Cancer Biol Med 2026. doi: 10.20892/j.issn.2095-3941.2025.0844

LETTER

Antibiotics and probiotics differentially shape immunotherapy outcomes in non-small cell lung cancer

Shuling Ma[1]*, Zijun Zhai[1,2]*, Rui Ding[1], Shijie Shang[1,3], Xinyi Liang[1], Shan Yin[1,2], Haofeng Lin[4], Guomeng Sha[1], Ran Zhang[1], Jinming Yu[1], Qian Song[1], Dawei Chen[1,2]

[1]Shandong Provincial Key Laboratory of Precision Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan 250117, China; [2]Cheeloo College of Medicine, Shandong University Cancer Center, Jinan 250000, China; [3]Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430023, China; [4]Guangdong Provincial Key Laboratory of Pharmaceutical Bioactive Substances, Guangdong Pharmaceutical University, Guangzhou 510006, China

The introduction of immune checkpoint inhibitors (ICIs) has substantially improved therapeutic outcomes in patients with non-small cell lung cancer (NSCLC) but marked inter-individual variability in treatment response persists[1]. Increasing evidence indicates that the gut microbiota has a critical role in regulating antitumor immune responses through the gut-organ axis, thereby influencing the efficacy and toxicity of systemic anticancer therapies[2]. Among microbiota-modulating strategies, antibiotics and probiotics have attracted considerable interest. Nevertheless, the clinical impact of antibiotics and probiotics on treatment outcomes in NSCLC has not been comprehensively and systematically evaluated. Against this background, a meta-analysis was performed to determine the associations between antibiotic exposure or probiotic supplementation and clinical outcomes in patients with NSCLC, focusing on the objective response rate (ORR), progression-free survival (PFS), and overall survival (OS).

Antibiotic exposure and survival outcomes

The studies included in the antibiotic cohort were predominantly retrospective real-world investigations and enrolled patients with NSCLC receiving ICIs, most of whom had advanced disease. The antibiotic-related analysis was comprised of 34 studies that included patients treated with ICIs mainly in the second-line or later settings (**Table 1**). Across studies, immunotherapy regimens were largely based on PD-1/PD-L1 inhibitors. Antibiotic exposure was generally defined as systemic antibiotic use within predefined peri-ICI time windows and the antibiotics administered were mainly broad-spectrum agents. The meta-analysis demonstrated that antibiotic use was significantly associated with unfavorable survival outcomes in patients with NSCLC receiving immunotherapy. As shown in **Figure 1A**, antibiotic exposure was associated with a significantly reduced ORR [pooled odds ratio (OR) = 0.60, 95% confidence interval (CI) = 0.43–0.84, $P = 0.002$]. Antibiotic exposure was also associated with a significantly shorter OS [pooled hazard ratio (HR) = 1.68, 95% CI = 1.49–1.91, $P < 0.001$] and PFS (pooled HR = 1.62, 95% CI = 1.37–1.92, $P < 0.001$; **Figure 1B, C**). These findings are biologically plausible. Antibiotics can markedly reduce gut microbial diversity, disrupt the homeostasis of beneficial commensal bacteria, and potentially alter the pulmonary immune microenvironment *via* the gut-lung axis. Consistent with this notion, clinical studies have reported that antibiotic administration, particularly when given before or during immunotherapy, is closely associated with diminished ICI efficacy[36]. Collectively, antibiotic exposure exhibited a trend toward a reduced ORR, while being consistently and significantly associated with an inferior OS and PFS, which indicated that the detrimental impact on immunotherapy outcomes in NSCLC is more robustly captured by long-term survival endpoints than by early response metrics. This finding also suggests that antibiotic-induced disruption of the gut microbiota may exert sustained and systemic inhibitory effects on antitumor immunity rather than merely delaying initial tumor control. In this context, antibiotic exposure during critical windows surrounding immunotherapy initiation may be particularly relevant, underscoring the need for careful consideration of antibiotic use in patients with NSCLC receiving ICIs[37].

*These authors contributed equally to this work.
Correspondence to: Qian Song and Dawei Chen
E-mail: sqoxaid@163.com and dave0505@yeah.net
ORCID ID: https://orcid.org/0000-0001-6545-8926 and https://orcid.org/0000-0002-6762-7997
Received February 11, 2026; accepted April 14, 2026;
published online May 29, 2026.
Available at www.cancerbiomed.org
©2026 The Authors. Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

Table 1 Detail characteristics of 34 antibiotic (ATB) cohort included in the meta-analysis

Author	Year	Country	Trial type	Median follow-up	Cancer type	*N*	Line of therapy	Antibiotics regimen in relation to ICI	Time frame	Immunotherapy	Antibiotic	References
Derosa et al.	2018	France	Retrospective cohort	-	Advanced NSCLC	239	Mixed line	≤30 days before ICI initiation; also ≤60 days explored	-	Anti-PD-(L)1 alone or anti-PD-(L)1 + anti-CTLA-4	β-lactams most common; also quinolones and sulfonamides	[3]
Huemer et al.	2018	Austria	Retrospective single-center study	-	NSCLC	30	All were 2nd-line or above	1 month before ICI to 1 month after ICI	2015.5–2017.11	Nivolumab (25), pembrolizumab (5)	Penicillins (64%), fluoroquinolones (36%), carbapenems (18%)	[4]
Zhao et al.	2019	China	Single-center retrospective study	-	Advanced NSCLC	109	Mixed lines; first line 28 (25.7%), ≥ second line 81 (74.3%)	Within 1 month before or after first ICI	2016.1–2018.5	Pembrolizumab, nivolumab, SHR-1210 (camrelizumab)	Most commonly β-lactam inhibitors and fluoroquinolones	[5]
Barrón et al.	2019	Multiple Latin American countries (Mexico, Colombia, Argentina, Peru, and Brazil)	Multicenter retrospective study	-	Advanced NSCLC	140	Mostly ≥2nd line	Within 30 days prior to ICI initiation	-	PD-1/PD-L1	β-lactams, quinolones	[6]
Bagley et al.	2019	USA	Retrospective cohort study using Flatiron Health EHR-derived database	-	Advanced NSCLC	1,960	First-line	Primary analysis: ABX from −6 to +4 weeks around ICI start; secondary analyses: within 6 weeks pre-ICI and within 4 weeks post-ICI	-	ICI	-	[7]
Hakozaki et al.	2019	Japan	Retrospective single-center study	-	Advanced NSCLC	90	Second or later line	Antibiotics for ≥3 days within 30 days of nivolumab therapy	2016.1–2017.4	Nivolumab monotherapy	TMP/SMX, amoxicillin/clavulanate, ceftriaxone, meropenem, piperacillin/tazobactam, ampicillin/sulbactam, cefazolin, levofloxacin	[8]
Kim et al.	2019	Korea	Retrospective single-center study	-	Advanced NSCLC	131	Mixed-line	Antibiotics within 60 days before ICI initiation; subgroup analyses for within 30 days and 31–60 days before ICI	2012.2–2018.5	Nivolumab, pembrolizumab, and others (anti-PD-1/PD-L1/CTLA-4-based regimens)	Cephalosporins; fluoroquinolones; beta-lactam/beta-lactamase inhibitors; carbapenems; glycopeptides; macrolides	[9]

