Scilight

*Translational Insights*

https://www.sciltp.com/journals/ti

*Article*

Check for updates

# Association of Antibiotic Exposure with Survival Outcomes in Patients with Advanced KRAS-Mutated NSCLC Receiving Immune Checkpoint Inhibitors

Qianlin Huang [1,2,†], Weichi Luo [1,†], Yichen Zhang [1,†], Zhihong Chen [1], Qiuyi Zhang [3], Fen Wang [4], Jianhua Chang [3,*] and Qing Zhou [1,2,*]

[1] Guangdong Lung Cancer Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Southern Medical University, Guangzhou 510080, China
[2] Guangdong Cardiovascular Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, China
[3] Department of Medical Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital & Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Shenzhen 518116, China
[4] Department of Oncology, Peking University Shenzhen Hospital, Shenzhen 518036, China
* Correspondence: changjianhua@163.com (J.C.); gzzhouqing@126.com (Q.Z.)
† These authors contributed equally to this work.

**How To Cite:** Huang, Q.; Luo, W.; Zhang, Y.; et al. Association of Antibiotic Exposure with Survival Outcomes in Patients with Advanced KRAS-Mutated NSCLC Receiving Immune Checkpoint Inhibitors. *Translational Insights* **2026**, *1*(1), 16. https://doi.org/10.53941/ti.2026.100016

Received: 25 June 2026
Revised: 6 August 2026
Accepted: 10 August 2026
Published: 24 August 2026

**Abstract:** Background: Antibiotic exposure has been associated with reduced benefit from immune checkpoint inhibitors (ICIs) in patients with non-small cell lung cancer (NSCLC). However, evidence in patients with KRAS-mutated NSCLC remains limited. This study evaluated the associations of antibiotic and probiotic exposure with survival outcomes in patients with advanced KRAS-mutated NSCLC receiving ICIs. Methods: This multicenter retrospective study included patients with advanced KRAS-mutated NSCLC who received at least one dose of ICI-based therapy. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method and compared using the log-rank test. Univariable and multivariable Cox proportional hazards regression analyses were performed to evaluate factors associated with survival outcomes. Results: A total of 101 patients were included, of whom 23 were exposed to antibiotics and 18 received probiotics. All patients had documented KRAS mutations. Eight patients received KRAS-targeted inhibitors after ICI progression, and none received KRAS-targeted inhibitors before or during ICI treatment. The median PFS was 9.0 months in the antibiotic-exposed group and 14.4 months in the non-antibiotic group ($p = 0.041$). The median OS was 23.5 and 38.4 months, respectively ($p = 0.017$). After multivariable adjustment, antibiotic exposure remained associated with shorter PFS (hazard ratio [HR], 2.04; 95% confidence interval [CI], 1.08–3.84; $p = 0.029$) and OS (HR, 2.85; 95% CI, 1.30–6.22; $p = 0.009$). Probiotic exposure was not significantly associated with PFS or OS. Conclusions: Antibiotic exposure was associated with shorter PFS and OS in patients with advanced KRAS-mutated NSCLC receiving ICIs, whereas probiotic exposure was not significantly associated with survival outcomes.

**Keywords:** non-small cell lung cancer; immune checkpoint inhibitors; KRAS mutation; antibiotics; probiotics

## 1. Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide and continues to impose a substantial global health burden [1]. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer cases and represents the major histological subtype [2]. Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations are among the most common oncogenic alterations in NSCLC, particularly in lung adenocarcinoma, and define a clinically important molecular subgroup [3]. KRAS-mutated NSCLC is biologically and clinically heterogeneous. Compared with EGFR-mutated or ALK-rearranged NSCLC, KRAS-mutated tumors are more frequently associated with smoking exposure, higher tumor mutational burden, increased neoantigen load, and relatively higher programmed death-ligand 1 (PD-L1) expression [3]. These features may favor tumor immune recognition and responsiveness to immune checkpoint inhibitors (ICIs) [4]. Although ICIs targeting the programmed cell death protein 1 (PD-1)/PD-L1 axis have substantially changed the treatment landscape of advanced NSCLC [2], clinical outcomes remain heterogeneous among patients with KRAS-mutated disease [5]. This heterogeneity is influenced by tumor-related factors, including PD-L1 expression, specific KRAS variants, and co-occurring genomic alterations such as TP53, STK11, and KEAP1 mutations [6–8]. Individual KRAS variants may also exhibit distinct immunological phenotypes; for example, KRAS G12D has been associated with lower tumor mutational burden and a more immunosuppressive tumor microenvironment, although the predictive value of individual variants remains incompletely defined [9]. In addition to these tumor-intrinsic determinants, host-related factors, particularly the gut microbiome, may further modify the efficacy of ICIs [10–13].

The prevalence and subtype distribution of KRAS mutations vary according to ethnicity, histological subtype, and smoking background [14–16]. KRAS G12C is less frequent in East Asian than in Western lung adenocarcinoma populations [15,16]. Because individual KRAS variants may be associated with distinct immunological phenotypes and responses to ICIs, differences in subtype composition may limit the direct generalizability of findings across populations. Moreover, previous studies of antibiotic exposure and ICI outcomes have largely involved molecularly unselected NSCLC cohorts or mixed cancer populations. These considerations provide a rationale for specifically evaluating the association between antibiotic exposure and ICI outcomes in a Chinese cohort with KRAS-mutated NSCLC. Growing evidence indicates that the gut microbiome is associated with systemic antitumor immunity and the efficacy of ICIs [10–13]. Gut microbial diversity, specific commensal taxa, and microbial metabolites may influence antigen presentation, cytokine signaling, T-cell priming, and effector immune responses required for effective PD-1/PD-L1 blockade [10,12,13]. Antibiotics can disrupt gut microbial composition and function, potentially attenuating microbiome-mediated immune activation and reducing ICI benefit [10,12]. Clinical studies and meta-analyses have linked antibiotic exposure before or around ICI initiation to shorter progression-free survival (PFS) and overall survival (OS) in patients with advanced NSCLC [17–20]. Probiotics are often used with the intention of restoring microbial balance, but available observational studies have reported heterogeneous findings, and their clinical impact on ICI outcomes remains uncertain [21–23]. Most evidence has been derived from unselected NSCLC cohorts or mixed cancer populations [17–23], leaving the associations of antibiotic and probiotic exposure with ICI outcomes in KRAS-mutated NSCLC insufficiently characterized.

This study aimed to explore the associations of antibiotic and probiotic exposure with clinical outcomes in patients with advanced KRAS-mutated NSCLC receiving ICIs. We further evaluated whether these associations remained after adjustment for clinicopathological factors and whether they were consistent across clinically relevant subgroups.

