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Impact of microbiome-modulating strategies in cancer patients receiving immunotherapy (MSIT): A systematic review and meta-analysis

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Abstract

The gut microbiota influences immune checkpoint inhibitors (ICIs) efficacy. Microbiome-modulating strategies (MMSs), including probiotics, synbiotics, and faecal microbiota transplantation (FMT), have emerged as promising adjuncts, but their clinical impact remains uncertain. We systematically reviewed PubMed, Embase, and CENTRAL to February 2025 for clinical cohorts evaluating MMS in cancer patients receiving ICIs.

Thirty-six studies (25 trials/cohorts; n=2,746) were included. Meta-analyses, and subgroup analyses were performed for efficacy along with microbiome shifts and safety. MMS plus ICIs achieved a pooled objective response rate (ORR) of 40% (95% CI: 31%–49%; $I^2 = 63.4\%$; $p = 0.0003$; 95% PI: 15%–72%). Descriptive proportions showed ORR of 45% (95% CI: 32%–58%; $I^2 = 72.5\%$; $p = 0.0058$) for probiotics and 33% (95% CI: 22%–48%; $I^2 = 60.7\%$; $p = 0.0064$) for FMT; however, these findings are non-comparative and confounded by study differences. Exploratory subgroup signals were noted for probiotics in NSCLC (ORR 55%; 95%CI: 45%–64%; $I^2 = 0\%$; $p = 0.3683$) and FMT in melanoma (ORR 39%; 95% CI: 15%–69%; $I^2 = 72.5\%$; $p = 0.0262$). Dual ICI regimens showed the highest point estimate for ORR (43%; 95% CI: 17%–73%; $I^2 = 68.5\%$; $p = 0.0747$) but increased toxicity. Microbiome analyses revealed enrichment of short-chain fatty acid-producing taxa and *Bifidobacterium* spp. among responders. Based on a limited pooled sample size (n=143), MMS-related adverse events were mostly grade 1–2 (42%; 95% CI: 14%–77%, $I^2 = 53.8\%$, $p = 0.0210$), with rare severe events (1%). Overall, MMS show promising, though preliminary, hypothesis-generating signals for modulating ICI response. Given high heterogeneity and reliance on early-phase, single-arm trials, the findings underscore urgent need for large, biomarker-driven randomized controlled trials to define optimal interventions and cautiously integrate microbiome modulation into immuno-oncology care.

Keywords: microbiome, probiotics, faecal microbiota transplantation, immunotherapy, immune checkpoint inhibitors.

1 Introduction

Cancer remains a significant global health burden, accounting for more than 9.7 million deaths in 2022 (1). Advances in immuno-oncology, particularly the development of immune checkpoint inhibitors (ICIs) targeting programmed death-1 (PD-1), programmed death ligand-1 (PD-L1), and cytotoxic T lymphocyte–associated antigen 4 (CTLA-4), have transformed the therapeutic landscape across multiple malignancies (2-4). Despite these advances, the proportion of patients who derive durable benefits remains modest. In non-small cell lung cancer (NSCLC), for example, fewer than one-third of patients respond to ICI monotherapy, and even in immunotherapy-responsive cancers such as melanoma, resistance inevitably develops in a substantial fraction of patients (5-7). This clinical reality underscores the urgent need for adjunctive strategies to increase the efficacy of ICIs.

One emerging and highly promising determinant of the immunotherapy response is the gut microbiome. The human intestinal microbiota consists of trillions of microorganisms that influence immune system development, epithelial barrier function, and systemic metabolism (8-10). Increasing evidence indicates that microbiome composition and function are key modulators of anticancer immunity (11). A fundamental aspect of this relationship is the ability of the gut microbiome to drive the maturation and priming of immune cells, including T lymphocytes, to elicit immune responses (**Figure 1**) (12). Conversely, favourable microbiome signatures, including enrichment of taxa such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Bifidobacterium longum*, have been linked to enhanced responses to ICIs (13-15). These associations suggest that targeted manipulation of the microbiome could represent a novel avenue to optimize cancer immunotherapy. Mechanistic studies support this rationale. In murine tumour models, antibiotic-induced microbiome disruption impaired ICI efficacy, whereas recolonization with specific commensals restored antitumour responses (16, 17).

To restore gut homeostasis and enhance antitumour immunity, several microbiome-modulating strategies (MMSs) have been explored as adjuncts to cancer treatment (16). Certain bacterial taxa promote dendritic cell maturation, enhance CD8$^+$ T-cell infiltration into tumours, and increase the production of short-chain fatty acids (SCFAs), which fuel effector T-cell metabolism and memory differentiation (18-20). Other commensals reinforce mucosal integrity and regulate bile acid and tryptophan metabolism, indirectly modulating systemic immune tone (21-23). Collectively, these findings provide strong biological plausibility that microbiome modulation can synergize with checkpoint blockade.

A variety of MMSs have therefore been explored in oncology. Faecal microbiota transplantation (FMT) aims to restore microbial homeostasis by transferring stool from healthy donors or immunotherapy responders into cancer patients (24-26). Several early-phase trials have shown that FMT can resensitize refractory melanoma patients to anti-PD-1 therapy, with associated increases in microbial diversity and the expansion of favourable taxa (24, 25). Probiotics, live microorganisms conferring health benefits when administered in adequate amounts, represent another widely investigated approach. Specific strains of *Lactobacillus, Bifidobacterium*, and mixed consortia have demonstrated immunostimulatory properties, including the augmentation of natural killer and T-cell activity (27, 28). Synbiotics combine probiotics with prebiotic substrates to increase colonization (29-31), and dietary interventions rich in fibre or polyphenols also influence microbiome composition and immune responses (32, 33). Together, these MMSs offer diverse and potentially complementary means of shaping host–tumour interactions through microbial pathways.

However, despite strong mechanistic rationales and early clinical signals, the clinical impact of MMS in patients receiving ICIs remains incompletely defined. The field faces several challenges. First, studies vary widely in design, ranging from small single-arm cohorts to randomized controlled trials, making it difficult to draw consistent conclusions. Second, heterogeneity in MMS type, dosage, and timing complicates comparisons across studies. Third, the effects of MMS appear to be cancer specific, with some interventions showing benefit in certain tumour types but not others. For example, probiotics have been associated with improved outcomes in patients with NSCLC, whereas FMT appears to be more effective in patients with melanoma and renal cell carcinoma (34-36). Finally, safety data are limited, particularly concerning the risks of introducing live microbes into immunocompromised oncology populations.

