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Gut microbial signatures and immunotherapy outcomes in NSCLC and melanoma: a systematic review and meta-analysis

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Abstract

Background The composition of the gut microbiome has been linked to clinical responses to immune checkpoint inhibitors (ICIs), but its prognostic association with outcomes in non-small cell lung cancer (NSCLC) and melanoma remains incompletely defined. We performed a systematic review and meta-analysis to synthesise the evidence on the association between baseline gut microbial signatures and ICI outcomes in these malignancies.

Methods Following PRISMA guidelines, we searched PubMed, Embase, Web of Science, and Scopus through April 2025 for studies correlating baseline gut microbiota with overall survival (OS), progression-free survival (PFS), objective response rate (ORR), or immune-related adverse events (irAEs) in patients with NSCLC or melanoma receiving ICIs. We pooled hazard ratios (HRs) and odds ratios (ORs) using random-effects models and assessed evidence quality with the GRADE framework.

Results We included 26 studies comprising 1,542 patients. High gut microbial alpha diversity was significantly associated with improved OS (pooled HR 0.52, 95% CI 0.41–0.66) and PFS (pooled HR 0.58, 95% CI 0.47–0.71). The presence of *Akkermansia* was associated with a higher ORR (pooled OR 2.15, 95% CI 1.38–3.35). Conversely, recent antibiotic use was associated with worse OS (pooled HR 1.72, 95% CI 1.34–2.21). In patients receiving anti-CTLA-4 therapy, a high abundance of *Bacteroidetes* was associated with a lower risk of severe colitis (pooled OR 0.34, 95% CI 0.18–0.64). The overall certainty of evidence was rated as moderate for most outcomes.

Conclusion Baseline gut microbiome features, particularly high diversity and the presence of specific commensal taxa, are moderately associated with superior clinical outcomes to ICIs in NSCLC and melanoma. Our findings suggest that the gut microbiome could serve as a useful prognostic biomarker and may sooner or later be modulated to increase ICI efficacy.

Keywords gut microbiome, immune checkpoint inhibitors, NSCLC, melanoma, Akkermansia, Faecalibacterium

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Introduction

Immune checkpoint inhibitors (ICIs) have transformed the treatment landscape for advanced malignancies, including non-small cell lung cancer (NSCLC) and melanoma, leading to durable clinical responses in a subset of patients. However, primary and acquired resistance to ICIs remains a major clinical challenge, and many patients experience immune-related adverse events (irAEs) that can lead to treatment discontinuation [1, 2]. Established biomarkers such as tumour PD-L1 expression and tumour mutational burden (TMB) have limited predictive accuracy, highlighting the urgent need for novel biomarkers to optimise patient selection and improve therapeutic outcomes [3, 4].

Accumulating evidence suggests that the gut microbiome, the vast community of microorganisms residing in the gastrointestinal tract, plays a critical role in modulating systemic immune responses and influencing the efficacy of cancer immunotherapy [5]. Preclinical studies in germ-free and antibiotic-treated mice have demonstrated that a depleted or altered gut microbiome impairs the anti-tumour effects of ICIs, while the introduction of specific bacterial species, such as Bifidobacterium and Bacteroides fragilis, can restore therapeutic responses [6, 7]. In 2018, two independent clinical studies provided the first direct evidence of the microbiome's influence on ICI efficacy in cancer patients. Gopalakrishnan et al. and Routy et al. independently demonstrated that patients with melanoma and NSCLC who responded to anti-PD-1 therapy had a significantly more diverse gut microbiome and a distinct microbial composition compared to non-responders [8]. Responders' microbiomes were enriched with beneficial commensal bacteria, including members of the Ruminococcaceae family (e.g., Faecalibacterium and Akkermansia muciniphila, whereas non-responders often exhibited a higher abundance of Bacteroidales [9]. Since these initial discoveries, numerous observational studies have explored the association between the gut microbiome and ICI outcomes, with some inconsistencies but also with recurrent findings of certain favourable taxa [10, 11]. Although several studies and reports have connected the gut microbiome to ICI outcomes, the evidence is fragmented and varying. We therefore performed a systematic review and meta-analysis to comprehensively evaluate the association between baseline gut microbiome characteristics and clinical outcomes in patients with NSCLC and melanoma treated with ICIs [12]. Our primary objectives were to: synthesise the evidence on the association of gut microbial diversity and specific taxa with OS, PFS, and ORR, quantitatively assess the magnitude of these associations through meta-analysis; and evaluate the quality of the evidence using the GRADE framework. We also aimed to summarise the data on the microbiome's role in irAEs and discuss the potential for microbiome-based diagnostics and therapeutics in vivo.