## 2. Methods

### *2.1. Study Design and Patients*

This multicenter retrospective study enrolled patients from August 2018 to July 2022 at the Guangdong Provincial People's Hospital, Cancer Hospital Chinese Academy of Medical Sciences, Shenzhen Center, and Peking University Shenzhen Hospital. The inclusion criteria were: (1) histologically confirmed NSCLC; (2) unresectable locally advanced, recurrent, or metastatic disease; (3) confirmed KRAS mutation detected by molecular testing; (4) treatment with at least one dose of ICI-based therapy. Accordingly, all patients included in the final analytic cohort had documented KRAS-mutated NSCLC.

Ethical approval for this study was granted by the Ethics Committee at Guangdong Provincial People's Hospital, No.GDREC2016175H(R2). The research methodology adhered to the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all patients.

### *2.2. Clinical Data Collection and Outcome Assessment*

The diagnosis of NSCLC was confirmed by histological examination of tumor specimens obtained from biopsy or surgical samples. Disease stage was determined based on imaging findings, pathological evaluation, and clinical records according to the American Joint Committee on Cancer staging system. Patients were considered to have advanced disease according to the documented clinical stage at the time of ICI initiation. KRAS mutation status was identified through molecular testing of tumor tissue and/or plasma samples. PD-L1 expression was assessed using the tumor proportion score (TPS), when available.

Clinical data were retrospectively collected from electronic medical records. The extracted variables included age at ICI initiation, sex, smoking status, Eastern Cooperative Oncology Group performance status (ECOG PS), histological subtype, brain metastasis status, PD-L1 expression, ICI treatment regimen, antibiotic exposure, probiotic exposure, date of ICI initiation, date of disease progression, date of death, date of last follow-up, detailed KRAS variant information, and the use of KRAS-targeted inhibitors. Baseline variables were defined according to the information available before or at the initiation of ICI therapy. Age was dichotomized at 63 years according to the median age of the study population. Smoking status was categorized as never-smoker or ever-smoker. ECOG PS was categorized as ≤1 or >1. Histological subtype was classified as adenocarcinoma, squamous cell carcinoma, or other histological subtypes. Brain metastasis status was determined according to imaging findings and clinical documentation before or at ICI initiation. PD-L1 expression was categorized as <1%, 1–49%, or ≥50%, and unavailable results were recorded as missing data. Detailed KRAS variant-level information was extracted from the available molecular testing reports. Variants were categorized as G12C, G12V, G12D, other recorded KRAS variants, or specific subtype unavailable. "Specific subtype unavailable" indicated that a KRAS mutation was documented, but the exact variant could not be retrieved from the available molecular report. Medical records were also reviewed for the use of KRAS-targeted inhibitors before, during, or after ICI treatment.

ICI-based therapy was defined as any systemic treatment regimen containing at least one immune checkpoint inhibitor, administered either as monotherapy or in combination with other anticancer agents. Treatment regimens were categorized as ICI monotherapy or ICI-based combination therapy. Treatment strategies were determined by the treating physicians according to disease characteristics, molecular profile, previous treatment history, performance status, and patient preference.

The primary outcomes were PFS and OS. PFS was defined as the interval from the first administration of ICIs to radiological or clinical disease progression or death from any cause, whichever occurred first. OS was defined as the interval from the first administration of ICIs to death from any cause. Patients who were alive at the last follow-up were censored for OS, and patients without documented disease progression were censored for PFS at the last available disease assessment or follow-up record.

### *2.3. Definitions of Antibiotic and Probiotic Exposure*

Antibiotic exposure was defined as the use of systemic antibacterial agents within 60 days before or after ICI initiation. Patients who received antibiotics during this predefined exposure window were assigned to the antibiotic-exposed group, whereas those without antibiotic use during this period were assigned to the non-antibiotic group. Antibiotic exposure was identified from prescription records, medication orders, and clinical documentation. When available, additional information was collected, including antibiotic class, indication for antibiotic treatment, route of administration, timing relative to ICI initiation, and duration of treatment. This exposure window was selected to capture antibiotic use occurring around the start of immunotherapy, a clinically relevant period during which host immune activation and early response to ICI treatment may be particularly important.

The use of probiotics was defined as the period from 60 days before the first ICI administration to the entire duration of immunotherapy. Patients were categorized into probiotic-exposed and non-probiotic groups according to probiotic use during this predefined period. Information on probiotic type, timing, and duration was collected when available. Because probiotic use in routine clinical practice may vary in formulation, dosage, duration, and indication, probiotic exposure was analyzed as a binary clinical variable in the main analyses.

### *2.4. Statistical Analysis*

Baseline characteristics were summarized using descriptive statistics and compared according to antibiotic and probiotic exposure status. Continuous variables were presented as the mean with standard deviation or the median with interquartile range, as appropriate. Categorical variables were reported as counts and percentages. Between-group comparisons were performed using Student's *t* test or the Mann-Whitney *U* test for continuous variables and the chi-square test or Fisher's exact test for categorical variables, as appropriate. For categorical variables with more than two categories and sparse cell counts, the Fisher–Freeman–Halton exact test was used.

PFS and OS were estimated using the Kaplan-Meier method, and survival distributions were compared using the log-rank test. Univariable Cox proportional hazards regression analyses were performed to evaluate the associations of clinical factors, antibiotic exposure, and probiotic exposure with PFS and OS. Variables with a $p$ value < 0.10 in the univariable analyses were included in the multivariable Cox proportional hazards regression models. Hazard ratios (HRs) and 95% confidence intervals (CIs) were reported. Analyses involving variables with missing data were performed using complete cases only.

Exploratory subgroup analyses were performed to evaluate the association between antibiotic exposure and survival outcomes across clinically relevant subgroups defined by sex, age, smoking status, ECOG PS, histological subtype, brain metastasis status, PD-L1 expression, probiotic exposure status, and treatment regimen. Exploratory subgroup analyses were also conducted to assess the association between probiotic exposure and survival outcomes. Given the limited sample size in some subgroups, these analyses were considered exploratory.

All statistical tests were two-sided, and a $p$ value < 0.05 was considered statistically significant. Statistical analyses were performed using R software, version 4.4.1.