Previous systematic reviews have addressed aspects of this topic, often focusing on either probiotics or FMT alone (37, 38). While valuable, these reviews do not provide an integrated comparison across the spectrum of MMS, nor do they incorporate the most recent clinical data. Furthermore, earlier analyses rarely explored disease- and regimen-specific signals, leaving important questions unanswered: Are certain microbiome interventions more effective in treating lung cancer than in treating melanoma? Do outcomes differ depending on whether MMSs are combined with PD-1 monotherapy or dual checkpoint blockade? Addressing these questions is essential for translating microbiome science into actionable clinical strategies.

To address these gaps, we conducted a systematic review and meta-analysis of clinical studies evaluating MMS, including probiotics, synbiotics, and FMT, in cancer patients receiving ICIs. Our objectives were: 1) to assess the pooled efficacy of MMS across cancers; 2) to identify intervention- and disease-specific patterns of benefit; and 3) to evaluate microbiome shifts and safety outcomes associated with these strategies. By integrating data from both interventional and real-world studies, this work provides the most comprehensive synthesis to date of the clinical impact of microbiome modulation in immuno-oncology. We aim to clarify whether MMSs represent merely experimental adjuncts or whether they have the potential to be incorporated into routine cancer care while outlining the key directions for future research.

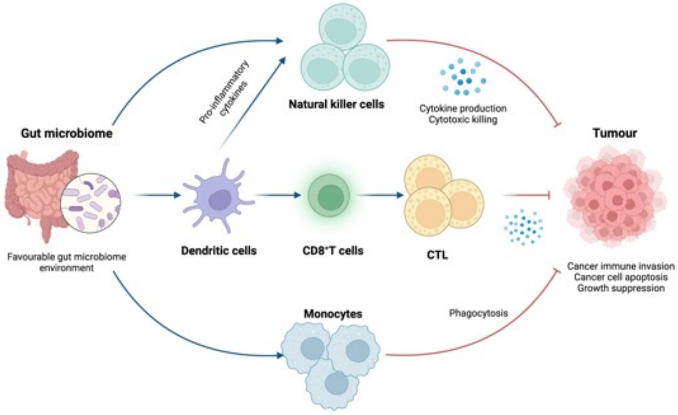

Figure 1. The gut microbiomes influence tumour survival via immune responses.

A favourable gut microbiome environment with high bacterial diversity can enable immune invasion of cancerous cells via several pathways. Beneficial bacteria promote dendritic cell maturation which promotes $CD8^+$ T cell activation, promoting cytotoxic T lymphocyte (CTL) invasion of tumour cells. Dendritic cells are also able to promote natural killer cell activation, leading to cytokine production and cytotoxic killing.

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2 Materials and methods

2.1 Study design and protocol registration

The systematic review was conducted in accordance with the PRISMA declaration guidelines and used the checklist provided (refer to **Supplementary file: PRISMA checklist S1**) (39). The study protocol was prospectively registered at PROSPERO under the registration number CRD420251028287.

Given the early and heterogeneous nature of clinical research on microbiome-modulating strategies in immunotherapy, we included all interventional and observational studies, including early-phase, single-arm, quasiexperimental, and conference abstract reports, if sufficient quantitative data could be extracted for analysis. This approach aligns with the PRISMA 2020 guidance to maximize comprehensiveness and avoid publication bias.

2.2 Literature search, study selection and data extraction

Regardless of whether they included active or placebo controls, cohort studies, interventions, and early-phase clinical trials on immunotherapy-receiving cancer patients along with MMS-related techniques (FMT, probiotics, synbiotics, prebiotics or dietary interventions) were included. Preclinical research, reviews, and editorials lacking original data were not considered, nor were studies that did not involve immune therapy or microbiome-modulating techniques.

Three databases were used to retrieve English-language literature: PubMed (pubmed.ncbi.nlm.nih.gov), Embase (www.embase.com), and the Cochrane Library (www.cochranelibrary.com) via a search strategy (refer to **Supplementary file: Search terms**). **S2**). Until February 26, 2025, papers identified in these databases were imported into the Covidence platform (www.covidence.org) for a comprehensive screening process.

Study selection and data management were performed using Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia; www.covidence.org). To ensure rigorous screening, four reviewers (MT, HL, ND, and VN) independently screened the titles and abstracts of all identified records and performed full-text eligibility assessment. Any discrepancies or conflicts identified during this real-time screening phase were resolved through discussion and consensus; if a resolution could not be reached, a third senior reviewer (NH) was consulted. Following selection, data extraction was carried out by two independent reviewers using study details (authors, study period, publication year, study type, trial identification if possible, and country), participant characteristics (sample size, age, sex, cancer type, immune treatment type), intervention details (type, dose, route of administration, duration, prior line of therapy), clinical and microbial outcome measures, and any other pertinent information. All extracted data were cross-checked to ensure accuracy and consistency across the review team.

2.3 Risk of bias analysis

The risk of bias (ROB) in the extracted studies was evaluated by two independent authors. The Cochrane Risk of Bias tool 2.0 (ROB2) was used for the randomized control trial studies (40). The methodological quality of nonrandomized interventional studies was assessed via the MINORS tool (41) at which the mean scores and ranges were examined to categorize the overall strength of evidence as low, moderate, or high quality.

2.4 Meta-analysis and subgroup analysis

For the meta-analysis, we included single-arm trials, as well as intervention arms of other comparative trials and cohort studies, to visualize the combined effects across different intervention types, cancer types, and immunotherapies. The primary outcome measures were the overall response rate (ORR), complete response rate (CR), partial response rate (PR), stable disease (SD), progressive disease (PD), and disease control rate (DCR). We omitted studies with a sample size of fewer than

10 from the meta-analysis because of insufficient outcome clarity and to minimize the potential risk of bias; however, these studies were included in the narrative synthesis to capture relevant contextual and exploratory findings.