Table 1 Continued

Author	Year	Country	Trial type	Median follow-up	Cancer type	*N*	Line of therapy	Antibiotics regimen in relation to ICI	Time frame	Immunotherapy	Antibiotic	References
Pinato et al.	2019	UK	Prospective multicenter cohort study	14.6 months	NSCLC subgroup from pan-cancer cohort	119	Mixed (mostly first-line metastatic)	Broad-spectrum antibiotics within 30 days prior to ICI initiation (pATB) or during ICI (cATB)	2015.1–2018.4	Anti-PD-1/PD-L1 ICIs	Mainly β-lactam based	[10]
Ouaknine Krief et al.	2019	France	Retrospective real-world cohort study	500 days (IQR 401–599)	NSCLC	72	Nivolumab used as 2nd-line or later therapy	Early use of antibiotics (EUA) = 2 months before to 1 month after ICI initiation	2014.7–2017.9	Nivolumab	Oral or iv antibiotics, median duration 9.5 days, mainly β-lactams (36/51) and vancomycin	[11]
Mielgo Rubio et al.	2019	Spain	Retrospective multicenter study	6.5 months	Advanced NSCLC, PD-L1 ≥50%	121	1st-line	Antibiotics 2 months before to 1 month after ICI start	2016.9–2019.3	Pembrolizumab monotherapy	Quinolones (40.7%), penicillins/derivatives (35.2%); iv 65.5%, oral 34.5%	[12]
Schett et al.	2020	Switzerland (two tertiary centers)	Retrospective observational cohort	5.5 months (95% CI 5–14)	Advanced NSCLC (EGFR WT, ALK–)	218	Across 1st–4th-lines (majority 2nd-line)	Within 2 months prior to ICI start	2013.1–2017.12	Nivolumab/ pembrolizumab/ atezolizumab	β-lactams, quinolones, macrolides (oral or iv)	[13]
Chalabi et al.	2020	Multiple countries (mainly Europe and America)	Pooled retrospective analysis of randomized Phase II/III trials (OAK + POPLAR)	19.2 months	NSCLC	1,512	Mixed line	30 days before ICI to 30 days after ICI (±30 days)	-	Atezolizumab	Quinolones, penicillins, cephalosporins	[14]
Cortellini et al.	2021	Europe multicenter	Retrospective multicenter real-world cohort	21.8 months	Metastatic NSCLC with PD-L1 ≥50%	950	First-line only	Systemic ATB within 30 days before first-line treatment initiation	2017.1–2020.5	Pembrolizumab monotherapy	-	[15]
Hamada et al.	2021	Japan	Single-center retrospective study	251.5 days	Advanced NSCLC	69	Mixed lines (1L/2L/≥3L)	Within 21 days before or after first anti-PD-1 dose	2016.1–2019.12	Nivolumab or pembrolizumab	Levofloxacin, macrolides, cephems, TMP-SMX, carbapenems, tazobactam/piperacillin	[16]

Table 1 Continued

Author	Year	Country	Trial type	Median follow-up	Cancer type	*N*	Line of therapy	Antibiotics regimen in relation to ICI	Time frame	Immunotherapy	Antibiotic	References
Verschueren et al.	2021	Belgium	Single-country real-world cohort	-	Stage IV NSCLC	442	Mixed lines: 1L, 2L, and 3L	Antibiotic use within 30 days before and 30 days after treatment initiation	-	Nivolumab, pembrolizumab, atezolizumab	-	[17]
Ren et al.	2021	China	Pooled analysis of 5 tislelizumab monotherapy studies	-	NSCLC	-	-	Within 30 days of day 1	-	Tislelizumab	-	[18]
Balado et al.	2021	Spain	Retrospective study	-	Advanced/ metastatic NSCLC, PD-L1 ≥50%	49	1st-line immunotherapy	ATB+: systemic antibiotics within ±30 days of pembrolizumab start	2017.7–2020.1	Pembrolizumab monotherapy	-	[19]
Castello et al.	2021	Italy	Prospective registration + retrospective analysis	12.4 months (9.7–15.2)	NSCLC	50	Mixed-line	12 patients received antibiotics 1 month before ICI initiation, and 11 patients received antibiotics within 1 month after ICI initiation (3 of whom received antibiotics in both time periods)	2015.12–2019.5	Nivolumab (62%), pembrolizumab (32%), nivolumab + ipilimumab (4%), atezolizumab (2%)	β-lactam ± inhibitor (8 cases), quinolone (8 cases), rifaximin (1 case), glycopeptide (1 case), 2 cases unspecified	[20]
Geum et al.	2021	South Korea	Retrospective single-center cohort study	-	Advanced/ recurrent NSCLC, predominantly adenocarcinoma (70%)	140	≥2nd-line	Any systemic antibiotic use from 30 days before nivolumab initiation until completion of nivolumab therapy	2015.7–2018.6	Nivolumab	Various systemic antibiotics, mainly broad-spectrum agents	[21]
Ochi et al.	2021	Japan (9 institutions)	Multicenter retrospective cohort study	-	Advanced/ metastatic NSCLC	531	Any line of therapy	Within 2 months before or 1 month after starting ICI	2015.12–2018.5	PD-1/PD-L1 (nivolumab/ pembrolizumab/ atezolizumab)	β-lactams most common, other classes (e.g., quinolones, macrolides) used at lower frequencies, not further detailed in the original report	[22]