## 3. Results

### *3.1. Patient Characteristics*

A total of 101 patients with advanced KRAS-mutated NSCLC who received ICIs were included in this study. All 101 patients had documented KRAS mutations. Detailed variant-level information was available for 54 patients (53.5%), whereas the specific KRAS subtype was unavailable for 47 patients (46.5%). In the overall cohort, KRAS G12C was identified in 22 patients (21.8%), G12V in 14 patients (13.9%), and G12D in 7 patients (6.9%). The remaining 11 patients (10.9%) with available variant-level information had other recorded KRAS variants. Eight patients (7.9%) had documented use of a KRAS-targeted inhibitor during the recorded treatment course. All eight patients were in the non-antibiotic group, corresponding to 10.3% of patients without antibiotic exposure, whereas none of the patients in the antibiotic-exposed group received a KRAS-targeted inhibitor. Baseline characteristics of the overall cohort, stratified according to probiotic exposure status and antibiotic exposure status, are summarized in Table 1. Among the 101 patients, 23 patients were classified as antibiotic-exposed and 78 patients had no documented antibiotic exposure. In addition, 18 patients received probiotics, whereas 83 patients had no documented probiotic exposure. In the antibiotic analysis, most baseline characteristics were comparable between patients with and without antibiotic exposure, including sex, age, smoking status, ECOG performance status, brain metastasis, PD-L1 expression, and treatment regimen. However, histological subtype differed significantly between the two groups. Baseline characteristics were generally balanced between patients with and without probiotic exposure.

**Table 1.** Baseline characteristics of patients with advanced KRAS-mutated NSCLC.

| | **Probiotics** | | | **Antibiotics** | | |
|---|---|---|---|---|---|---|
| | **No (n = 83)** | **Yes (N = 18)** | ***p* Value** | **No (N = 78)** | **Yes (N = 23)** | ***p* Value** |
| Sex | | | | | | |
| Female | 9 (10.8%) | 1 (5.6%) | 0.686 | 8 (10.3%) | 2 (8.7%) | 1.000 |
| Male | 74 (89.2%) | 17 (94.4%) | | 70 (89.7%) | 21 (91.3%) | |
| Age (year) | | | | | | |
| Mean (SD) | 62.0 (8.13) | 61.6 (7.40) | 0.861 | 61.9 (8.18) | 62.1 (7.38) | 0.905 |
| Smoking | | | | | | |
| No | 20 (24.1%) | 4 (22.2%) | 1.000 | 19 (24.4%) | 5 (21.7%) | 0.480 |
| Yes | 63 (75.9%) | 14 (77.8%) | | 59 (75.6%) | 18 (78.3%) | |
| ECOG PS | | | | | | |
| ＞1 | 1 (1.2%) | 1 (5.6%) | 0.326 | 0 (0.0%) | 2 (8.7%) | 0.050 |
| ≤1 | 82 (98.8%) | 17 (94.4%) | | 78 (100.0%) | 21 (91.3%) | |
| Histology | | | | | | |
| Adeno | 75 (90.4%) | 18 (100.0%) | 0.769 | 73 (93.6%) | 20 (87.0%) | 0.007 |
| SCC | 3 (3.6%) | 0 (0.0%) | | 0 (0.0%) | 3 (13.0%) | |
| Other | 5 (6.0%) | 0 (0.0%) | | 5 (6.4%) | 0 (0%) | |
| Brain Metastases | | | | | | |
| No | 62 (74.7%) | 14 (77.8%) | 1.000 | 60 (76.9%) | 16 (69.6%) | 0.472 |
| Yes | 21 (25.3%) | 4 (22.2%) | | 18 (23.1%) | 7 (30.4%) | |

**Table 1.** *Cont.*

| | Probiotics | | | Antibiotics | | |
|---|---|---|---|---|---|---|
| | **No (n = 83)** | **Yes (N = 18)** | ***p* Value** | **No (N = 78)** | **Yes (N = 23)** | ***p* Value** |
| PD-L1 | | | | | | |
| <1% | 16 (19.3%) | 3 (16.7%) | 0.812 | 13 (16.7%) | 6 (26.1%) | 0.130 |
| 1–49% | 24 (28.9%) | 4 (22.2%) | | 25 (32.1%) | 3 (13.0%) | |
| ≥50% | 26 (31.3%) | 8 (44.4%) | | 23 (29.5%) | 11 (47.8%) | |
| NA | 17 (20.5%) | 3 (16.7%) | | 17 (21.8%) | 3 (13.0%) | |
| Treatment Regimen | | | | | | |
| ICI monotherapy | 14 (16.9%) | 4 (22.2%) | 0.734 | 15 (19.2%) | 3 (13.0%) | 0.757 |
| ICI-based combination therapy | 69 (83.1%) | 14 (77.8%) | | 63 (80.8%) | 20 (87.0%) | |
| KRAS mutation subtype | | | | | | |
| G12C | 20 (24.1%) | 2 (11.1%) | 0.436 | 17 (21.8%) | 5 (21.7%) | 0.163 |
| G12V | 13 (15.7%) | 1 (5.6%) | | 13 (16.7%) | 1 (4.3%) | |
| G12D | 5 (6.0%) | 2 (11.1%) | | 3 (3.8%) | 4 (17.4%) | |
| Other or rare variants | 9 (10.8%) | 2 (11.1%) | | 8 (10.3%) | 3 (13.0%) | |
| Subtype unavailable | 36 (43.4%) | 11 (61.1%) | | 37 (47.4%) | 10 (43.5%) | |

Adeno, adenocarcinoma; ECOG PS, Eastern Cooperative Oncology Group performance status; ICI, immune checkpoint inhibitor; NA, not available; NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand 1; SCC, squamous cell carcinoma; SD, standard deviation; Other or rare KRAS variants comprised G12A, G12S, G13C, G13D, Q61H, D154N, and cases with multiple recorded KRAS variants; "Subtype unavailable" indicates that a KRAS mutation was documented, but detailed variant-level information was unavailable.

### *3.2. Association of Antibiotic and Probiotic Exposure with Survival Outcomes*

Kaplan–Meier analysis showed that antibiotic exposure was associated with shorter PFS and OS in patients with advanced KRAS-mutated NSCLC receiving ICIs. The median PFS was 9.0 months in the antibiotic group and 14.4 months in the non-antibiotic-exposed group (log-rank $p = 0.041$; Figure 1A). The median OS was 23.5 months in the antibiotic-exposed group and 38.4 months in the non-antibiotic group (log-rank $p = 0.017$; Figure 1B). Probiotic exposure was not significantly associated with either PFS or OS (Figure 2A,B).