Subgroup analysis was conducted by immune checkpoint inhibitors type, cancer group, and microbiome modulation strategy. The immune blockade options include PD-1 inhibitors, PD-L1 inhibitors, generic ICIs, and dual ICIs. In this study, dual ICIs target and block two different immune checkpoint proteins, PD-1 and CTLA-4 inhibitors. The MMSs included in the analysis were FMT, probiotics, and synbiotics. There are 6 cancer types and groups, such as lung cancer (non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC)), GI cancer comprising metastatic colorectal cancer (mCRC), gastric cancer (GC), hepatocellular carcinoma (HCC), esophageal squamous-cell carcinoma (ESCC), pancancer (mixed/unspecified solid tumours), metastatic renal cell carcinoma (mRCC), melanoma, and head and neck squamous cell carcinoma (HNSCC). To resolve heterogeneity and determine the effectiveness of immune therapy in cancer patients receiving FMT or probiotics, a second-level subgroup analysis was also conducted.

The safety profile was assessed by evaluating adverse event (AE) data in relation to microbiome-modulating strategies (MMS-AEs) or immune responses (irAEs), with extractable information included when available. Safety outcomes were categorized according to the AE grade and their attribution. The percentage of patients who experienced an adverse event was calculated as the number of events divided by the total number of patients investigated.

2.5 Quality of evidence

Publication bias was assessed via a Doi plot and the Luis Furuya–Kanamori index (LFK), which are recommended for single-arm proportional meta-analyses (42). Leave-one-out sensitivity analysis was conducted for the major asymmetry found in the Doi plot.

2.6 Statistics

Study and participant characteristics were compiled via descriptive statistics, reported as means and standard deviations or medians and ranges as appropriate. In accordance with the DerSimonian and Laird method (43), a random-effects meta-analysis was conducted to take into consideration potential statistical heterogeneity among studies. As most datasets were single-arm studies, we synthesized proportions (ORR, CR, PR, SD, PD, DCR) rather than relative effects. Study-level proportions were logit-transformed and pooled with inverse-variance weighting; pooled logits were then back-transformed to proportions and reported with 95% confidence intervals (95% CIs) (44). To accurately reflect the impact of the observed heterogeneity on clinical predictability, 95% prediction intervals (95% PIs) (45) were consistently reported for all primary outcomes alongside the 95% CIs. Heterogeneity was quantified with I^2 and τ^2 (40).

For safety outcomes, we also reported random effect estimates as a sensitivity analysis to assess robustness to model assumptions.

Subgroup analyses were performed by intervention type, cancer type, and ICI treatment. While pooled proportions were calculated for all identified subgroups to ensure transparency, formal interpretations and conclusions regarding efficacy were restricted to subgroups comprising two or more independent studies to ensure a minimum level of replicability. With statistical significance set at $p < 0.05$, all the statistical analyses were performed via the R (version 2025.05.0) package with the meta-analysis packages 'meta' (version 8.2), 'metafor' (version 4.8-0), and 'metasens' (version 1.5-3).

3 Results

3.1 Study selection

A total of 2,141 records were identified from the database searches, including 1,521 from Embase, 567 from PubMed, and 53 from CENTRAL. After removing 637 duplicate records prior to screening, 1,704 unique records remained for initial title and abstract screening. Of these, 1,592 records were excluded because they did not meet the inclusion criteria.

A total of 112 reports were sought for retrieval and subsequently assessed for eligibility through full-text review. Ultimately, 36 studies (24, 25, 46-79), comprising 25 clinical trials and cohort studies, were included in the systematic review, and the detailed study selection process is presented in **Figure 2**.

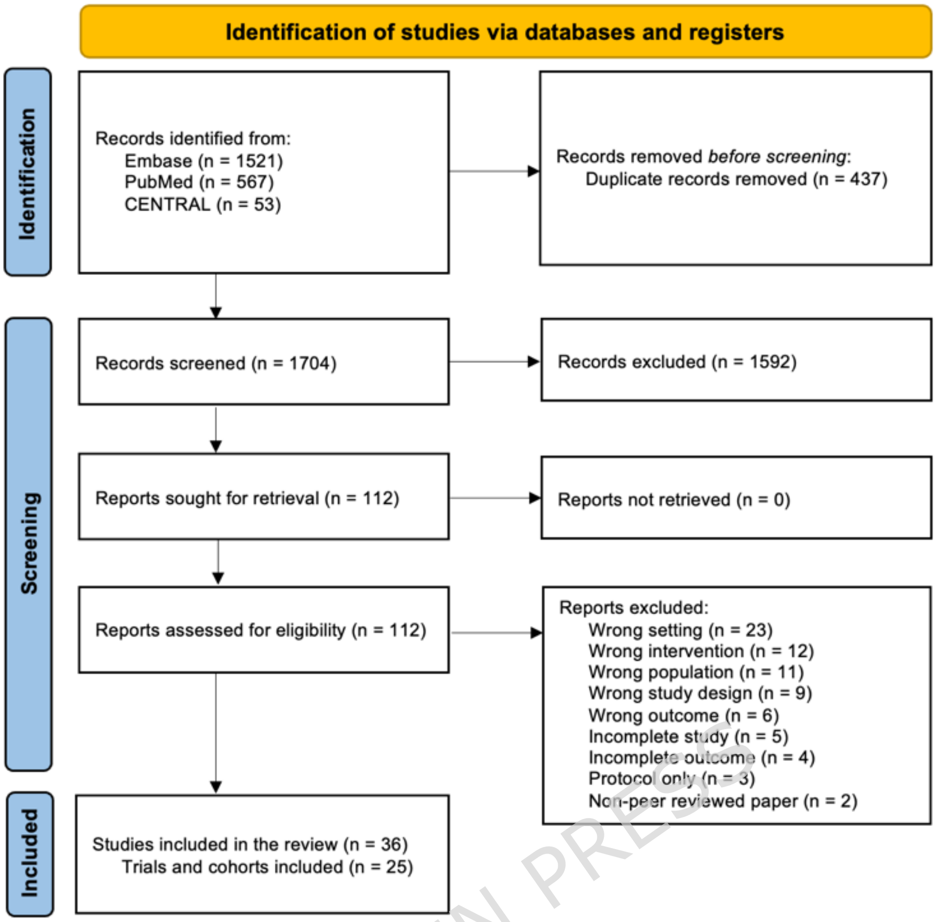


Figure 2. Flow diagram for the identification of studies in the systematic literature review

3.2 Study characteristics

The included studies, published from 2019–2025, were conducted across various countries, including Canada, China, France, Israel, Italy, Japan, Korea, Mexico, Norway, and the USA. Fourteen clinical trials were identified: 10 single-arm trials (24, 25, 47, 56-58, 66, 74, 75, 77), three (50, 51, 68) randomized controlled trials (RCTs) and one (72) quasiexperimental prospective study. We also observed 6 cohort studies comprising 5 retrospective studies (61, 62, 67, 69, 73) and one prospective approach (70). Sample sizes in observational studies are relatively large, ranging from over 100 to nearly 930 participants, providing complementary real-world evidence regarding the impact of microbiome modulation on outcomes such as PFS, OS, response rates, gut microbiome composition, and safety. One study (56) evaluated prebiotic intervention (inulin), and another assessed synbiotics (59), adding to the spectrum of microbiome-modulating strategies investigated in real-world settings. Five trials (53-55, 64, 71) with insufficient or preliminary data were included as narrative studies and were not considered for the meta-analysis. The baseline characteristics of each study extracted are shown in **supplementary file 3, Table S1**.