Table 1 Continued

Author	Year	Country	Trial type	Median follow-up	Cancer type	*N*	Line of therapy	Antibiotics regimen in relation to ICI	Time frame	Immunotherapy	Antibiotic	References
Lu et al.	2021	Taiwan, China	Retrospective cohort	-	NSCLC	340	1st/2nd-line: 70%; ≥3rd-line: 30%	Systemic antibiotics within 30 days before ICI	2016.1–2019.3	ICI: ipilimumab, nivolumab, pembrolizumab, atezolizumab, durvalumab (96% PD-(L)1 monotherapy)	Broad-spectrum antibiotics: fluoroquinolones 39%, penicillins 38%, 1st/2nd-gen cephalosporins 33%, 3rd/4th-gen cephalosporins 30%, carbapenems 7%, macrolides 5%	[23]
Nyein et al.	2022	USA	Retrospective single-center study	-	NSCLC (Stage III/IV)	256	-	From 60 days before ICI to 30 days after ICI	2011.1–2017.3	PD-1/PD-L1/CTLA-4 (monotherapy or combined with chemotherapy/ targeted therapy)	β-lactams, fluoroquinolones, macrolides, cephalosporins, tetracyclines; Most commonly used: levofloxacin (15), cefazolin (14), azithromycin (8)	[24]
Qiu et al.	2022	China	Retrospective study	-	NSCLC	148	1st-line: 48.0%, 2nd-line: 37.8%, 3rd-line: 14.2%	ATB exposure was defined as use within 60 days before or after ICI initiation, and was further classified as 30–60 days before ICI, within 30 days before ICI, concurrent with ICI, within 30 days after ICI, and 30–60 days after ICI.	2018.1–2021.6	Camrelizumab, pembrolizumab, toripalimab, sintilimab, tislelizumab, nivolumab, durvalumab	β-lactams, quinolones, and their combination. Subclasses of β-lactams: Penicillins, cephalosporins, carbapenems	[25]
Joshi et al.	2022	USA	Prospective real-world cohort study	-	NSCLC	136	First-line only	1 month before ICI	2014.1–2019.5	Pembrolizumab (1st-line, monotherapy or combined with chemotherapy)	β-lactams, fluoroquinolones, macrolides, cephalosporins, tetracyclines	[26]
Stokes et al.	2022	USA	Nested cohort study		NSCLC	3,634		pAbx (prior antibiotics): antibiotic exposure within 30 days before initiation of ICI	2010–2018	Nivolumab 59.1%, pembrolizumab 35.1% (total >94%)	The most common were β-lactams (30.1%); other ATBs were not subdivided	[27]

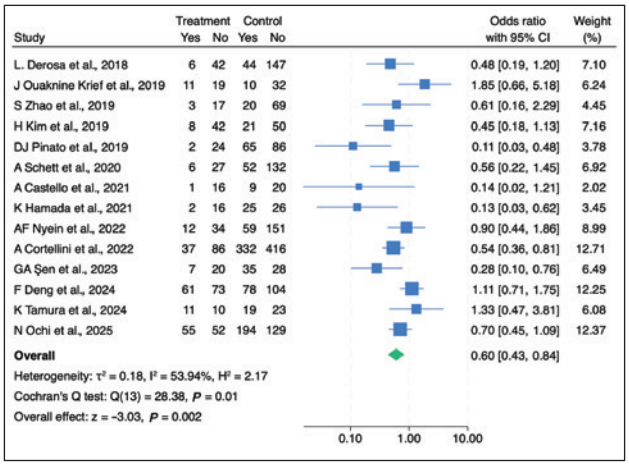
Table 1 Continued

Author	Year	Country	Trial type	Median follow-up	Cancer type	*N*	Line of therapy	Antibiotics regimen in relation to ICI	Time frame	Immunotherapy	Antibiotic	References
Stokes et al.	2022	USA	Nested cohort study	-	NSCLC	3,634	-	cAbx (concurrent antibiotics): antibiotic exposure within 60 days after initiation of ICI	2010–2018	Nivolumab 59.1%, pembrolizumab 35.1% (total >94%)	The most common were β-lactams (30.1%); other ATBs were not subdivided	[27]
Şen et al.	2023	Turkey	Retrospective cohort	-	NSCLC (Stage IV)	90	81.1% 1st-line, 18.9% 2nd-line	Within 12 weeks after ICI initiation (primary analysis), the first 4/8 weeks were also analyzed	-	PD-1/PD-L1	Outpatient: moxifloxacin, clarithromycin, amoxicillin-clavulanate; Inpatient: piperacillin-tazobactam, meropenem, vancomycin, colistin, TMP-SMX	[28]
Deng et al.	2024	China	Retrospective cohort study	25.2 months (95% CI 22.99–27.34)	NSCLC, locally advanced or metastatic (Stage III–IV)	316	1st-line 238/316 (75.3%), ≥2nd-line 78/316 (24.7%); ATB group: 1st-line 108/134 (80.6%), ≥2nd-line 26/134 (19.4%); N-ATB group: 1st-line 130/182 (71.4%), ≥2nd-line 52/182 (28.6%)	Within 30 days before ICI initiation (p-ATB) or during ICI treatment	2018.01–2023.10	PD-1/PD-L1 inhibitors: tislelizumab (138 cases), camrelizumab (85 cases), sintilimab (61 cases), pembrolizumab (14 cases), and other ICIs (18 cases).	β-lactams (68 cases, 50.7%), quinolones (31 cases, 23.1%), quinolones + β-lactams (32 cases, 23.9%), macrolides (3 cases, 2.3%; β-lactams were further divided into BLBLI (β-lactam/β-lactamase inhibitor combinations, 54 cases, 40.3%) and non-BLBLI; common BLBLI included cefoperazone-sulbactam, mezlocillin-sulbactam, piperacillin-tazobactam, and amoxicillin-clavulanate.	[29]
Tamura et al.	2024	Japan	Retrospective study [propensity score matching (PSM)]	ABx: 45.9 months *vs.* Non-ABx: 47.2 months	NSCLC	201	First-line only	30 days before ICI	2018.12–2020.12	Pembrolizumab or atezolizumab	β-lactam/β-lactamase inhibitor combinations [AMPC/CVA 63.6%, PIPC/TAZ 15.2%, ABPC/SBT 9.1%], fluoroquinolones [LVFX, MFLX], sulfonamide/trimethoprim, macrolide [CAM], penicillin [AMPC], cephalosporin [CEZ], and glycopeptide [VCM]	[30]

Table 1 Continued

Author	Year	Country	Trial type	Median follow-up	Cancer type	*N*	Line of therapy	Antibiotics regimen in relation to ICI	Time frame	Immunotherapy	Antibiotic	References
Luo et al.	2024	China	Prospective cohort study	-	EGFR+ advanced NSCLC	74	Mixed line	2 months before and after ICI	2019.3–2022.9	PD-1/PD-L1 inhibitors	-	[31]
Xie et al.	2024	China West China Hospital of Sichuan University	Retrospective cohort study	-	NSCLC accounted for 74.8% (≈472/631)	472	-	Use of antibiotics within 3 months before initiation of ICI therapy	2018.12–2023.6	ICI	Cephalosporins	[32]
Rousseau et al.	2025	France	Retrospective cohort + target trial emulation	-	Advanced/metastatic NSCLC	41,529	1st-line therapy	≥2 prescriptions of ATC J01 antibiotics, with prescription dates falling between 60 days prior to and 42 days after pembrolizumab initiation	2015.1–2022.12	Pembrolizumab monotherapy or pembrolizumab combined with chemotherapy (1st-line)	Macrolides, penicillins, penicillins plus penicillinase inhibitors, β-lactams other than penicillin, sulfonamides, fluoroquinolones, and combinations of several antibiotic types	[33]
Ochi et al.	2025	Japan	Multicenter retrospective study	17.0 months (95% CI 15.8–18.2)	NSCLC	-	1st-line therapy	2 months before therapy to 1 month after therapy	2018.12–2020.12	Anti–PD-1, anti–PD-L1	-	[34]
Hu et al.	2025	China	Single-center retrospective	33.79 months	mNSCLC (Stage IV)	199	-	±30 days before and after ICI	2017.12–2021.10	PD-1/PD-L1	β-lactams 83.3% (penicillins ± β-lactamase inhibitors, cephalosporins, carbapenems), quinolones 57.8%, aminoglycosides, glycopeptides, and macrolides	[35]