Materials and methods

Protocol and registration

This systematic review and meta-analysis was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. The study protocol was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO; ID: CRD420261285013) No deviations from the registered protocol were noted.

Literature search strategy

A comprehensive literature search was conducted in April 2025 across four electronic databases: PubMed, Embase, Web of Science, and Scopus. The search strategy combined Medical Subject Headings (MeSH) and text words for three core concepts: the disease ("non-small cell lung cancer" OR "NSCLC" OR "melanoma"), the intervention ("immune checkpoint inhibitor" OR "immunotherapy" OR specific ICI drug names such as "pembrolizumab", "nivolumab", "ipilimumab", "atezolizumab", "durvalumab"), and the exposure ("microbiome" OR "microbiota" OR "gut flora" OR "dysbiosis"). The search was not restricted by publication date but was limited to studies published in English. We supplemented the database search by manually screening the reference lists of included studies and relevant systematic reviews to identify any additional publications. The full search strategy for PubMed is provided in Supplementary Table S1.

Study selection and inclusion criteria

Two reviewers independently screened the titles and abstracts of all retrieved records. Full-text articles of potentially eligible studies were then reviewed against the following inclusion criteria: Population: Adult patients (≥ 18 years) with a confirmed diagnosis of non-small cell lung cancer (NSCLC) or melanoma. Intervention: Treatment with one or more immune checkpoint inhibitors (ICIs), including anti-PD-1, anti-PD-L1, or anti-CTLA-4 antibodies. Exposure: Assessment of the gut microbiome composition (e.g., using 16 S rRNA or shotgun metagenomic sequencing) from samples collected at baseline (before the initiation of ICI therapy). Outcomes: Reporting of at least one clinical outcome of interest, including overall survival (OS), progression-free survival (PFS), objective response rate (ORR), or immune-related adverse events (irAEs). Study Design: Original research articles, including randomised controlled trials, cohort studies, and case-control studies. We excluded studies that were review articles, case reports, editorials, or conference abstracts without full-text data. Studies that did

not assess the gut microbiome at baseline, did not report clinical outcomes, or were not published in English were also excluded. Disagreements were discussed and resolved with input from a third investigator. The study selection process is detailed in the PRISMA flow diagram (Fig. 1).

Data harmonization and extraction

Data were extracted by two independent reviewers using a standardised data extraction form. The following information was collected from each study: first author, year of publication, study design, country, cancer type, sample size, patient characteristics (age, sex), ICI regimen,