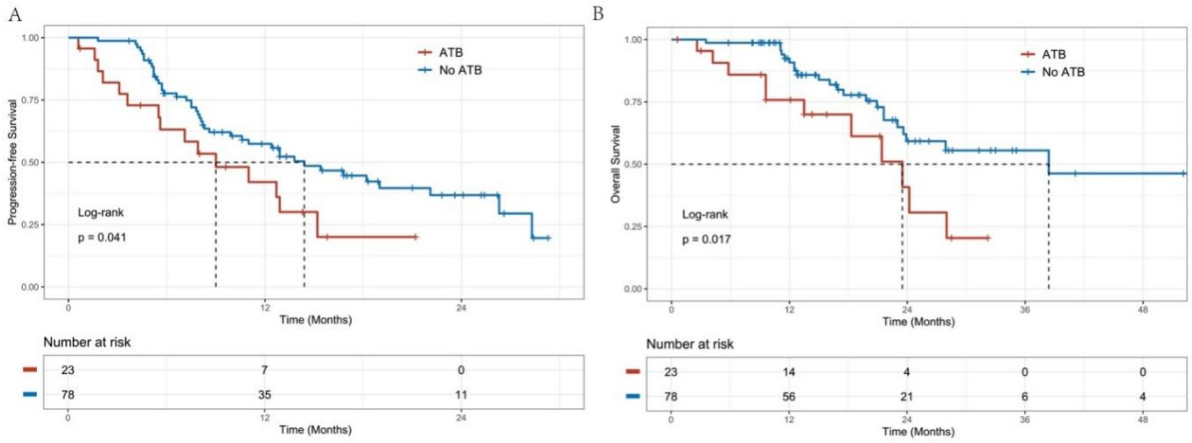


**Figure 1.** Kaplan-Meier survival curves illustrating the association of antibiotic use with PFS (**A**) and OS (**B**) in advanced KRAS-mutated NSCLC receiving ICIs. ATB, antibiotic exposure; OS, overall survival; PFS, progression-free survival; NSCLC, non-small cell lung cancer; ICIs, immune checkpoint inhibitors.

Univariable and multivariable Cox proportional hazards regression analyses of factors associated with PFS and OS are presented in Table 2. In the univariable analysis for PFS, antibiotic exposure was associated with an increased risk of disease progression or death (HR = 1.85; 95% CI: 1.02–3.37; $p = 0.044$), and this association remained statistically significant after multivariable adjustment (HR = 2.04; 95% CI: 1.08–3.84; $p = 0.029$). Histology subtype showed a marginal association with PFS in the univariable analysis but was not significantly associated with PFS after multivariable adjustment. For OS, antibiotic exposure was associated with an increased risk of death in univariable analysis (HR = 2.37; 95% CI: 1.14–4.91; $p = 0.021$) and remained statistically significant after multivariable adjustment (HR = 2.85; 95% CI: 1.30–6.22; $p = 0.009$). Ever-smoking status was

associated with a lower risk of death in the univariable analysis but not after multivariable adjustment. PD-L1 expression ≥ 50% showed a nonsignificant trend toward longer OS in the univariable analysis but was not independently associated with OS in the adjusted model. Probiotic exposure was not significantly associated with PFS (HR = 0.89; 95% CI: 0.45–1.77; $p = 0.741$) or OS (HR = 1.40; 95% CI: 0.65–3.03; $p = 0.387$). These findings indicate that antibiotic exposure, but not probiotic exposure, remained independently associated with shorter PFS and OS after multivariable adjustment.

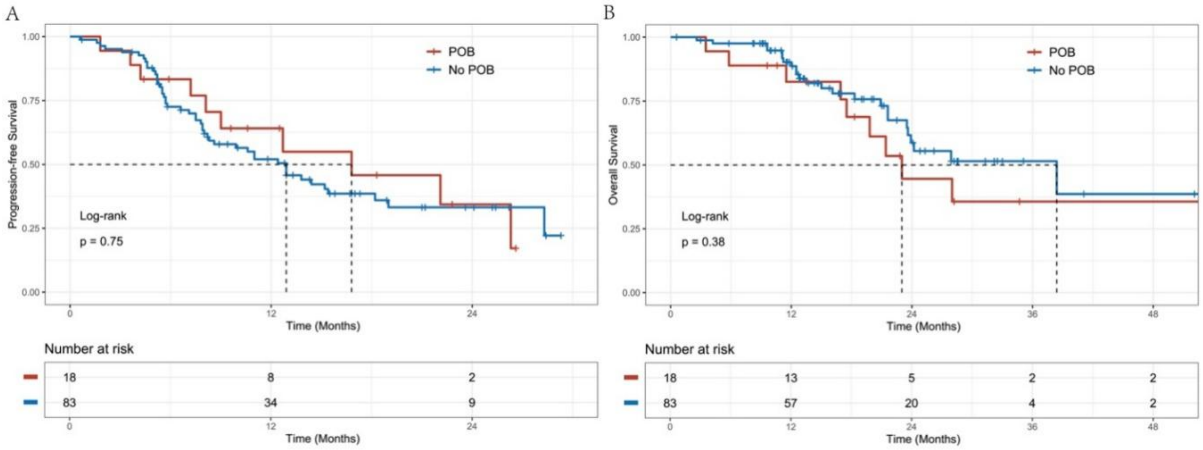


**Figure 2.** Kaplan-Meier survival curves illustrating the association of probiotic use with PFS (**A**) and OS (**B**) in advanced KRAS-mutated NSCLC receiving ICIs. POB, probiotic exposure; OS, overall survival; PFS, progression-free survival; NSCLC, non-small cell lung cancer; ICIs, immune checkpoint inhibitors.

**Table 2.** Univariable and multivariable Cox proportional hazards regression analyses of factors associated with PFS and OS.

| Factors | PFS | | | | OS | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariable Analysis | | Multivariable Analysis | | Univariable Analysis | | Multivariable Analysis | |
| | HR (95% CI) | *p* Value | HR (95% CI) | *p* Value | HR (95% CI) | *p* Value | HR (95% CI) | *p* Value |
| Sex: Male | 0.52 (0.24–1.16) | 0.109 | - | - | 0.76 (0.27–2.17) | 0.606 | - | - |
| Age ≤ 63 years | 1.15 (0.68–1.94) | 0.593 | - | - | 0.98 (0.49–1.95) | 0.958 | - | - |
| Ever-smoker | 0.64 (0.35–1.16) | 0.138 | - | - | 0.46 (0.22–0.96) | 0.038 | 0.52 (0.24–1.13) | 0.098 |
| ECOG PS > 1 | 1.50 (0.21–10.93) | 0.687 | - | - | 2.66 (0.36–19.79) | 0.340 | - | - |
| Brain metastasis | 1.31 (0.73–2.37) | 0.368 | - | - | 0.97 (0.42–2.25) | 0.950 | - | - |
| Histology: SCC vs. Adeno | 1.06 (0.26–4.37) | 0.072 | 0.60 (0.14–2.68) | 0.508 | 0.77 (0.11–5.68) | 0.801 | - | - |
| PD-L1 expression ≥ 50% | 0.54 (0.26–1.14) | 0.105 | - | - | 0.36 (0.13–1.04) | 0.058 | 0.41 (0.14–1.22) | 0.111 |
| PD-L1 expression 1–49% | 1.12 (0.55–2.28) | 0.754 | - | - | 1.02 (0.40–2.61) | 0.960 | 1.56 (0.56–4.33) | 0.392 |
| Probiotic exposure | 0.89 (0.45–1.77) | 0.741 | - | - | 1.40 (0.65–3.03) | 0.387 | - | - |
| Antibiotic exposure | 1.85 (1.02–3.37) | 0.044 | 2.04 (1.08–3.84) | 0.029 | 2.37 (1.14–4.91) | 0.021 | 2.85 (1.30–6.22) | 0.009 |
| ICI-based combination therapy | 1.09 (0.57–2.08) | 0.796 | - | - | 1.40 (0.60–3.29) | 0.437 | - | - |

CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; ICI, immune checkpoint inhibitor; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; SCC, squamous cell carcinoma.

### *3.3. Exploratory Subgroup Analyses*

Exploratory subgroup analyses were performed to evaluate the association between antibiotic exposure and survival outcomes across clinically relevant subgroups (Figure 3). In the overall cohort, antibiotic exposure was associated with an increased risk of disease progression or death for PFS (HR = 1.85; 95% CI: 1.02–3.37; $p$ = 0.044 and an increased risk of death for OS (HR = 2.37; 95% CI: 1.14–4.91; $p$ = 0.021), consistent with the primary analyses. For PFS, nominally significant associations were observed among patients older than 63 years, ever-smokers, patients with ECOG PS ≤ 1, patients with adenocarcinoma, and patients with PD-L1 expression < 1% (Figure 3A). Directionally unfavorable estimates were also observed among male patients, in both brain metastasis strata, and in both treatment regimen strata, although none of these estimates reached nominal statistical significance. Estimates for patients with ECOG PS > 1 and non-adenocarcinoma histology could not be reliably calculated because of the small numbers of patients and events. For OS, nominally significant associations were observed among patients older than 63 years, ever-smokers, patients with ECOG PS ≤ 1, patients with adenocarcinoma, patients with brain metastases, patients with PD-L1 expression < 1%, patients without probiotic exposure, and patients receiving ICI-based combination therapy (Figure 3B). The estimate among male patients was directionally unfavorable but narrowly missed statistical significance ($p$ = 0.051), whereas the estimate among female patients was imprecise because of the small sample size and wide confidence interval. Several subgroup estimates, particularly that for patients with brain metastases, were accompanied by wide confidence intervals and should therefore be interpreted cautiously.

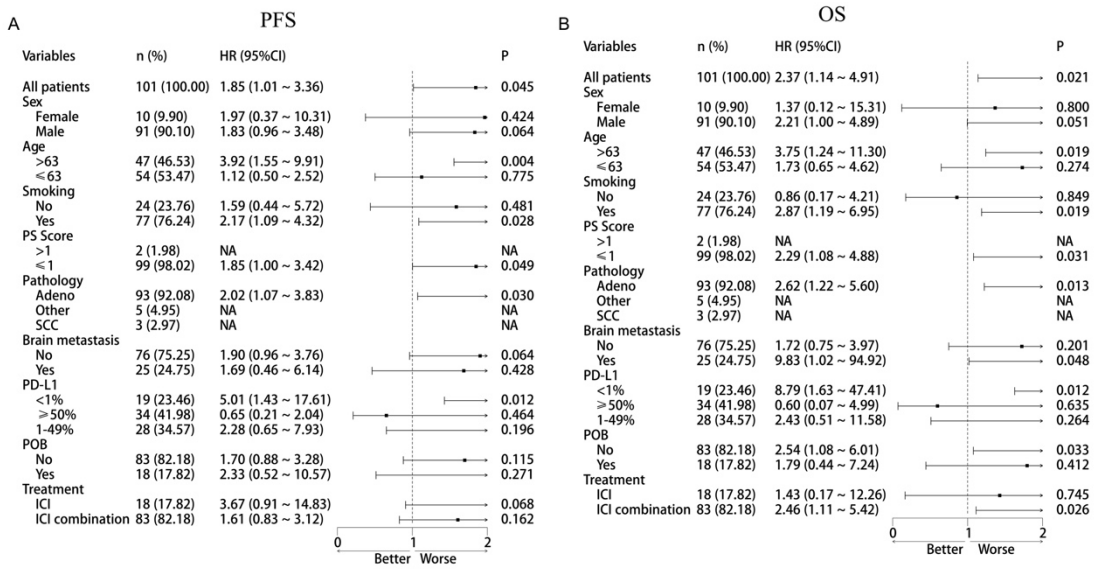


**Figure 3.** Exploratory subgroup analyses of the associations of antibiotic exposure with PFS (**A**) and OS (**B**) in advanced KRAS-mutated NSCLC receiving ICIs. Hazard ratios (HRs) greater than 1 indicate a higher risk of disease progression or death among patients with antibiotic exposure relative to patients without antibiotic exposure. Horizontal lines represent 95% CIs. CI, confidence interval; HR, hazard ratio; ICI, immune checkpoint inhibitor; NA, not available; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; POB, probiotic exposure; SCC, squamous cell carcinoma.

Exploratory subgroup analyses were also performed to evaluate the association between probiotic exposure and survival outcomes. Overall, probiotic exposure showed no consistent association with either PFS or OS across most clinical subgroups. Although a nominally significant association was observed in the ICI monotherapy subgroup for OS, this result should be interpreted cautiously because of the small sample size and wide confidence interval (Supplementary Figure S1). These exploratory findings should be interpreted cautiously and require validation in larger independent cohorts.

## 4. Discussion

In this study, antibiotic exposure within 60 days before or after ICI initiation was associated with shorter PFS and OS in patients with advanced KRAS-mutated NSCLC receiving ICIs. These associations remained statistically significant after multivariable adjustment. Probiotic exposure was not significantly associated with either survival outcome. Exploratory subgroup analyses showed unfavorable associations in several clinically relevant subgroups.

These findings suggest that antibiotic exposure may represent a clinically relevant factor associated with reduced ICI benefit in this molecularly defined population.