3.3 Sensitivity analysis

For statistical robustness, publication bias was evaluated via a funnel plot with Egger's test (p = 0.201; **Supplementary file 3, Figure S1**), which revealed no statistically significant asymmetry. Since most of the included trials were single-arm studies, the proportional data were assessed via Doi plots, which revealed major asymmetry (LFK = 2.58; **Supplementary file 3, Figure S2**). Leave-one-out sensitivity analysis revealed that the pooled ORR fluctuated between 0.29 and 0.32 depending on the exclusion of specific trials (**Supplementary file 3, Table S2**). This range, coupled with high heterogeneity, suggests that the overall effect estimate is sensitive to individual study characteristics and should be interpreted as an exploratory range rather than a definitive point estimate.

3.4 Subject characteristics

All the studies included a total of 2746 participants, with ages ranging from 34 to 90 years. While 37% and 12% of the recorded data indicated the male and female prevalence, respectively, in the present study, several studies did not report the sex distribution. The median follow-up duration varied widely, ranging from approximately 3 months to over 34 months, depending on the study (**Supplementary file 3, Table S1**).

3.5 Risk of bias

Interventional studies included three randomized controlled trials (50, 51, 68), one quasiexperimental prospective study (72), and ten single-arm interventions (24, 25, 47, 56-58, 66, 74, 75, 77). Among the randomized controlled trials, no studies reported a high risk of bias (ROB) (**Supplementary file 3, Figure S3**). Some concerns of ROB were encountered owing to a lack of full paper to evaluate all the outcomes there.

Assessment of methodological quality via the MINORS tool revealed that most studies did not clearly define a study endpoint and lacked a sample size calculation (0.19 ± 0.60, range: 0–2). The overall average MINORS score was 12.14 ± 4.91 (range: 8–22), indicating moderate methodological quality with considerable variability (**Supplementary file 3, Table S2**).

3.6 Efficacy of MMS combined with immunotherapy

The overall pooled ORR for all cancer patients receiving MMS combined with immunotherapy was 40% (95% CI: 31%–49%; $I^2 = 63.4\%$; $p = 0.0003$; 95% Prediction Intervals (PI): 14%–72%) (Figure 3A). Sensitivity analysis using the leave-one-out method showed that the pooled ORR fluctuated between 29% and 32% upon removal of individual studies, suggesting a level of instability in the estimate due to the exploratory nature of the included trials.

In the subgroup analysis (Table 1), proportions of ORR across cancer types and intervention types were reported for all identified cohorts, focusing on subgroups represented by at least two studies for efficacy interpretations. Regarding intervention modality, the point estimate for the probiotics subgroup was 45% (95% CI: 32%–58%; $I^2 = 72.5\%$; $p = 0.0058$) compared to 33% (95% CI: 22%–48%; $I^2 = 60.7\%$; $p = 0.0064$) for the FMT subgroup (Table 1). Among cancer-specific subgroups, lung cancer and mRCC showed pooled ORRs of 55% (95% CI: 45%–64%; $I^2 = 0\%$; $p = 0.3683$) and 56% (95% CI: 41%–70%; $I^2 = 0\%$; $p = 0.8069$), respectively, while patients with melanoma revealed a pooled ORR of 48% (95% CI: 23%–74%; $I^2 = 71.6\%$; $p = 0.0143$) (Table 1). Dual ICI therapy showed an ORR of 43% (95% CI: 17%–73%; $I^2 = 68.5\%$; $p = 0.0747$).

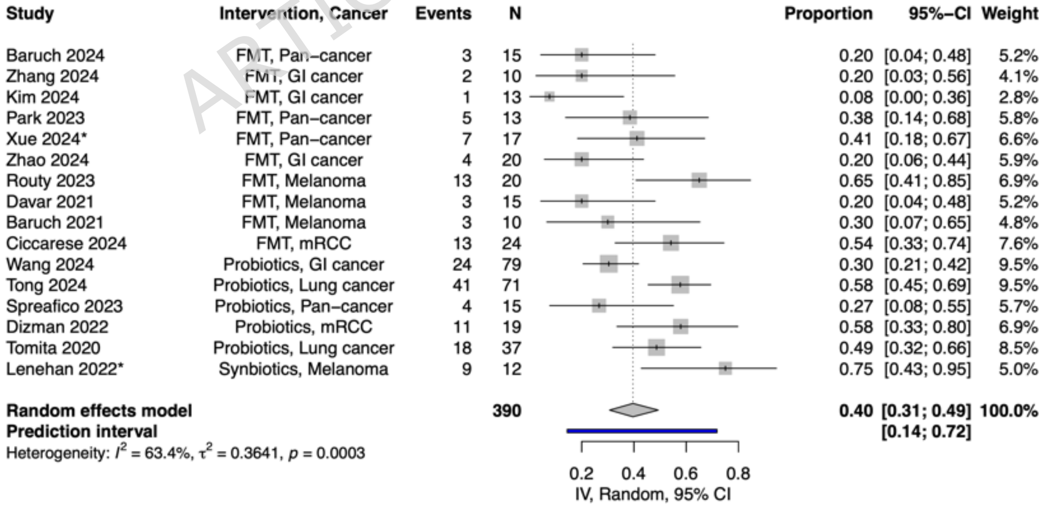
In the second-level subgroup analysis (Table 2), efficacy was further examined by specific intervention-cancer combinations. Among patients receiving FMT, melanoma patients had an ORR of 39% (95% CI: 15%–69%; $I^2 = 72.5\%$; $p = 0.0262$), the pancancer group had an ORR of 29% (95% CI: 15%–50%; $I^2 = 11.7\%$; $p = 0.2873$), and the GI cancer group had an