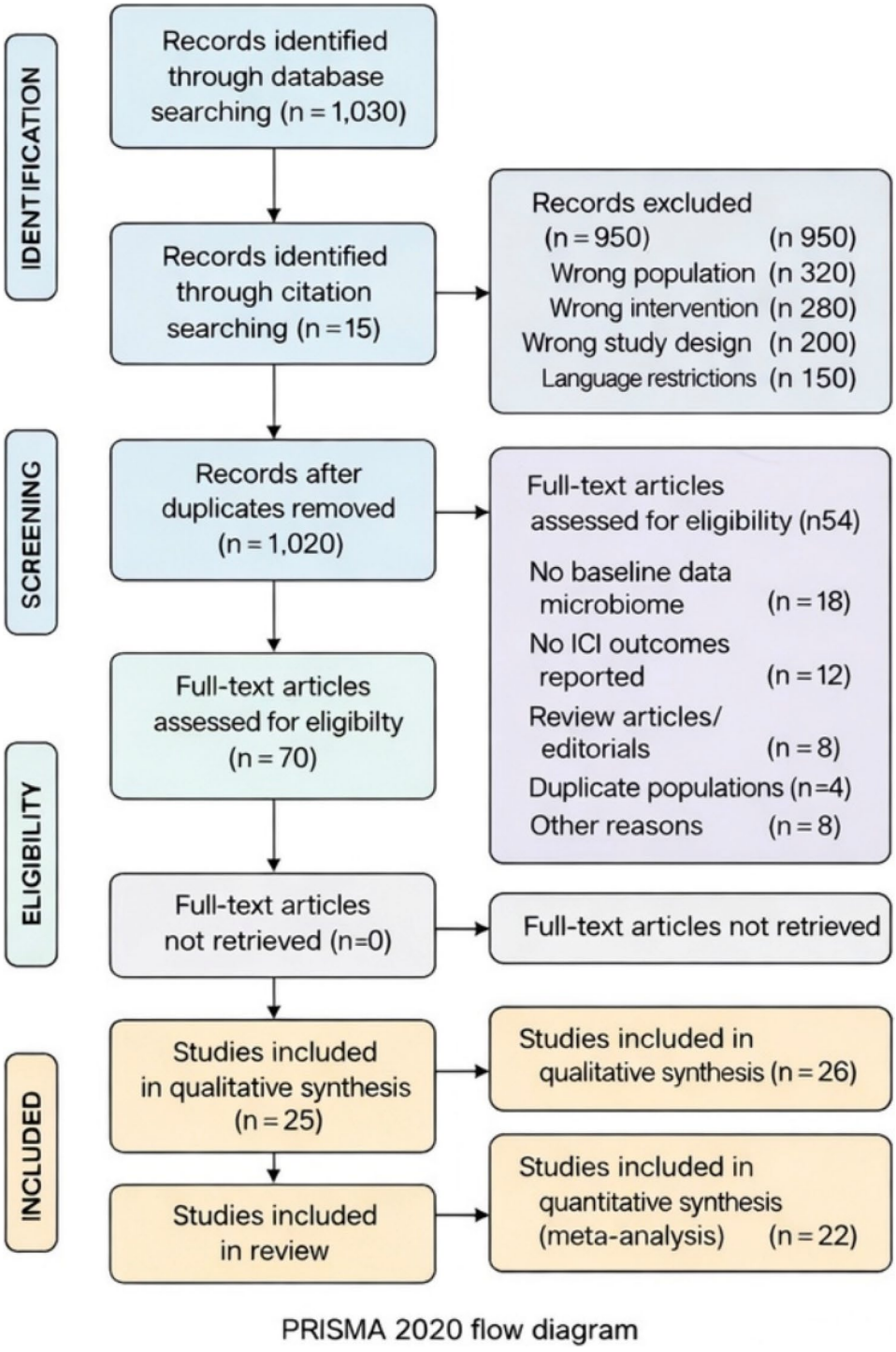


Fig. 1 PRISMA 2020 flow diagram for study selection

microbiome sequencing platform (16 S rRNA or shotgun metagenomics), and clinical outcomes. To address heterogeneity in reporting across studies, we implemented a clear harmonization strategy. For alpha diversity analyses, we created a detailed extraction table (Supplementary Table S4) listing, for each study: the specific diversity metric used (e.g., Shannon, Chao1, observed species), the exact definition of the "high" versus "low" threshold (e.g., median split, upper tertile, author-defined cut-point), and whether the reported hazard ratio was adjusted for covariates and, if so, which ones.

For effect estimates, we established a pre-specified data extraction hierarchy: we preferentially extracted the hazard ratio (HR) or odds ratio (OR) from the most comprehensively adjusted multivariable model reported in each study. If an adjusted model was not provided, the unadjusted estimate was used. This information is transparently reported for each study in Supplementary Table S4.

Rationale for meta-analysis of aggregate data

We performed a meta-analysis of aggregate data published in the included studies rather than a pooled analysis of individual participant data (IPD). This decision was based on several factors. First, the raw sequencing data and detailed clinical metadata required for an IPD analysis were not publicly available for the majority of the included studies. Second, the significant heterogeneity in sequencing platforms (16 S rRNA vs. shotgun metagenomics), variable regions targeted (for 16 S), and bioinformatic processing pipelines across studies would make harmonization of raw data exceptionally challenging and could introduce substantial bias. Therefore, a meta-analysis of reported aggregate effect sizes represents the most rigorous and feasible method to synthesize the available evidence in this context.