Our findings align with previous evidence linking antibiotic exposure to reduced benefit from ICIs in advanced NSCLC. Zhao et al. reported that antibiotic use within one month before or after anti-PD-1/PD-L1 therapy was associated with significantly shorter PFS and OS in a Chinese NSCLC cohort [17]. Derosa et al. similarly found that antibiotic exposure around ICI initiation was associated with inferior clinical outcomes in patients with advanced NSCLC [18]. A systematic review and meta-analysis subsequently confirmed that antibiotic-treated patients with NSCLC receiving immunotherapy had significantly poorer survival outcomes [19]. Consistent findings have also been reported in pooled analyses of clinical-trial populations and in real-world cohorts receiving chemoimmunotherapy [20,24]. Beyond NSCLC, Pinato et al. reported a negative association between prior antibiotic treatment and ICI outcomes across multiple tumor types, suggesting that the potential impact of antibiotics may not be restricted to a single cancer type [25]. Hamada et al. also observed lower response rates and shorter PFS and OS among antibiotic-exposed patients with NSCLC [26]. These studies collectively support the hypothesis that antibiotic exposure near the initiation of immunotherapy may compromise the clinical benefit of ICIs. Nevertheless, not all studies have reported statistically significant associations; Nyein et al. observed numerically worse outcomes among antibiotic-exposed patients, but the differences were not statistically significant [27]. Ochi et al. suggested that the impact of antibiotics may vary according to PD-L1 expression [28].

These inconsistencies may be explained by differences in study population, cancer type, treatment regimen, timing of antibiotic exposure, antibiotic class, duration of treatment, indication for antibiotic use, and adjustment for confounding variables. In this context, our study adds to the existing literature by focusing specifically on advanced KRAS-mutated NSCLC, a clinically and biologically distinct subgroup in which antibiotic-related effects remain insufficiently characterized. The persistence of the association after multivariable adjustment suggests that antibiotic exposure may be relevant even in this molecularly defined population, although causality cannot be inferred from the present retrospective data.

The magnitude of the association between antibiotic exposure and OS was greater than that observed for PFS. A similar pattern was observed in pooled analyses of the OAK and POPLAR trials, in which antibiotic exposure was associated with shorter OS but not PFS among patients receiving atezolizumab [20]. Several nonexclusive explanations may account for this pattern. Antibiotic-associated disruption of the gut microbiome may exert sustained effects on systemic immune homeostasis beyond the initial period of radiographic disease control [10,12,13]. However, OS is influenced by a broader range of factors than PFS, including baseline clinical vulnerability, pulmonary function, the extent of pulmonary involvement, lactate dehydrogenase (LDH) levels, infection severity, hospitalization, and subsequent systemic treatment. Antibiotic exposure may therefore partly serve as a marker of underlying infection or poorer general health, resulting in confounding by indication [29]. Although the multivariable models adjusted for the available clinicopathological variables, these additional factors were not consistently available or standardized in the retrospective dataset and could not be comprehensively incorporated. Accordingly, the stronger association with OS should not be interpreted as evidence of a direct causal effect of antibiotics.

Eight patients (7.9%) received a KRAS-targeted inhibitor after progression on ICI-based therapy, all of whom were in the non-antibiotic group. Because KRAS-targeted therapy was initiated only after ICI progression, it could not have affected PFS as defined in this study. However, subsequent KRAS inhibitor use may have influenced post-progression survival and, consequently, OS. Given the small number of KRAS inhibitor-treated patients and the absence of such patients in the antibiotic-exposed group, its contribution to the observed OS difference could not be reliably evaluated. Therefore, the association between antibiotic exposure and shorter PFS is unlikely to be explained by subsequent KRAS inhibitor use, whereas the OS association should be interpreted cautiously because of potential residual confounding from post-progression treatment.

The present findings should be interpreted within the substantial molecular heterogeneity of KRAS-mutated NSCLC. PD-L1 expression, individual KRAS variants, and co-occurring genomic alterations involving TP53, STK11, or KEAP1 may influence the tumor immune microenvironment and the clinical efficacy of ICIs [5–9]. In the present study, PD-L1 expression was evaluated in baseline comparisons, Cox regression models, and exploratory subgroup analyses. However, detailed KRAS variant-level information was unavailable for 47 patients, and complete co-mutation data were not uniformly available. Antibiotic exposure should therefore be interpreted as a potential host-related modifier alongside these established tumor-related determinants.

Previous research on gut microbiota in KRAS-mutated NSCLC has been limited. Nevertheless, studies in NSCLC and other solid tumors support a biologically plausible link between gut microbiota, systemic antitumor immunity, and responsiveness to immune checkpoint blockade [30–33]. ICIs depend on the presence of a functional host immune system capable of antigen recognition, T-cell priming, effector-cell expansion, and tumor infiltration. The gut microbiota may influence these processes through multiple interconnected mechanisms,

including modulation of innate immune activation, regulation of antigen-presenting cells, production of immunologically active metabolites, and maintenance of systemic inflammatory tone. Therefore, disruption of the gut microbial ecosystem during the critical period around ICI initiation could plausibly weaken the immune activation required for effective PD-1/PD-L1 blockade. Mechanistically, antibiotics may disrupt the gut ecosystem by reducing microbial diversity, depleting beneficial commensal bacteria, and altering microbial metabolic functions [10,12,34]. Such dysbiosis may impair dendritic-cell activation, antigen presentation, cytokine signaling, CD8+ T-cell priming, and effector T-cell trafficking, thereby attenuating the immune activation required for effective PD-1/PD-L1 blockade [10,12]. Favorable ICI responses have been associated with specific bacterial taxa, including *Akkermansia*, *Bifidobacterium* [13,35].

Importantly, the relationship between the gut microbiome and ICI efficacy cannot be attributed solely to antibiotic exposure. Derosa et al. reported that the presence of fecal Akkermansia muciniphila at baseline was associated with increased objective response rates and longer OS independently of PD-L1 expression, antibiotic exposure, and performance status [13]. These findings suggest that baseline microbial composition may influence ICI outcomes independently of antibiotic use and that antibiotic exposure cannot be regarded as a direct surrogate for a favorable or unfavorable microbiome. Because stool samples were not available in our study, we could not determine whether the observed associations were mediated by Akkermansia muciniphila or other specific microbial taxa. Cross-cohort analyses have also shown that microbiome signatures are clinically relevant but may vary across populations and geographic settings [36,37]. Therefore, antibiotic-related dysbiosis may act as a host-level factor that perturbs systemic antitumor immunity and may help explain the shorter PFS and OS observed in antibiotic-exposed patients in our KRAS-mutated NSCLC cohort. However, because the present study did not include microbiome sequencing, metabolomic profiling, or immune microenvironment analyses, this mechanistic interpretation remains hypothesis-generating and requires validation in prospective studies integrating antibiotic exposure data with gut microbiota composition, microbial metabolites, and immune profiling.