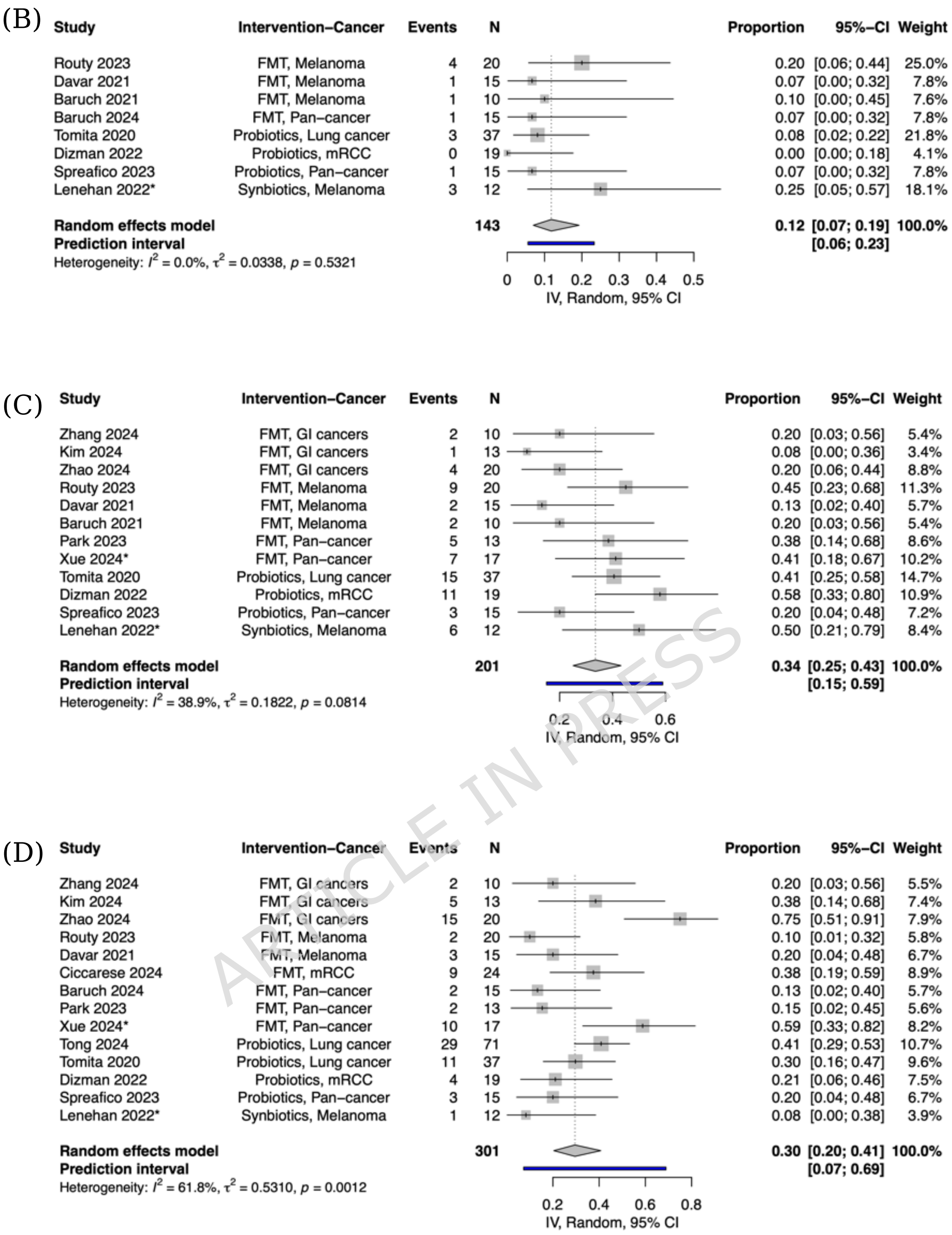
ORR of 17% (95% CI: 8%–32%; $I^2 = 0\%$; $p = 0.6268$). When stratified by immunotherapy type, FMT combined with a PD-1 inhibitor had a 31% ORR (95% CI: 20%–46%; $I^2 = 59.3\%$; $p = 0.0118$), whereas probiotics with a PD-1 inhibitor had a 43% ORR (95% CI: 17%–73%; $I^2 = 68.5\%$; $p = 0.0747$).

Secondary outcomes including complete response (CR), partial response (PR), and disease control rate (DCR) were also assessed. The pooled CR was 12% (95% CI: 7%–19%; $I^2 = 0\%$; $p = 0.5321$) (Figure 3B), with subgroup rates of 13% (95% CI: 6%–25%; $I^2 = 0\%$; $p = 0.5842$) for the FMT group and 7% (95% CI: 3%–16%; $I^2 = 0\%$; $p = 0.7286$) for the probiotics. The PR rate was 34% (95% CI: 25%–43%; $I^2 = 38.9\%$; $p = 0.0814$) (Figure 3C), including 33% (95% CI: 17%–53%; $I^2 = 46.9\%$; $p = 0.1297$) for melanoma and 34% (95% CI: 22%–50%; $I^2 = 0\%$; $p = 0.4158$) for the pancancer dataset. The pooled Disease Control Rate (DCR) was 66% (95% CI: 50%–80%; $I^2 = 70.6\%$; $p = 0.0002$; 95% PI: 16%–95%) (Figure 3F). Subgroup analyses indicated DCRs of 70% in the probiotic group and 63% in the FMT group. By cancer type, mRCC exhibited the highest point estimate for DCR at 85%, while GI cancer and pancancer cohorts showed DCRs of 66% and 48%, respectively. Consistent with the ORR findings, the wide confidence intervals for these secondary outcomes reflect the limited sample sizes of the early-phase trials.