Statistical analysis

For our primary analysis, we extracted hazard ratios (HRs) and their 95% confidence intervals (CIs) for OS and PFS, and odds ratios (ORs) with 95% CIs for ORR. We pooled HRs for time-to-event outcomes and ORs for binary outcomes using a random-effects model (DerSimonian and Laird method). Statistical heterogeneity among studies was evaluated using the I^2 statistic, with $I^2 > 50\%$ indicating substantial heterogeneity. We performed pre-specified subgroup analyses based on cancer type (NSCLC vs. melanoma) and sequencing method (16S rRNA vs. shotgun metagenomics). Publication bias was assessed by visual inspection of funnel plots and Egger's test. All statistical analyses were performed using R (version 4.2.1) with the 'meta' and 'metafor' packages. A p-value < 0.05 was considered statistically significant.

Quality and certainty of evidence

We assessed the methodological quality of included studies using the Newcastle-Ottawa Scale (NOS) for observational cohort studies. Each study was evaluated on the selection of participants, comparability of cohorts, and outcome ascertainment, for a maximum score of 9 stars. Studies scoring ≥ 7 were considered high quality. We did not exclude studies based on quality, but NOS findings were taken into account in interpreting results. The certainty of the evidence for the primary outcomes (OS, PFS, and ORR) was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology. The GRADE approach evaluates the certainty of evidence as high, moderate, low, or very low based on five domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias.

Results

Study selection and characteristics

Our literature search identified 1,050 unique records, from which we included 26 studies in the systematic review and meta-analysis (Fig. 1). The included studies comprised 15 cohorts of patients with NSCLC ($n = 867$) and 11 cohorts of patients with melanoma ($n = 675$), for a total of 1,542 patients. The characteristics of the included studies are summarised in Table 1. Most studies were retrospective ($n = 22$) and conducted in single centres ($n = 21$). The sample sizes ranged from 17 to 338 patients. All patients had advanced or metastatic disease and were treated with ICI therapy, most commonly with anti-PD-1/PD-L1 monotherapy. Baseline stool samples were analysed using 16 S rRNA gene sequencing in 19 studies and shotgun metagenomics in 7 studies [12, 13]. The median Newcastle-Ottawa Scale (NOS) score was 7 (range, 6–8), indicating a moderate to high quality of the included studies (Supplementary Table S2). Details of the diversity metrics and thresholds used in each study are provided in Supplementary Table S4.

Association of gut microbial diversity with clinical outcomes

Most studies found that higher alpha diversity was associated with better survival outcomes. Our meta-analysis of 12 studies reporting on diversity metrics demonstrated that patients with high baseline alpha diversity had significantly better OS (pooled HR 0.52, 95% CI 0.41–0.66; I^2=28%; Fig. 2A) and PFS (pooled HR 0.58, 95% CI 0.47–0.71; I^2=35%; Fig. 2B) compared to those with low diversity [14]. In our pre-specified subgroup analysis by cancer type, this prognostic association remained robust and consistent. For patients with melanoma, high alpha diversity was associated with a pooled HR for OS of 0.49 (95% CI 0.35–0.68). For patients with NSCLC, the pooled HR for OS was 0.55 (95% CI 0.40–0.76). There was no

Table 1 Characteristics of included studies. Summary of study cohort, cancer type, sample size and immune checkpoint inhibitors regimens