In contrast to antibiotic exposure, probiotic use was not significantly associated with PFS or OS in our cohort. This finding should be interpreted cautiously. Previous observational studies suggest that the effects of probiotics on ICI outcomes may depend on strain composition and concurrent antibiotic exposure [21–23]. The limited number of probiotic-exposed patients and the heterogeneity of probiotic use in routine practice, including variations in formulation, dosage, duration, timing, and adherence, may have reduced our ability to detect a clinically relevant association. Moreover, conventional probiotic supplementation may be insufficient to restore the complex microbial ecosystem required for favorable ICI responses. Therefore, our findings do not exclude a potential role for microbiome modulation but suggest that nonspecific probiotic use may have limited or inconsistent clinical effects in patients with advanced KRAS-mutated NSCLC receiving ICIs.

From a clinical perspective, our results support careful antibiotic stewardship in patients initiating ICI therapy. Antibiotics remain essential when clinically indicated, particularly in patients with infections or treatment-related complications. The present findings should not be interpreted as a recommendation to withhold necessary antimicrobial treatment. Rather, unnecessary antibiotic exposure should be avoided, and the spectrum and duration of treatment should be optimized whenever feasible.

This study has several limitations. First, its retrospective design limits causal inference, and residual confounding cannot be excluded. Second, the sample size was limited, particularly in the antibiotic- and probiotic-exposed groups. Third, detailed KRAS variant-level information was unavailable for 47 patients, and the small numbers within individual variant categories precluded reliable subtype-specific survival analyses. Fourth, stool samples, microbiome sequencing, and metabolomic profiling were unavailable, and the proposed microbiome-mediated mechanisms remain hypothesis-generating.

## 5. Conclusions

Antibiotic exposure around ICI initiation was associated with shorter PFS and OS in patients with advanced KRAS-mutated NSCLC receiving ICIs, whereas probiotic exposure was not significantly associated with survival outcomes. These findings do not establish a causal effect and require validation in larger prospective studies incorporating detailed molecular profiles, antibiotic indications, subsequent treatments, and longitudinal gut microbiome assessment.

## Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2608211545467352/TI-26060226-SI.pdf.

## Author Contributions

(I) Conception and design: Q.H., W.L., Y.Z. and Q.Z. (Qing Zhou). (II) Administrative support: Q.H., Z.C., F.W., Q.Z. (Qiuyi Zhang), J.C. and Q.Z. (Qing Zhou). (III) Provision of study materials or patients: Q.H., W.L. and Y.Z. (IV) Collection and assembly of data: Q.H., W.L., Y.Z., Z.C., F.W. and Q.Z. (Qing Zhou). (V) Data analysis and interpretation: Q.H. (VI) Manuscript writing: Q.H., W.L., Y.Z. and Q.Z. (Qing Zhou). (VII) All authors have read and agreed to the published version of the manuscript.

## Funding

This work was supported by Guangzhou Science and Technology Program (Grant No. 2025A03J4506 to Q.Z. (Qing Zhou)), the National Natural Science Foundation of China (Grant No. 82373349 to Q.Z. (Qing Zhou)), and MOE Changjiang Distinguished Professor Supporting Project (Grant No. KY0120240205 to Q.Z. (Qing Zhou)).

## Institutional Review Board Statement

The study was approved by the Ethics Committee at Guangdong Provincial People's Hospital, No.GDREC2016175H(R2).

## Informed Consent Statement

All patients were informed of the study and consented to the enrollment. Written informed consent was obtained from all patients.

## Data Availability Statement

All data relevant to this study are included in the article and its supplementary materials. Additional de-identified data are available from the corresponding author upon reasonable request.

## Acknowledgments

We would like to thank the patients, their families and the study personnel who participated in this study.

## Conflicts of Interest

The authors declare no conflict of interest.

## Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

## References

1. Bray, F.; Laversanne, M.; Sung, H.; et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin*. **2024**, *74*, 229–263. https://doi.org/10.3322/caac.21834.
2. Assi, H.I.; Kamphorst, A.O.; Moukalled, N.M.; et al. Immune checkpoint inhibitors in advanced non-small cell lung cancer. *Cancer* **2018**, *124*, 248–261. https://doi.org/10.1002/cncr.31105.
3. Adderley, H.; Blackhall, F.H.; Lindsay, C.R. KRAS-mutant non-small cell lung cancer: Converging small molecules and immune checkpoint inhibition. *EBioMedicine* **2019**, *41*, 711–716. https://doi.org/10.1016/j.ebiom.2019.02.049.
4. Mazieres, J.; Drilon, A.; Lusque, A.; et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: Results from the IMMUNOTARGET registry. *Ann. Oncol.* **2019**, *30*, 1321–1328. https://doi.org/10.1093/annonc/mdz167.
5. West, H.J.; McCleland, M.; Cappuzzo, F.; et al. Clinical efficacy of atezolizumab plus bevacizumab and chemotherapy in *KRAS*-mutated non-small cell lung cancer with *STK11*, *KEAP1,* or *TP53* comutations: Subgroup results from the phase III IMpower150 trial. *J. Immunother. Cancer* **2022**, *10*, e003027. https://doi.org/10.1136/jitc-2021-003027.
6. Skoulidis, F.; Byers, L.A.; Diao, L.; et al. Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. *Cancer Discov.* **2015**, *5*, 860–877. https://doi.org/10.1158/2159-8290.CD-14-1236.
7. Skoulidis, F.; Goldberg, M.E.; Greenawalt, D.M.; et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. *Cancer Discov*. **2018**, *8*, 822–835. https://doi.org/10.1158/2159-8290.CD-18-0099.