A **ORR: antibiotics *vs.* no antibiotics**



B **OS: antibiotics *vs.* no antibiotics**

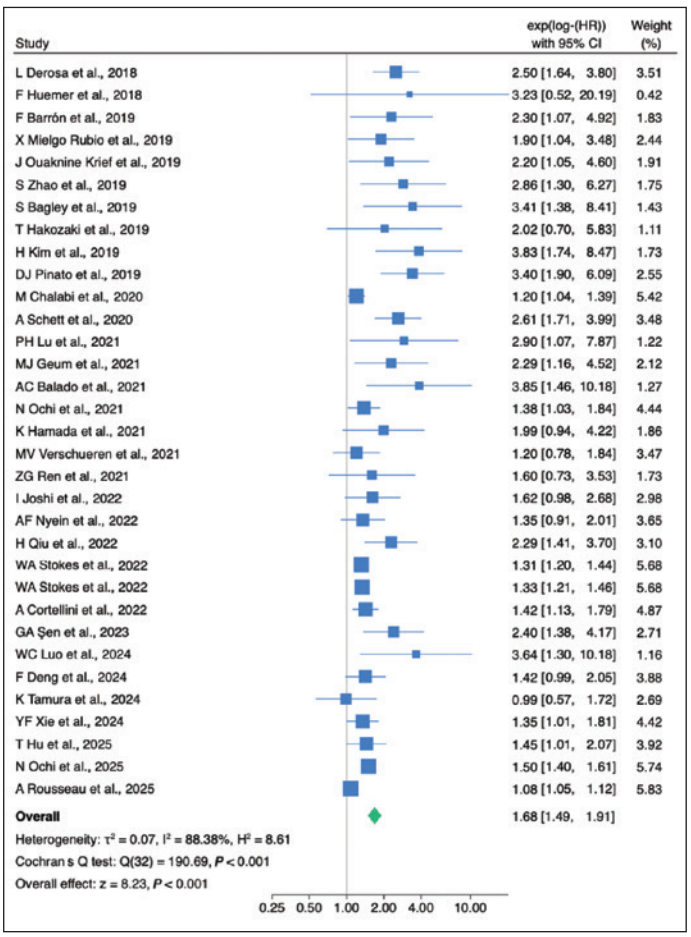
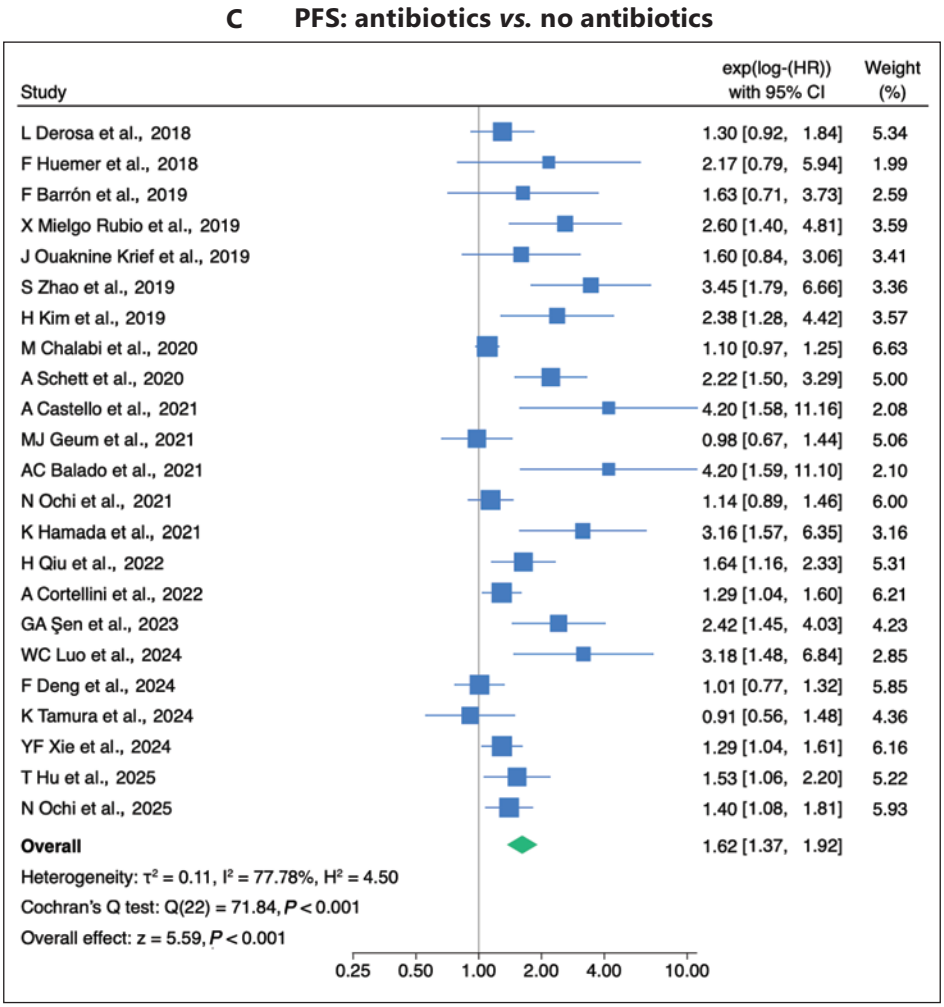


Figure 1 Continued

Subgroup analyses by line of therapy showed a broadly consistent association between antibiotic exposure and worse outcomes. The association was more evident in first-line and mixed-line settings for the ORR with pooled ORs of 0.65 (95% CI = 0.48–0.87; I^2 = 1%) and 0.42 (95% CI = 0.25–0.70; I^2 = 56%), whereas the estimate in second-line



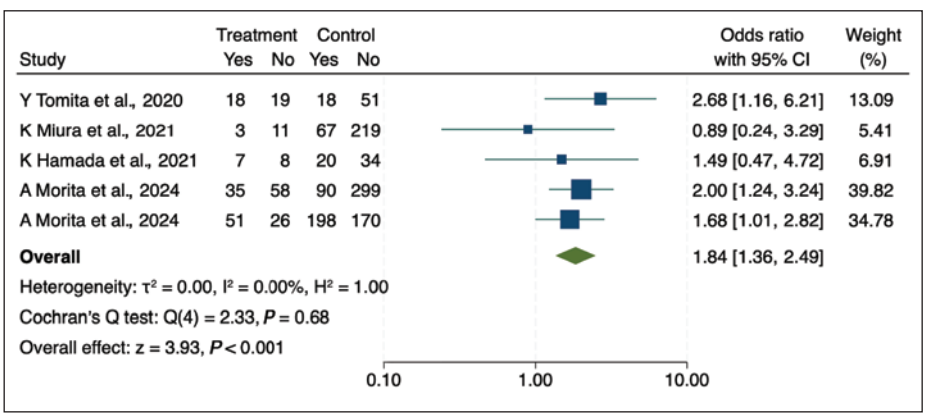


Figure 1 Continued

and later studies was not significant and showed substantial heterogeneity (OR = 1.85, 95% CI = 0.66–5.18). However, no significant subgroup difference was observed (P = 0.103). Antibiotic exposure was associated with shorter OS across first-line, second-line and later therapies, and mixed-line populations with pooled HRs of 1.47 (95%