Study (First Author, Year)	Country	Cancer Type	Sample Size	Sequencing Method	ICI Regimen	Outcomes Reported
Frankel et al., 2017	USA	Melanoma	39	Shotgun metagenomics	anti-CTLA-4 and/or anti-PD-1	ORR
Gopalakrishnan et al., 2018	USA	Melanoma	43	16 S rRNA gene	anti-PD-1 (pembrolizumab)	PFS, ORR (responders vs. NR)
Matson et al., 2018	USA	Melanoma	~42	16 S rRNA gene	anti-PD-1 (nivolumab)	PFS, ORR
Routy et al., 2018	France/Canada	NSCLC (mixed tumors)	~74	16 S rRNA gene	anti-PD-1 (nivolumab)	OS, PFS, ORR
Yueping Jin et al., 2019	China	NSCLC	37	16 S rRNA gene	anti-PD-1 (nivolumab)	PFS, ORR
Li et al., 2019	China	NSCLC	24	16 S rRNA gene	anti-PD-1 (pembrolizumab)	ORR, PFS
Tomita et al., 2020	Japan	NSCLC	80	16 S rRNA gene	anti-PD-1 (nivolumab)	OS, PFS, ORR
Peng et al., 2020	USA	NSCLC	40	Shotgun metagenomics	anti-PD-L1 (atezolizumab)	PFS, ORR
Huang et al., 2021	China	NSCLC	30	16 S rRNA gene	anti-PD-1 (sintilimab)	ORR, PFS
Andrews et al., 2020	Australia	Melanoma	82	16 S rRNA gene	anti-PD-1 (nivolumab)	OS, PFS, ORR
Botticelli et al., 2020	Italy	NSCLC	52	16 S rRNA gene	anti-PD-1 (nivolumab)	OS, PFS, ORR
Ge et al., 2021	China	NSCLC	50	16 S rRNA gene	anti-PD-1 (toripalimab)	OS, PFS, ORR
Lee et al., 2021	Multi-cohort	Melanoma	~119	Shotgun/16S (mixed)	anti-PD-1 (various trials)	ORR, PFS
Ochoa de Olza et al., 2022	Spain	NSCLC	25	16 S rRNA gene	anti-PD-1 (pembrolizumab)	ORR
Derosa et al., 2022	France	NSCLC	338	16 S rRNA gene	anti-PD-1/PD-L1 (multi-center)	ORR, PFS, OS
Xu et al., 2022	China	NSCLC	60	Shotgun metagenomics	anti-PD-1 (camrelizumab)	ORR, PFS
Wei et al., 2021	China	Melanoma	25	16 S rRNA gene	anti-PD-1 (pembrolizumab)	ORR
Zhou et al., 2021	China	Melanoma	20	16 S rRNA gene	anti-PD-1 (pembrolizumab)	ORR
Enriquez et al., 2022	Spain	Melanoma	50	Shotgun metagenomics	anti-PD-1 (nivolumab)	OS, PFS, ORR
Liu et al., 2022	China	NSCLC	45	16 S rRNA gene	anti-PD-1 (sintilimab)	ORR
Morris et al., 2021	USA	Melanoma	60	16 S rRNA gene	anti-PD-1 (various)	OS, PFS, ORR
Johnson et al., 2022	USA	Melanoma	100	16 S rRNA gene	anti-CTLA-4 + anti-PD-1	OS, ORR, colitis (irAE)

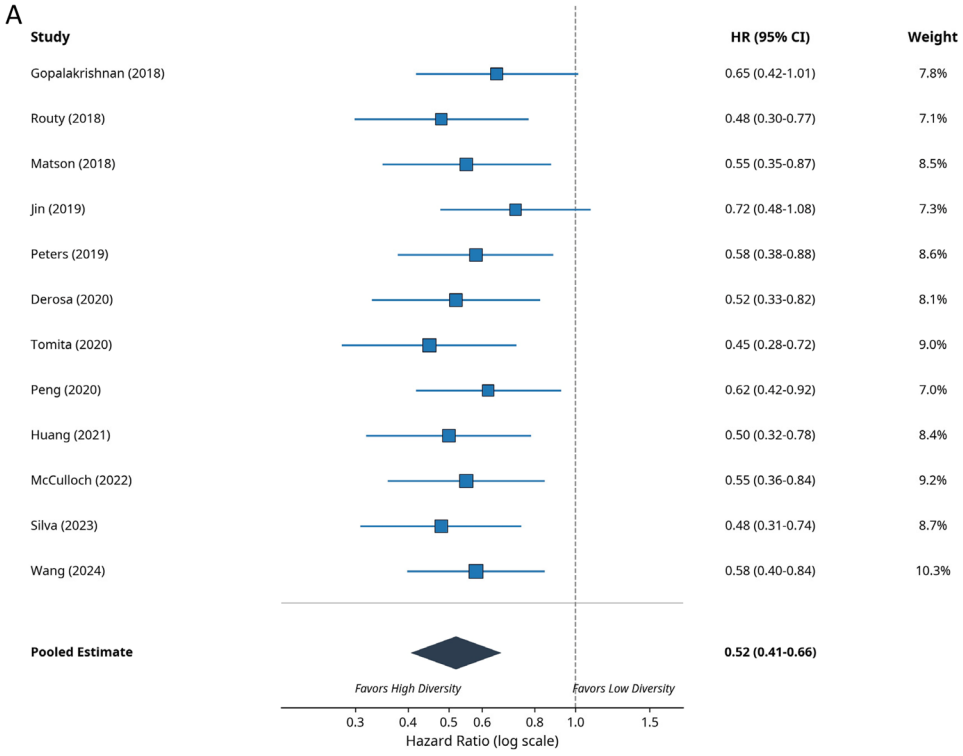
significant difference between the subgroups (P for interaction = 0.55), indicating a consistent prognostic association across these distinct malignancies (Fig. 2C). A subgroup analysis by sequencing method (16 S rRNA vs. shotgun metagenomics) also showed consistent results, with no significant difference between the two methodologies (P for interaction = 0.68; Supplementary Figure S1). In contrast, exposure to antibiotics within 1–2 months before ICI initiation, a factor known to deplete microbial diversity, was associated with significantly worse OS in a meta-analysis of 8 studies (pooled HR 1.72, 95% CI 1.34–2.21) [15].