Finally, the meta-analysis found a stable disease (SD) rate of 30% (95% CI: 20%–41%; $I^2 = 61.8\%$; $p = 0.0012$; 95% PI: 7%–69%) and a progressive disease (PD) rate of 22% (95% CI: 13%–35%; $I^2 = 64.9\%$; $p = 0.0015$; 95% PI: 3%–70%) across the total population (Figure 3D-E). Subgroup analysis showed SD rate of 31% for both probiotics and FMT while PD rate was 17% in the probiotics group and 26% in the FMT group. When examining response patterns by specific cancer types, GI cancer exhibited the highest SD rate at 46% (95% CI: 17%–78%; $I^2 = 75.8\%$; $p = 0.0161$), accompanied by a PD rate of 22% (95% CI: 1%–85%; $I^2 = 85.8\%$; $p = 0.0079$). This was followed by lung cancer, which showed an SD rate of 37% (95% CI: 27%–48%; $I^2 = 21.8\%$; $p = 0.0261$), and mRCC, which had an SD rate of 30% (95% CI: 17%–49%; $I^2 = 24.8\%$; $p = 0.0816$). Notably, the pancancer dataset showed the highest PD rate of 40% (95% CI: 27%–55%; $I^2 = 0\%$; $p = 0.5334$). The coexistence of relatively high PR (34%) and PD (40%) rates within this small subgroup reflects the data instability and high variance inherent in small cumulative sample sizes (n=60), leading to "all-or-nothing" response patterns in these heterogeneous cohorts.

(A)





(B)

Study	Intervention–Cancer	Events	N	Proportion	95%–CI	Weight
Routy 2023	FMT, Melanoma	4	20	0.20	[0.06; 0.44]	25.0%
Davar 2021	FMT, Melanoma	1	15	0.07	[0.00; 0.32]	7.8%
Baruch 2021	FMT, Melanoma	1	10	0.10	[0.00; 0.45]	7.6%
Baruch 2024	FMT, Pan–cancer	1	15	0.07	[0.00; 0.32]	7.8%
Tomita 2020	Probiotics, Lung cancer	3	37	0.08	[0.02; 0.22]	21.8%
Dizman 2022	Probiotics, mRCC	0	19	0.00	[0.00; 0.18]	4.1%
Spreafico 2023	Probiotics, Pan–cancer	1	15	0.07	[0.00; 0.32]	7.8%
Lenehan 2022*	Synbiotics, Melanoma	3	12	0.25	[0.05; 0.57]	18.1%
Random effects model			**143**	**0.12**	**[0.07; 0.19]**	**100.0%**
Prediction interval					**[0.06; 0.23]**	

Heterogeneity: $I^2 = 0.0\%$, $\tau^2 = 0.0338$, $p = 0.5321$

(C)

Study	Intervention–Cancer	Events	N	Proportion	95%–CI	Weight
Zhang 2024	FMT, GI cancers	2	10	0.20	[0.03; 0.56]	5.4%
Kim 2024	FMT, GI cancers	1	13	0.08	[0.00; 0.36]	3.4%
Zhao 2024	FMT, GI cancers	4	20	0.20	[0.06; 0.44]	8.8%
Routy 2023	FMT, Melanoma	9	20	0.45	[0.23; 0.68]	11.3%
Davar 2021	FMT, Melanoma	2	15	0.13	[0.02; 0.40]	5.7%
Baruch 2021	FMT, Melanoma	2	10	0.20	[0.03; 0.56]	5.4%
Park 2023	FMT, Pan–cancer	5	13	0.38	[0.14; 0.68]	8.6%
Xue 2024*	FMT, Pan–cancer	7	17	0.41	[0.18; 0.67]	10.2%
Tomita 2020	Probiotics, Lung cancer	15	37	0.41	[0.25; 0.58]	14.7%
Dizman 2022	Probiotics, mRCC	11	19	0.58	[0.33; 0.80]	10.9%
Spreafico 2023	Probiotics, Pan–cancer	3	15	0.20	[0.04; 0.48]	7.2%
Lenehan 2022*	Synbiotics, Melanoma	6	12	0.50	[0.21; 0.79]	8.4%
Random effects model			**201**	**0.34**	**[0.25; 0.43]**	**100.0%**
Prediction interval					**[0.15; 0.59]**	

Heterogeneity: $I^2 = 38.9\%$, $\tau^2 = 0.1822$, $p = 0.0814$

(D)

Study	Intervention–Cancer	Events	N	Proportion	95%–CI	Weight
Zhang 2024	FMT, GI cancers	2	10	0.20	[0.03; 0.56]	5.5%
Kim 2024	FMT, GI cancers	5	13	0.38	[0.14; 0.68]	7.4%
Zhao 2024	FMT, GI cancers	15	20	0.75	[0.51; 0.91]	7.9%
Routy 2023	FMT, Melanoma	2	20	0.10	[0.01; 0.32]	5.8%
Davar 2021	FMT, Melanoma	3	15	0.20	[0.04; 0.48]	6.7%
Ciccarese 2024	FMT, mRCC	9	24	0.38	[0.19; 0.59]	8.9%
Baruch 2024	FMT, Pan–cancer	2	15	0.13	[0.02; 0.40]	5.7%
Park 2023	FMT, Pan–cancer	2	13	0.15	[0.02; 0.45]	5.6%
Xue 2024*	FMT, Pan–cancer	10	17	0.59	[0.33; 0.82]	8.2%
Tong 2024	Probiotics, Lung cancer	29	71	0.41	[0.29; 0.53]	10.7%
Tomita 2020	Probiotics, Lung cancer	11	37	0.30	[0.16; 0.47]	9.6%
Dizman 2022	Probiotics, mRCC	4	19	0.21	[0.06; 0.46]	7.5%
Spreafico 2023	Probiotics, Pan–cancer	3	15	0.20	[0.04; 0.48]	6.7%
Lenehan 2022*	Synbiotics, Melanoma	1	12	0.08	[0.00; 0.38]	3.9%
Random effects model			**301**	**0.30**	**[0.20; 0.41]**	**100.0%**
Prediction interval					**[0.07; 0.69]**	