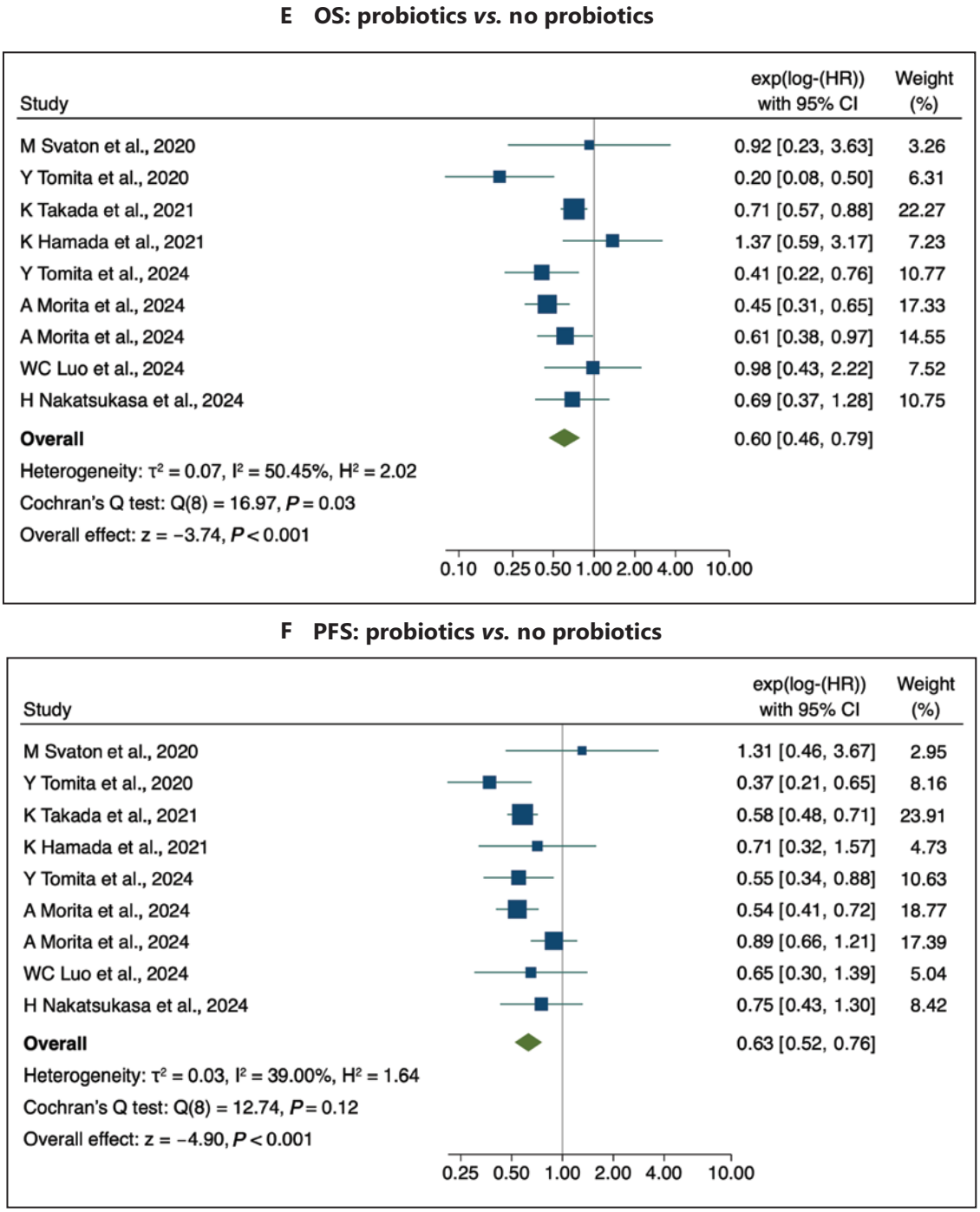


Figure 1 Effects of antibiotics and probiotics on immunotherapy outcomes in patients with non–small cell lung cancer. (A–C) Antibiotics: ORR, OS, and PFS. Squares indicate study-specific effects; diamonds show pooled estimates with 95% CIs. (D–F) Probiotics: ORR, OS, and PFS. Squares indicate study-specific effects; diamonds show pooled estimates with 95% CIs. CI, confidence interval; HR, hazard ratio; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

CI = 1.19–1.82; I^2 = 91%), 2.27 (95% CI = 1.55–3.31; I^2 = 0%), and 2.00 (95% CI = 1.59–2.52; I^2 = 73%), respectively. PFS had a similar pattern with pooled HRs of 1.58 (95% CI = 1.04–2.39; I^2 = 82%), 1.33 (95% CI = 0.91–1.96; I^2 = 29%), and 1.83 (95% CI = 1.39–2.40; I^2 = 85%) in the corresponding subgroups. Neither OS nor PFS differed significantly

across treatment-line strata (P = 0.173 and P = 0.648, respectively).

The results were similar when stratified by immune checkpoint blockade strategy. Antibiotic exposure remained associated with worse OS in anti-PD-1-only studies (HR = 1.56, 95% CI = 1.29–1.88; I^2 = 89%), anti-PD-L1-only studies (HR = 1.20, 95% CI = 1.04–1.39), anti-PD-1/anti-PD-L1 studies (HR = 1.76, 95% CI = 1.38–2.23; I^2 = 75%), and mixed-strategy studies (HR = 1.93, 95% CI = 1.48–2.51; I^2 = 47%) without a significant subgroup difference (P = 0.390). Pooled HRs for PFS were 2.00 (95% CI = 1.37–2.93; I^2 = 74%), 1.10 (95% CI = 0.97–1.25), 1.58 (95% CI = 1.22–2.05; I^2 = 68%), and 1.61 (95% CI = 1.09–2.37; I^2 = 76%) across these strata with no significant interaction (P = 0.536). The ORR was less consistent. Specifically, the reduction in response was most apparent in anti-PD-1/anti-PD-L1 studies (OR = 0.45, 95% CI = 0.23–0.88; I^2 = 62%), whereas the estimates in anti-PD-1-only (OR = 0.59, 95% CI = 0.23–1.48; I^2 = 70%) and mixed-strategy studies (OR = 0.77, 95% CI = 0.49–1.22; I^2 = 39%) were not significant; no subgroup difference was identified (P = 0.601). Overall, the adverse association of antibiotic exposure was more consistent for OS and PFS than the ORR.

