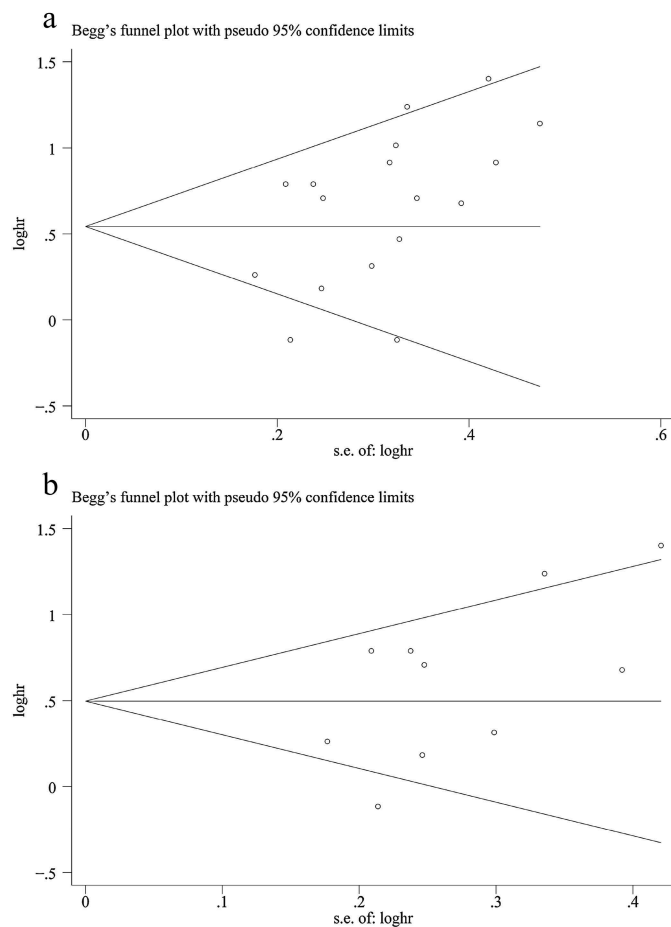
In conclusion, the results of this meta-analysis indicated that ATB use was negatively associated with OS and PFS in cancer patients treated with ICI immunotherapy. Therefore, ATBs should be used with caution and strict indications to avoid unnecessary ATB use in cancer patients treated with ICIs. For patients who require ATB, careful ATB selection should be employed to avoid reducing the anti-tumor immune response. In addition to studying the underlying mechanism, future studies should identify which specific gut microbiota phenotypes can enhance or reduce anti-tumor immune responses, and elucidate whether it is feasible to modulate the gut microbiota to a more favorable phenotype to promote a synergistic effect with ICI immunotherapy through fecal microbiota transplantation, probiotic administration, or the reduction of unfavorable

microbiota phenotypes through ABT use. Future multicenter randomized clinical studies are also required to explore which favorable interventions can promote gut microbiota recovery and resolve the deleterious effects of ATB-induced gut microbiota dysbiosis. Moreover, the association between ATB and chemo-immunotherapy combinations are also warranted in future studies.

## Materials and methods

### Literature search

We performed a systematical literature search for relevant studies that had evaluated the association between ATB use and ICI efficacy in cancer patients using the PubMed, Embase, American Society of Clinical Oncology, and European Society of Medical Oncology databases (up to May 2019) using the following search terms: “nivolumab”, “pembrolizumab”, “avelumab”, “atezolizumab”, “lambrolizumab”, “pidilizumab”, “durvalumab”, “ipilimumab”, “tremelimumab”, “immune checkpoint inhibitor”, “PD-1 inhibitor”, “PD-L1 inhibitors”, “CTLA-4 inhibitors”, “antibiotic”, “anti-infectious”, “anti-infection”, “cancer”, “tumor”, “neoplasm”, and “carcinoma”. Moreover, we manually



**Figure 5.** Funnel plot of progression-free survival. A: overall analysis; B: sample size  $\geq 100$  subgroup.

searched the references of the relevant studies to identify other potentially eligible studies.

### Eligibility criteria

Studies that met all of the following inclusion criteria were included in our meta-analysis: 1) patient: eligible patients were diagnosed with a solid cancer and treated with ICIs alone (PD-1, PD-L1, or CTLA-4 inhibitors) or in combination with systemic chemotherapy, whereas patients treated with ICIs alongside loco-regional therapy were excluded; 2) intervention: ATBs were administered before and/or after the initiation of ICI therapy, irrespective of the duration and dosage; 3) comparison: the control group did not receive treatment with ATBs; 4) outcome: the two primary outcomes were OS and PFS, and the outcome measures could be extracted. Furthermore, if there were several eligible duplicated studies identified, the most recent study was included in the meta-analysis.

### Data extraction

The data from the included studies was independently reviewed and extracted by two authors (Xuan-Zhang Huang and Peng Gao). The following data were extracted from each included study: first author, publication year and country,

study design, cancer type, definition of ATB use, type of ICI drug, sample size, age, and outcome measures. Any problems with the data extraction were resolved by discussion.

### Statistical analysis

This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement (Supplementary File 1).<sup>36</sup> The primary outcome was OS and secondary outcome was PFS, and the association between ATB use and ICI efficacy was measured by HR with the corresponding 95% CI. The overall analysis was conducted by including all studies, and subgroup analyses were conducted based on the time of ATB use, cancer type, sample size, type of ICI drug, publication country, and study type.

We used  $I^2$  statistics and a Cochran Q test to evaluate the heterogeneity among the studies, and the heterogeneity was considered statistically significant when the  $I^2$  was greater than 50% and/or a  $P$  value less than 0.10. A random effect model was used to pool the HRs if the heterogeneity was significant; otherwise, a fixed effects model was used.<sup>37</sup> Begg's and Egger's tests were used to evaluate publication bias, and a trim-and-fill analysis was performed to evaluate the effect of potential publication bias on an outcome if the  $p$  value for Begg's or Egger's tests was low (i.e.,  $p < .15$ ).<sup>38-40</sup> To assess the bias risk of an individual study on the results and to investigate the stability and consistency of our results, sensitivity analyses were conducted to investigate whether a single study dominated the results by a leave-one-out approach (individually omitting each study).

All statistical analyses were performed using Stata software (Version 12.0, Stata Corporation, College Station, TX, USA). A two-sided  $P$  value of  $< 0.05$  was considered statistically significant.

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### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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