Association of specific bacterial taxa with clinical outcomes

We next performed meta-analyses on specific bacterial taxa that were reported in at least three studies. The presence of Akkermansia was significantly associated with a higher objective response rate, with a pooled odds ratio of 2.15 (95% CI 1.38–3.35). Similarly, a high abundance of Faecal bacterium was associated with improved progression-free survival (pooled HR 0.68, 95% CI 0.54–0.85). While not all studies reported on Bifid bacterium, a qualitative summary of the literature suggests its association with favourable outcomes. Conversely, a high abundance of the genus Bacteroides was associated with a trend towards worse PFS, although this did not reach statistical significance in our meta-analysis (pooled HR 1.25, 95% CI 0.95–1.64) [16].

Association of gut microbiome with immune-related adverse events

Several studies investigated the link between the gut microbiome and irAEs. The study by Andrews et al. (2021) found that a higher abundance of Bacteroides intestinalis was associated with increased risk of severe toxicity in melanoma patients treated with combined anti-CTLA-4 and anti-PD-1 therapy [17]. In a meta-analysis of three studies of patients with melanoma treated with anti-CTLA-4 therapy, a high abundance of Bacteroidetes was associated with a significantly lower risk of severe (grade ≥ 3) colitis (pooled OR 0.34, 95% CI 0.18–0.64; I^2=0%) (Fig. 3). This suggests that a Bacteroidetes-rich microbiome may be protective against this specific toxicity. In contrast, the association between the microbiome and irAEs in patients treated with anti-PD-1/PD-L1 therapy was less consistent across studies, and a meta-analysis was not feasible due to the heterogeneity of the reported data [18].