Probiotic supplementation and therapeutic benefit

Probiotic-related evidence is comprised of 10 studies, most of which were retrospective cohort or real-world comparative analyses (**Table S1**). These studies encompassed patients receiving ICIs across first- to third-line treatment settings. The formulations varied across studies with respect to probiotic interventions but preparations based on *Clostridium butyricum MIYAIRI 588* (CBM588) were most frequently used with *Bifidobacterium* spp. and mixed probiotic formulations. Probiotic interventions were typically administered orally as long-term adjuncts, like CBM588, to immunotherapy with control groups consisting of patients receiving identical immunotherapy regimens without probiotic supplementation. Probiotic supplementation was significantly associated with more favorable clinical outcomes in contrast to the unfavorable associations observed with antibiotics[38]. Probiotic use was associated with a significantly higher ORR compared to no probiotic use (pooled OR = 1.84, 95% CI = 1.36–2.49, P < 0.001), as well as a significantly prolonged OS (pooled HR = 0.60, 95% CI = 0.46–0.79, P < 0.001) and PFS (pooled HR = 0.63, 95% CI = 0.52–0.76, P < 0.001), as illustrated in **Figure 1D–F**. Although the probiotic formulations and administration strategies varied across studies, the consistent improvement in the ORR and survival outcomes suggested that microbiota-supportive interventions (especially CBM588) may enhance antitumor treatment responses and improve long-term prognosis in NSCLC. These findings provided a rationale for considering microbiota modulation as an adjunctive strategy to optimize therapeutic outcomes in clinical practice.

Assessment of publication bias

Potential publication bias was assessed using funnel plots (**Figure S1**). Visual inspection of the funnel plots showed no obvious marked asymmetry in the probiotic analyses, whereas some asymmetry was observed in the antibiotic analyses, particularly for OS and PFS. Nevertheless, the number of studies was limited for several comparisons and these findings should be interpreted with caution. Further large-scale prospective studies are warranted to confirm the present results.

Sensitivity analyses

Sensitivity analyses using a leave-one-out approach showed that the pooled estimates remained largely unchanged after sequential exclusion of individual studies (**Figure S2**). The associations with OS and PFS were consistently maintained for antibiotic exposure with minimal variation in effect size and statistical significance. A similar pattern was observed for ORR, indicating that the overall findings were not disproportionately influenced by any single dataset.

Sensitivity analyses likewise demonstrated stable effect directions across all evaluated outcomes for probiotic supplementation. Despite the limited number of studies, the pooled estimates remained broadly consistent, supporting the relative robustness of the observed associations.

Discussion

This meta-analysis synthesized currently available real-world evidence to evaluate the impact of microbiota-modulating exposures on immunotherapy outcomes in patients with NSCLC. The results showed that antibiotic exposure is consistently associated with poorer survival outcomes and is unfavorably associated with a reduction in the ORR. In contrast, probiotic supplementation demonstrated a favorable trend in treatment outcomes, although the current evidence base remains limited. Collectively, these observations underscore the emerging importance of host-related determinants, particularly gut microbiota composition, in shaping the durability of benefit from ICI.

The differential strength of the associations observed for survival outcomes and objective response suggests that early tumor regression and long-term immunotherapy benefit may be driven by partially distinct biological mechanisms. Initial tumor response is influenced by tumor-intrinsic characteristics,

including tumor immunogenicity and the baseline immune microenvironment, whereas durable clinical benefit depends on sustained activation and functional maintenance of antitumor immune responses. Antibiotic-associated disruption of gut microbial homeostasis may interfere with antigen presentation, impair dendritic cell maturation, and weaken memory T-cell responses, thereby contributing to both reduced treatment response and less durable immune-mediated tumor control[39].

Subgroup analyses indicated that the adverse impact of antibiotic exposure on immunotherapy outcomes was generally consistent across different lines of therapy and immune checkpoint inhibitor strategies with no significant differences observed between subgroups. This pattern suggests that the observed variability may partly reflect methodologic heterogeneity across studies rather than true biological differences. Because most included studies did not systematically report key antibiotic-related variables, such as antibiotic class, exposure timing, and indication for use, more refined stratified analyses could not be performed.

Current treatment recommendations for NSCLC are primarily based on disease stage, resectability, oncogenic driver status, and PD-L1 expression. Although ICIs have been incorporated into standard treatment algorithms across multiple clinical settings, current clinical guidelines do not provide explicit recommendations regarding antibiotic use or probiotic supplementation during immunotherapy, and microbiota-related factors are not included in routine treatment stratification.

NSCLC-specific evidence indicates that antibiotic exposure during immunotherapy is associated with clinically meaningful adverse outcomes. Peri-treatment antibiotic use has been linked to a significantly shorter PFS (pooled HR = 1.47, 95% CI = 1.13–1.90) and OS (pooled HR = 1.69, 95% CI = 1.25–2.29) in patients with advanced or metastatic NSCLC treated predominantly with ICI monotherapy, which corresponded to an absolute reduction of approximately 6–7 months in median survival. The negative association appears most pronounced when antibiotics are administered within the peri-immunotherapy window, supporting a potential interaction between early microbiota disruption and immune activation[40].

Similar trends have been reported in first-line chemo-immunotherapy (chemo-IO), which represents a current standard treatment paradigm for advanced NSCLC without actionable oncogenic drivers. Antibiotic exposure within 60 days before treatment initiation was associated with a lower ORR (27% *vs*. 40%), shorter PFS (3.9 *vs*. 5.9 months; HR = 1.35, 95% CI = 1.10–1.60), and reduced OS (10 *vs*. 15 months; HR = 1.50, 95% CI = 1.20–1.80) in a large multicenter cohort. Consistent findings have also been demonstrated in meta-analytic evaluations, demonstrating inferior survival in the overall IO-treated population (HR = 1.93, 95% CI = 1.52–2.45) and in patients receiving chemo-IO specifically (HR = 1.54, 95% CI = 1.28–1.84)[41].

These observations suggested that the adverse impact of antibiotic exposure may be particularly relevant in advanced-stage disease, in which durable immune-mediated tumor control is critical. From a clinical perspective, a more cautious and indication-driven approach to antibiotic use during the peri-ICI period may therefore be warranted. In contrast, although microbiota modulation through probiotic supplementation has been proposed as a potential strategy to enhance immunotherapy efficacy, current evidence remains insufficient to support routine implementation in unselected patients.

Several limitations to this meta-analysis should be acknowledged. First, most included studies were retrospective observational analyses and causal inference remains limited. Second, the overall number of eligible studies, particularly for probiotic interventions, was relatively small, which may have affected the statistical power and stability of pooled estimates. Although probiotic formulations were broadly comparable in the immunomodulatory profiles, methodologic variability in dosing strategies, treatment duration, and study design may still introduce residual confounding. Third, heterogeneity across studies and the lack of detailed stratified data precluded more refined analyses of specific microbiota-related intervention strategies.