Heterogeneity: $I^2 = 61.8\%$, $\tau^2 = 0.5310$, $p = 0.0012$

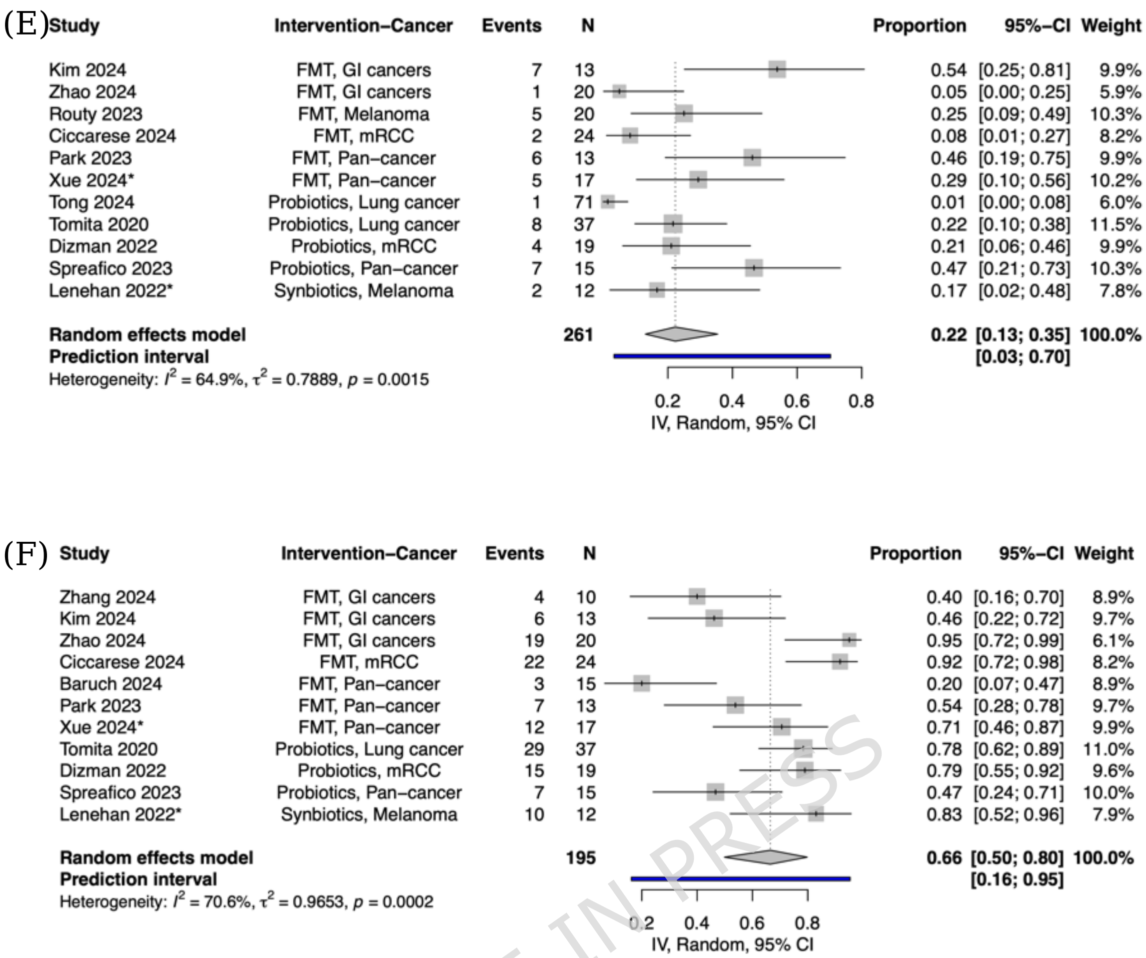
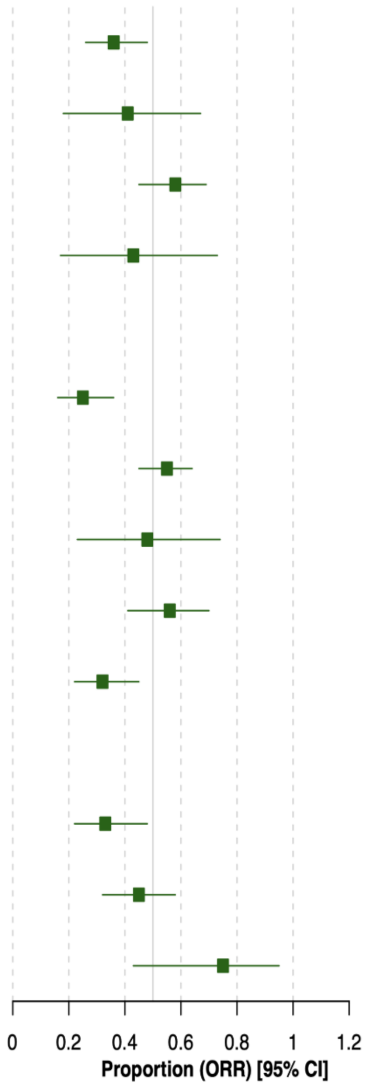


Figure 3. Meta-analysis forest plot for (A) Objective Response Rate, (B) Complete Response Rate, (C) Partial Response Rate, (D) Stable Disease, (E) Progressive Disease, and (F) Disease Control Rate.

Abbreviations: FMT = Faecal Microbiota Transplantation; Pan-Cancer = Aggregated data across multiple cancer types; mRCC = Metastatic renal cell carcinoma; GI Cancer = Gastrointestinal cancers.

Table 1. First-level subgroup analysis of objective response rate (ORR) in included studies.

Subgroup	**No. of Studies**	**ORR [95% CI [b]]**	**[95% PI [b]]**	**P value [c] [I^2]**	**Random-effect model [a]**
Immunotherapy		**0.40 [0.31; 0.49]**	[0.14; 0.72]	0.0003 [63.4%]	
1. PD-1 inhibitor	12	0.36 [0.26; 0.48]	-	0.0021 [62.3%]	
2. PD-L1 inhibitor	1	0.41 [0.18; 0.67]	-	-	
3. generic ICIs	1	0.58 [0.45; 0.69]	-	-	
4. dual ICIs	2	0.43 [0.17; 0.73]	-	0.0747 [68.5%]	
Cancer type		**0.40 [0.31; 0.49]**	[0.14; 0.72]	0.0003 [63.4%]	
1. GI cancer	4	0.25 [0.16; 0.36]	-	0.3572 [7.2%]	
2. Lung cancer	2	0.55 [0.45; 0.64]	-	0.3683 [0.0%]	
3. Melanoma	4	0.48 [0.23; 0.74]	-	0.0143 [71.6%]	
4. mRCC	2	0.56 [0.41; 0.70]	-	0.8069 [0.0%]	
5. Pancancer	4	0.32 [0.22; 0.45]	-	0.5625 [0.0%]	
Microbiome Modulation		**0.40 [0.31; 0.49]**	[0.14; 0.72]	0.0001 [65.5%]	
1. FMT	10	0.33 [0.22; 0.48]	-	0.0064 [60.7%]	
2. Probiotics	5	0.45 [0.32; 0.58]	-	0.0058 [72.5%]	
3. Synbiotics	1	0.75 [0.43; 0.95]	-	-	