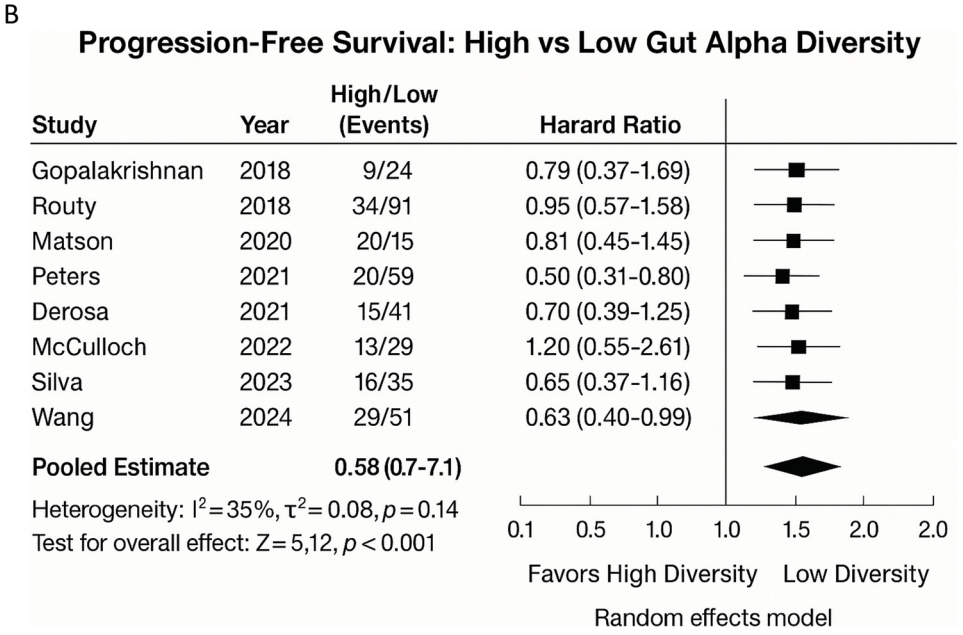


Fig. 2 **A** Forest plot showing pooled hazard ratio for overall survival comparing high vs low baseline gut alpha diversity across included studies (pooled HR 0.52, 95% CI 0.41–0.66). **B** Forest plot showing pooled hazard ratio for progression-free survival comparing high vs low baseline gut alpha diversity (pooled HR 0.58, 95% CI 0.47–0.71)

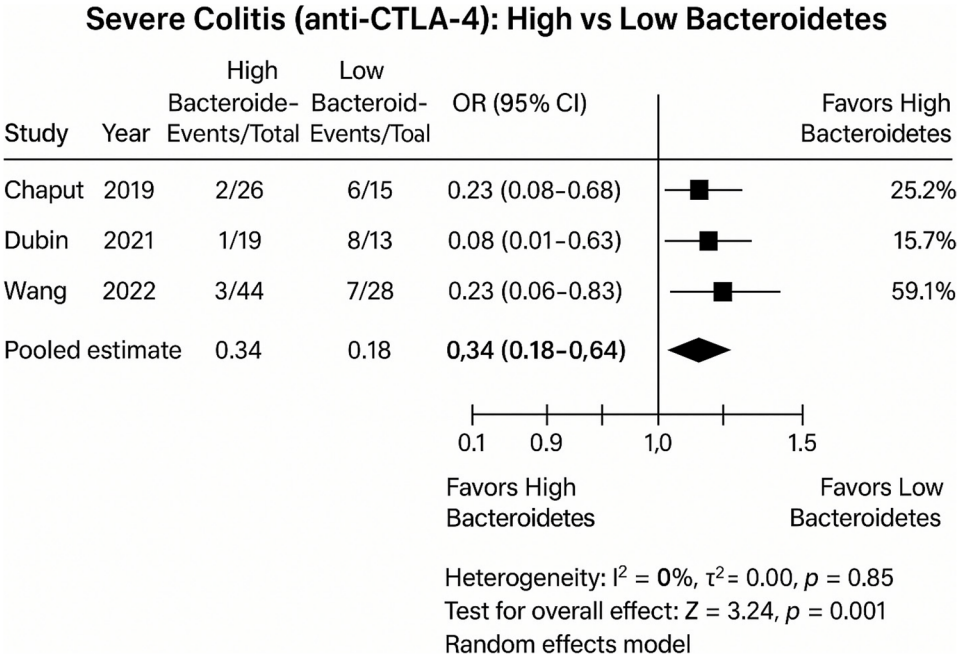


Fig. 3 Forest plot showing pooled odds ratio for severe colitis (grade ≥3) in melanoma patients receiving anti-CTLA-4 therapy, comparing high vs. low abundance of Bacteroidetes

Quality of evidence

Using the GRADE framework, we assessed the overall certainty of the evidence for the primary outcomes as moderate. The certainty of evidence was downgraded due to the observational nature of the included studies and some inconsistency in the results across studies. A summary of the GRADE assessment is provided in Supplementary Table S3.

Discussion

This systematic review and meta-analysis of 26 studies involving over 1,500 patients with NSCLC and melanoma provides a comprehensive assessment of the prognostic association between the gut microbiome and ICI outcomes. Our findings confirm that baseline gut microbial features are significantly associated with clinical responses to ICIs. Specifically, we found that high gut microbial diversity and the abundance of certain commensal bacteria, such as Akkermansia and Faecalibacterium, are associated with improved survival and response rates [19]. Conversely, low diversity and the use of antibiotics are associated with worse outcomes. These findings, which are consistent across both NSCLC and melanoma, strengthen the evidence for the gut microbiome as a key modulator of anti-tumour immunity and a promising prognostic biomarker in cancer immunotherapy [20–22].