8. Ricciuti, B.; Arbour, K.C.; Lin, J.J.; et al. Diminished efficacy of programmed death-(ligand)1 inhibition in STK11- and KEAP1-mutant lung adenocarcinoma is affected by KRAS mutation status. *J. Thorac. Oncol.* **2022**, *17*, 399–410. https://doi.org/10.1016/j.jtho.2021.10.013.
9. Gao, G.; Liao, W.; Ma, Q.; et al. KRAS G12D mutation predicts lower TMB and drives immune suppression in lung adenocarcinoma. *Lung Cancer* **2020**, *149*, 41–45. https://doi.org/10.1016/j.lungcan.2020.09.004.
10. Routy, B.; Le Chatelier, E.; Derosa, L.; et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **2018**, *359*, 91–97. https://doi.org/10.1126/science.aan3706.
11. Hakozaki, T.; Richard, C.; Elkrief, A.; et al. The Gut Microbiome Associates with Immune Checkpoint Inhibition Outcomes in Patients with Advanced Non-Small Cell Lung Cancer. *Cancer Immunol. Res.* **2020**, *8*, 1243–1250. https://doi.org/10.1158/2326-6066.CIR-20-0196.
12. Finlay, B.B.; Goldszmid, R.; Honda, K.; et al. Can we harness the microbiota to enhance the efficacy of cancer immunotherapy? *Nat. Rev. Immunol*. **2020**, *20*, 522–528. https://doi.org/10.1038/s41577-020-0374-6.
13. Derosa, L.; Routy, B.; Thomas, A.M.; et al. Intestinal Akkermansia muciniphila predicts clinical response to PD-1 blockade in patients with advanced non-small-cell lung cancer. *Nat. Med*. **2022**, *28*, 315–324. https://doi.org/10.1038/s41591-021-01655-5.
14. Fathi, Z.; Mousavi, S.A.J.; Roudi, R.; et al. Distribution of KRAS, DDR2, and TP53 gene mutations in lung cancer: An analysis of Iranian patients. *PLoS ONE* **2018**, *13*, e0200633. https://doi.org/10.1371/journal.pone.0200633.
15. Dearden, S.; Stevens, J.; Wu, Y.L.; et al. Mutation incidence and coincidence in non-small-cell lung cancer: Meta-analyses by ethnicity and histology (mutMap). *Ann. Oncol*. **2013**, *24*, 2371–2376. https://doi.org/10.1093/annonc/mdt205.
16. Loong, H.H.F.; Du, N.; Cheng, C.; et al. KRAS G12C mutations in Asia: A landscape analysis of 11,951 Chinese tumor samples. *Transl. Lung Cancer Res*. **2020**, *9*, 1759–1769. https://doi.org/10.21037/tlcr-20-455.
17. Zhao, S.; Gao, G.; Li, W.; 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*, 10–17. https://doi.org/10.1016/j.lungcan.2019.01.017.
18. Derosa, L.; Hellmann, M.D.; Spaziano, M.; 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*, 1437–1444. https://doi.org/10.1093/annonc/mdy103.
19. Abdelhamid, A.; Tuminello, S.; Ivic-Pavlicic, T.; et al. Antibiotic treatment and survival in non-small cell lung cancer patients receiving immunotherapy: A systematic review and meta-analysis. *Transl. Lung Cancer Res*. **2023**, *12*, 2427–2439. https://doi.org/10.21037/tlcr-23-597.
20. Chalabi, M.; Cardona, A.; Nagarkar, D.R.; 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*, 525–531. https://doi.org/10.1016/j.annonc.2020.01.006.
21. Takada, K.; Shimokawa, M.; Takamori, S.; et al. Clinical impact of probiotics on the efficacy of anti-PD-1 monotherapy in patients with nonsmall cell lung cancer: A multicenter retrospective survival analysis study with inverse probability of treatment weighting. *Int. J. Cancer* **2021**, *149*, 473–482. https://doi.org/10.1002/ijc.33557.
22. Tomita, Y.; Ikeda, T.; Sakata, S.; et al. Association of Probiotic *Clostridium butyricum* Therapy with Survival and Response to Immune Checkpoint Blockade in Patients with Lung Cancer. *Cancer Immunol. Res*. **2020**, *8*, 1236–1242. https://doi.org/10.1158/2326-6066.CIR-20-0051.
23. Takada, K.; Buti, S.; Bersanelli, M.; et al. Antibiotic-dependent effect of probiotics in patients with non-small cell lung cancer treated with PD-1 checkpoint blockade. *Eur. J. Cancer* **2022**, *172*, 199–208. https://doi.org/10.1016/j.ejca.2022.06.002.
24. Taioli, E.; Flores, R.M.; Abdelhamid, A.; et al. Antibiotic use and survival in patients with late-stage NSCLC treated with chemoimmunotherapy. *JTO Clin. Res. Rep*. **2024**, *5*, 100710. https://doi.org/10.1016/j.jtocrr.2024.100710.
25. Pinato, D.J.; Howlett, S.; Ottaviani, D.; et al. Association of Prior Antibiotic Treatment with Survival and Response to Immune Checkpoint Inhibitor Therapy in Patients with Cancer. *JAMA Oncol*. **2019**, *5*, 1774–1778. https://doi.org/10.1001/jamaoncol.2019.2785.
26. Hamada, K.; Yoshimura, K.; Hirasawa, 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*, 4985–4993. https://doi.org/10.21873/anticanres.15312.
27. Nyein, A.F.; Bari, S.; Hogue, S.; et al. Effect of prior antibiotic or chemotherapy treatment on immunotherapy response in non-small cell lung cancer. *BMC Cancer* **2022**, *22*, 101. https://doi.org/10.1186/s12885-022-09210-2.
28. Ochi, N.; Ichihara, E.; Takigawa, N.; 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. https://doi.org/10.1016/j.ejca.2021.02.040.

29. Metselaar-Albers, M.; Meijerman, I.; Engels, F.; et al. 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. https://doi.org/10.1136/jitc-2023-008269.
30. Gopalakrishnan, V.; Spencer, C.N.; Nezi, L.; et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **2018**, *359*, 97–103. https://doi.org/10.1126/science.aan4236.
31. Matson, V.; Fessler, J.; Bao, R.; et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* **2018**, *359*, 104–108. https://doi.org/10.1126/science.aao3290.
32. Zhang, C.; Wang, J.; Sun, Z.; et al. Commensal microbiota contributes to predicting the response to immune checkpoint inhibitors in non-small-cell lung cancer patients. *Cancer Sci*. **2021**, *112*, 3005–3017. https://doi.org/10.1111/cas.14979.
33. Vétizou, M.; Pitt, J.M.; Daillère, R.; et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* **2015**, *350*, 1079–1084. https://doi.org/10.1126/science.aad1329.
34. Hersi, F.; Elgendy, S.M.; Al Shamma, S.A.; et al. Cancer immunotherapy resistance: The impact of microbiome-derived short-chain fatty acids and other emerging metabolites. *Life Sci*. **2022**, *300*, 120573. https://doi.org/10.1016/j.lfs.2022.120573.
35. Sivan, A.; Corrales, L.; Hubert, N.; et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* **2015**, *350*, 1084–1089. https://doi.org/10.1126/science.aac4255.
36. Lee, K.A.; Thomas, A.M.; Bolte, L.A.; et al. Cross-cohort gut microbiome associations with immune checkpoint inhibitor response in advanced melanoma. *Nat. Med*. **2022**, *28*, 535–544. https://doi.org/10.1038/s41591-022-01695-5.
37. McCulloch, J.A.; Davar, D.; Rodrigues, R.R.; et al. Intestinal microbiota signatures of clinical response and immune-related adverse events in melanoma patients treated with anti-PD-1. *Nat. Med*. **2022**, *28*, 545–556. https://doi.org/10.1038/s41591-022-01698-2.