In conclusion, antibiotic exposure is associated with inferior survival outcomes in patients with NSCLC receiving immunotherapy, whereas probiotic supplementation shows a potential but yet unconfirmed benefit. These findings highlight the emerging role of gut microbiota as a host-associated determinant of immunotherapy efficacy and support the need for well-designed prospective studies to define microbiome-informed therapeutic strategies.

Grant support

This work was funded by the National Natural Science Foundation of China (Grant Nos. 82030082, 82172676, 82373217, and 82403455), Natural Science Foundation of Shandong (Grant Nos. ZR2023ZD26, ZR2024QH527), Non-communicable Chronic Diseases-National Science and Technology Major Project, the Distinguished Young Scholars of Shandong Provincial Science Fund (Grant No. ZR2024JQ032), Shandong Province International Science and Technology Cooperation Project (Grant No. 2025KJHZ012), and the China Postdoctoral Science Foundation (Grant Nos. 2024M761869 and 2025T180617).

Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Dawei Chen, Qian Song, Jinming Yu.
Collected the data: Shuling Ma, Zijun Zhai, Shijie Shang, Xinyi Liang, Shan Yin.
Contributed data or analysis tools: Shuling Ma, Zijun Zhai, Rui Ding, Ran Zhang, Guomeng Sha.
Performed the analysis: Shuling Ma, Zijun Zhai, Haofeng Lin.
Wrote the paper: Shuling Ma, Zijun Zhai.

Data availability statement

The data generated in this study are available upon request from the corresponding author.

References

1. Mountzios G, Remon J, Hendriks LEL, García-Campelo R, Rolfo C, Van Schil P, et al. Immune-checkpoint inhibition for resectable non-small-cell lung cancer — opportunities and challenges. Nat Rev Clin Oncol. 2023; 20: 664-77.
2. Song Q, Li X, Li Q, Shang S, Ma S, Zhai Z, et al. Bifidobacterium animalis suppresses non-small cell lung cancer progression and modulates tumor immunity through indole-3-acetic acid. Cell Rep. 2025; 44: 116132.
3. Derosa L, Hellmann MD, Spaziano M, Halpenny D, Fidelle M, Rizvi H, 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. 2018; 29: 1437-44.
4. Huemer F, Rinnerthaler G, Westphal T, Hackl H, Hutarew G, Gampenrieder SP, et al. Impact of antibiotic treatment on immune-checkpoint blockade efficacy in advanced non-squamous non-small cell lung cancer. Oncotarget. 2018; 9: 16512.
5. Zhao S, Gao G, Li W, Li X, Zhao C, Jiang T, et al. Antibiotics are associated with attenuated efficacy of anti-PD-1/PD-L1 therapies in Chinese patients with advanced non-small cell lung cancer. Lung Cancer. 2019; 130: 10-7.
6. Barrón F, Arrieta OG, Cardona A, Ruiz-Patiño A, Barrón Z, Rojas L. Relevance of antibiotic use on clinical activity of immune checkpoint inhibitors in Hispanic patients with advanced NSCLC (CLICAP-ABs). Technol Cancer Res Treat. 2019; 4.
7. Bagley SJ, Dhopeshwarkar N, Narayan V, Meropol NJ, Mamtani R, Boursi B. Impact of antibiotics (ABX) on overall survival (OS) in patients (pts) with advanced non-small-cell lung cancer (aNSCLC) and melanoma (aMel) treated with first-line immune checkpoint inhibition (ICI). J Clin Oncol. 2019; 37: e20643.
8. Hakozaki T, Okuma Y, Omori M, Hosomi Y. Impact of prior antibiotic use on the efficacy of nivolumab for non-small cell lung cancer. Oncol Lett. 2019; 17: 2946-52.
9. Kim H, Lee JE, Hong SH, Lee MA, Kang JH, Kim IH. The effect of antibiotics on the clinical outcomes of patients with solid cancers undergoing immune checkpoint inhibitor treatment: a retrospective study. BMC Cancer. 2019; 19: 1100.
10. Pinato DJ, Howlett S, Ottaviani D, Urus H, Patel A, Mineo T, et al. Association of prior antibiotic treatment with survival and response to immune checkpoint inhibitor therapy in patients with cancer. JAMA Oncol. 2019; 5: 1774-8.
11. Ouaknine Krief J, Helly de Tauriers P, Dumenil C, Neveux N, Dumoulin J, Giraud V, et al. Role of antibiotic use, plasma citrulline and blood microbiome in advanced non-small cell lung cancer patients treated with nivolumab. J Immunother Cancer. 2019; 7: 176.
12. Mielgo Rubio X, Aguado C, Sereno M, Chara L, Cabezón L, Velastegui A, et al. Early antibiotic use affects the efficacy of first line immunotherapy in lung cancer patients but route of administration seems to be decisive. J Thorac Oncol. 2019; 14: S445.
13. Schett A, Rothschild SI, Curioni-Fontecedro A, Krähenbühl S, Früh M, Schmid S, et al. Predictive impact of antibiotics in patients with advanced non small-cell lung cancer receiving immune checkpoint inhibitors: antibiotics immune checkpoint inhibitors in advanced NSCLC. Cancer Chemother Pharmacol. 2020; 85: 121-31.
14. Chalabi M, Cardona A, Nagarkar DR, Dhawahir Scala A, Gandara DR, Rittmeyer A, et al. Efficacy of chemotherapy and atezolizumab in patients with non-small-cell lung cancer receiving antibiotics and proton pump inhibitors: pooled post hoc analyses of the OAK and POPLAR trials. Ann Oncol. 2020; 31: 525-31.
15. Cortellini A, Di Maio M, Nigro O, Leonetti A, Cortinovis DL, Aerts JG, et al. Differential influence of antibiotic therapy and other medications on oncological outcomes of patients with non-small cell lung cancer treated with first-line pembrolizumab versus cytotoxic chemotherapy. J Immunother Cancer. 2021; 9: e002421.
16. Hamada K, Yoshimura K, Hirasawa Y, Hosonuma M, Murayama M, Narikawa Y, et al. Antibiotic usage reduced overall survival by over 70% in non-small cell lung cancer patients on anti-PD-1 immunotherapy. Anticancer Res. 2021; 41: 4985-93.
17. Verschueren MV, van der Welle CMC, Tonn M, Schramel FMNH, Peters BJM, van de Garde EMW. The association between gut microbiome affecting concomitant medication and the effectiveness of immunotherapy in patients with stage IV NSCLC. Sci Rep. 2021; 11: 23331.
18. Ren ZG, Gao Y, Wei C, Li X, Liu S, Zhang J, et al. 968P Association between use of antibiotics (ATB) and clinical outcomes with tislelizumab (tisle) monotherapy. Ann Oncol. 2021; 32: S835.
19. Balado AC, Lores MT, Sánchez RÁ, Ferrán BB, Montero EL, Torre AM, et al. 4CPS-301 Association of antibiotics and proton pump inhibitors on clinical activity of firstline pembrolizumab for non-small cell lung cancer: 2 years of real world data. Eur J Hosp Pharm. 2021; 28: A65.
20. Castello A, Rossi S, Toschi L, Lopci E. Impact of antibiotic therapy and metabolic parameters in non-small cell lung cancer patients receiving checkpoint inhibitors. J Clin Med. 2021; 10: 1251.
21. Geum MJ, Kim C, Kang JE, Choi JH, Kim JS, Son ES, et al. Broad-spectrum antibiotic regimen affects survival in patients receiving