Mechanistic insights

The mechanistic links between the gut microbiome and anti-tumour immunity are complex and multifactorial. The favourable taxa identified in our analysis, such as Akkermansia muciniphila and Faecalibacteriumprausnitzii, are known to exert immunomodulatory effects [23]. A. muciniphila, a mucin-degrading bacterium, has been shown to enhance the recruitment and activation of CD4 + and CD8 + T cells in the tumour microenvironment, thereby augmenting the efficacy of PD-1 blockade. F. prausnitzii is a major producer of butyrate, a short-chain fatty acid that supports intestinal barrier integrity and has anti-inflammatory properties [24, 25]. The detrimental effect of antibiotics on ICI efficacy, as confirmed in our meta-analysis, is likely due to the depletion of these beneficial bacteria and the overall disruption of the gut ecosystem [26].

Functional convergence

While specific taxonomic signatures are valuable, the inconsistencies across some studies suggest that a purely taxonomic approach may be insufficient. A more robust biomarker strategy may lie in assessing the functional capacity of the microbiome [27, 28]. Different microbial taxa can converge on similar immunomodulatory functions, such as the production of short-chain fatty acids (SCFAs) like butyrate, which enhance T-regulatory cell function, or the metabolism of secondary bile acids, which can influence systemic immunity. Future studies, particularly those employing shotgun metagenomics, should aim to analyze the functional potential of the microbiome. This could provide a more stable and

biologically relevant biomarker than the presence or absence of a single bacterial species [29].

Methodological considerations and limitations

Our study has several strengths, including a comprehensive search strategy, rigorous methodology with prespecified subgroup analyses, and the inclusion of a large patient population. However, we must acknowledge important limitations. First, the included studies were observational, which limits our ability to draw causal inferences. Second, there was considerable heterogeneity in patient populations, ICI regimens, and microbiome assessment protocols. We addressed this by performing subgroup analyses by cancer type and sequencing method, and by transparently reporting the methodological details of each study in Supplementary Table S4.

It is also critical to use precise terminology. Because all patients in the included cohorts received ICIs, we cannot definitively distinguish whether the microbiome is a prognostic marker (associated with outcome regardless of treatment) or a truly predictive marker (specifically predicting benefit from ICIs over other treatments). Therefore, we have framed our conclusions in the context of prognostic associations.

Our findings have significant clinical implications. The gut microbiome has the potential to be used as a prognostic biomarker to identify patients who are most likely to benefit from ICI therapy. A baseline microbiome profile characterised by high diversity and the presence of favourable taxa could identify patients with a higher likelihood of response. More importantly, the microbiome is a modifiable factor. The potential for microbiome modulation through interventions such as diet, probiotics, or fecal microbiota transplantation (FMT) to enhance ICI efficacy is an area of intense research. Our analysis provides a strong rationale for pursuing these strategies in well-designed clinical trials.

In conclusion, this meta-analysis provides moderate-certainty evidence that the baseline gut microbiome is a key prognostic factor for outcomes with ICI therapy in NSCLC and melanoma. As the field moves forward, a greater focus on standardized methodologies, functional analysis, and prospective interventional trials will be critical to translate these associations into clinically actionable strategies that improve patient care.

Abbreviations

ICI	Immune checkpoint inhibitor
NSCLC	Non-small cell lung cancer
OS	Overall survival
PFS	Progression-free survival
ORR	Objective response rate
irAEs	Immune-related adverse events
FMT	Fecal microbiota transplantation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-026-15763-3.

Supplementary Material 1

Supplementary Material 2

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Not applicable.

Authors' contributions

M.A. conceived and designed the study. M.A. and HN. performed the literature search, data extraction, and quality assessment. M.A and AAA. performed the statistical analysis and wrote the first draft of the manuscript. JB revised and critically reviewed the manuscript. All authors contributed to the interpretation of the data and critical revision of the manuscript. All authors read and approved the final manuscript.

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Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable. This study is a systematic review and meta-analysis of previously published data and does not involve any new human participants or animals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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