nivolumab for non-small cell lung cancer. Pharmaceuticals (Basel). 2021; 14: 445.
22. Ochi N, Ichihara E, Takigawa N, Harada D, Inoue K, Shibayama T, et al. The effects of antibiotics on the efficacy of immune checkpoint inhibitors in patients with non–small-cell lung cancer differ based on PD-L1 expression. Eur J Cancer. 2021; 149: 73-81.
23. Lu PH, Tsai TC, Chang JWC, Deng ST, Cheng CY. Association of prior fluoroquinolone treatment with survival outcomes of immune checkpoint inhibitors in Asia. J Clin Pharm Ther. 2021; 46: 408-14.
24. Nyein AF, Bari S, Hogue S, Zhao Y, Maller B, Sha S, et al. Effect of prior antibiotic or chemotherapy treatment on immunotherapy response in non-small cell lung cancer. BMC Cancer. 2022; 22: 101.
25. Qiu H, Ma QG, Chen XT, Wen X, Zhang N, Liu WM, et al. Different classes of antibiotics exhibit disparate negative impacts on the therapeutic efficacy of immune checkpoint inhibitors in advanced non-small cell lung cancer patients. Am J Cancer Res. 2022; 12: 3175-84.
26. Joshi I, Peravali M, Geng X, Rao S, Chen KY, Veytsman I, et al. Impact of baseline clinical biomarkers on treatment outcomes in patients with advanced NSCLC receiving first-line pembrolizumab-based therapy. Clin Lung Cancer. 2022; 23: 438-45.
27. Stokes WA, Behera M, Jiang R, Gutman D, Giuste F, Burns A, et al. Effect of antibiotic therapy on immunotherapy outcomes for non-small cell lung cancer: analysis from the Veterans Health Administration Database. J Clin Oncol. 2021; 39: 9017.
28. Alkan Şen G, Şentürk Öztaş N, Değerli E, Can G, Turna H, Özgüroğlu M. Effect of antibiotic treatment on immune checkpoint inhibitors efficacy in patients with advanced non-small cell lung cancer. Lung Cancer. 2023; 184: 107347.
29. Deng F, Du X, Zhang P, Xu J, Li Y, Yang Z. Impact of antibiotic on efficacy and adverse reactions of chemoimmunotherapy in non-small cell lung cancer patients: a retrospective cohort study. Thorac Cancer. 2024; 15: 2560-9.
30. Tamura K, Okuma Y, Nomura S, Fukuda A, Masuda K, Matsumoto Y, et al. Efficacy and safety of chemoimmunotherapy in advanced non-small cell lung cancer patients with antibiotics-induced dysbiosis: a propensity-matched real-world analysis. J Cancer Res Clin Oncol. 2024; 150: 216.
31. Luo WC, Mei SQ, Huang ZJ, Chen ZH, Zhang YC, Yang MY, et al. Correlation of distribution characteristics and dynamic changes of gut microbiota with the efficacy of immunotherapy in EGFR-mutated non-small cell lung cancer. J Transl Med. 2024; 22: 326.
32. Xie Y. A study on the impact of antibiotics on the efficacy and prognosis of advanced lung cancer patients undergoing immune checkpoint inhibitor therapy. Eur Respir J. 2024; 64: PA5129.
33. Rousseau A, Simon-Tillaux N, Michiels S, Derosa L, Laparra A, Planchard D, et al. Concomitant comedications and survival with first-line pembrolizumab in advanced non–small-cell lung cancer. JAMA Netw Open. 2025; 8: e2529225.
34. Ochi N, Ichihara E, Yokoyama T, Inoue K, Tamura T, Watanabe H, et al. Impact of antibiotic use on survival in patients with advanced non-small cell lung cancer treated with immune checkpoint inhibitor and chemotherapy. Int J Clin Oncol. 2025; 30: 2277-86.
35. Hu T, Li L, Cui J, Song X, Zhu H, Hou ZW, et al. Effects of antibiotics on immunotherapy in patients with metastatic nonsmall cell lung cancer. Eur J Cancer Prev. 2025; 34: 363-71.
36. Eng L, Sutradhar R, Niu Y, Liu N, Liu Y, Kaliwal Y, et al. Impact of antibiotic exposure before immune checkpoint inhibitor treatment on overall survival in older adults with cancer: a population-based study. J Clin Oncol. 2023; 41: 3122-34.
37. Metselaar-Albers M, Meijerman I, Engels F, Haanen J, Beijnen J, Lalmohamed A. No detrimental association between antibiotic use and immune checkpoint inhibitor therapy: an observational cohort study comparing patients with ICI-treated and TKI-treated melanoma and NSCLC. J Immunother Cancer. 2024; 12: e008269.
38. Nakatsukasa H, Takahashi M, Shibano M, Ishigami Y, Kawaguchi T, Nakamura Y, et al. Clinical impact of concomitant BIO-three use in advanced or recurrent non-small cell lung cancer treated with immune-checkpoint inhibitor. Int J Clin Oncol. 2024; 29: 1840-9.
39. Wang X, Fang Y, Liang W, Wong CC, Qin H, Gao Y, et al. Fusobacterium nucleatum facilitates anti-PD-1 therapy in microsatellite stable colorectal cancer. Cancer Cell. 2024; 42: 1729-46.e8.
40. Lurienne L, Cervesi J, Duhalde L, de Gunzburg J, Andremont A, Zalcman G, et al. NSCLC immunotherapy efficacy and antibiotic use: a systematic review and meta-analysis. J Thorac Oncol. 2020; 15: 1147-59.
41. Elkrief A, Méndez-Salazar EO, Maillou J, Vanderbilt CM, Gogia P, Desilets A, et al. Antibiotics are associated with worse outcomes in lung cancer patients treated with chemotherapy and immunotherapy. NPJ Precis Oncol. 2024; 8: 143.

Cite this article as: Ma S, Zhai Z, Ding R, Shang S, Liang X, Yin S, et al. Antibiotics and probiotics differentially shape immunotherapy outcomes in non-small cell lung cancer. Cancer Biol Med. 2026; 23: 703-716. doi: 10.20892/j.issn.2095-3941.2025.0844