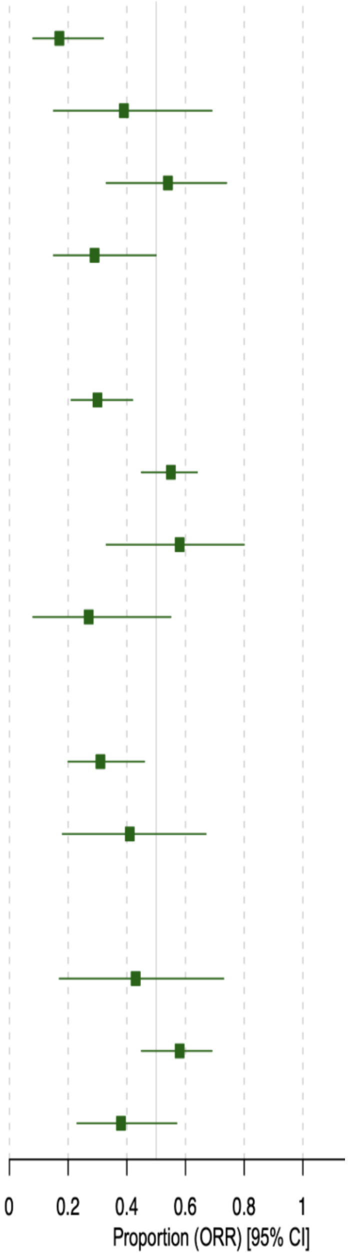
Abbreviations: 95% CI = 95% confidence interval, 95% PI = 95% prediction interval, FMT = faecal microbiota transplantation, GI = gastrointestinal, ICIs = immune checkpoint inhibitors, mRCC = metastatic renal cell carcinoma, NA = not available, ORR = objective response rate.

[a] Pooled proportions were calculated using a random-effects model (DerSimonian-Laird method) with inverse-variance weighting.

[b] The 95% CI reflects the precision of the mean estimate, while the 95% PI accounts for observed heterogeneity (I^2) to predict the potential response range in a new clinical setting. P-value refers to Cochran's Q test for heterogeneity ($P < 0.05$ indicates significant heterogeneity).

[c] *P-value refers to the test for statistical heterogeneity within the specific subgroup.*

Table 2. Second-level subgroup analysis of objective response rate (ORR) in cancer patients who received FMT or probiotics

Subgroup	**No. of Studies**	**ORR [95% CI [b]]**	**[95% PI [b]]**	**P value [c] [I^2]**	**Random-effect model [a]**
FMT-Cancer		**0.31 [0.20; 0.46]**	[0.07; 0.73]	0.0118 [59.3%]	
1. GI cancer	3	0.17 [0.08; 0.32]	-	0.6268 [0.0%]	
2. Melanoma	3	0.39 [0.15; 0.69]	-	0.0262 [72.5%]	
3. mRCC	1	0.54 [0.33; 0.74]	-	-	
4. Pancancer	3	0.29 [0.15; 0.50]	-	0.2873 [11.7%]	
Probiotics-Cancer		**0.45 [0.32; 0.58]**	[0.14; 0.80]	0.0058 [72.5%]	
1. GI cancer	1	0.30 [0.21; 0.42]	-	-	
2. Lung cancer	2	0.55 [0.45; 0.64]	-	0.3683 [0.0%]	
3. mRCC	1	0.58 [0.33; 0.80]	-	-	
4. Pancancer	1	0.27 [0.08; 0.55]	-	-	
FMT-Immunotherapy		**0.33 [0.22; 0.46]**	[0.09; 0.70]	0.0191 [54.6%]	
1. PD-1 inhibitor	9	0.31 [0.20; 0.46]	-	0.0118 [59.3%]	
2. PD-L1 inhibitor	1	0.41 [0.18; 0.67]	-	-	
Probiotics-Immunotherapy		**0.45 [0.32; 0.58]**	[0.14; 0.80]	0.0058 [72.5%]	
1. PD-1 inhibitor	2	0.43 [0.17; 0.73]	-	0.0747 [68.5%]	
2. generic ICIs	1	0.58 [0.45; 0.69]	-	-	
3. dual ICIs	2	0.38 [0.23; 0.57]	-	0.0586 [72.0%]	

Abbreviations: 95% CI = 95% confidence interval, 95% PI = 95% prediction interval, FMT = faecal microbiota transplantation, GI = gastrointestinal, ICIs = immune checkpoint inhibitors, mRCC = metastatic renal cell carcinoma, NA = not available, ORR = objective response rate.

[a] Pooled proportions were calculated using a random-effects model (DerSimonian-Laird method) with inverse-variance weighting.

[b] The 95% CI reflects the precision of the mean estimate, while the 95% PI accounts for observed heterogeneity (I^2) to predict the potential response range in a new clinical setting. P-value refers to Cochran’s Q test for heterogeneity ($P < 0.05$ indicates significant heterogeneity).

[c] P-value refers to the test for statistical heterogeneity within the specific subgroup.

3.7 Alterations in the microbiota following MMS across different cancer types

Figure 4 illustrates the reported changes in the abundance of key bacterial groups following microbiome-modulating interventions in cancer patients undergoing immunotherapy across multiple cancer types. Each circle represents the observed direction and magnitude of change for a given bacterial group within a cancer category across the included studies.

Across most cancer types, an increase in SCFA-producing bacteria (e.g., *Faecalibacterium*, *Eubacterium*, *Ruminococcus*) was consistently observed after intervention, notably in NSCLC (aggregate sample n=125), GI cancers (n=10), melanoma (n=331), and across pancancer groups (n=62). *Bifidobacterium* spp. also showed an increased abundance postintervention across all major cancer categories represented, with the largest change reported in NSCLC (n=407).

Other beneficial taxa, such as *Prevotella* spp. and *Alistipes* spp., were increased in select settings, whereas potentially unfavourable groups, such as *Bacteroides* spp. and oral-origin taxa (*Streptococcus mitis* and *Mogibacterium*), generally presented decreased relative abundances, particularly following interventions in NSCLC, GI cancer, and pancancer datasets. Notably, the prevalence of Desulfovibrio/Proteobacteria and certain oral-origin bacteria was also reduced following intervention, suggesting a shift toward a microbiome composition previously associated with improved immunotherapy outcomes.

Overall, the observed changes were directionally consistent with those hypothesized to predict favourable responses to immune checkpoint inhibition, and these trends were preserved across multiple cancer types and intervention strategies.

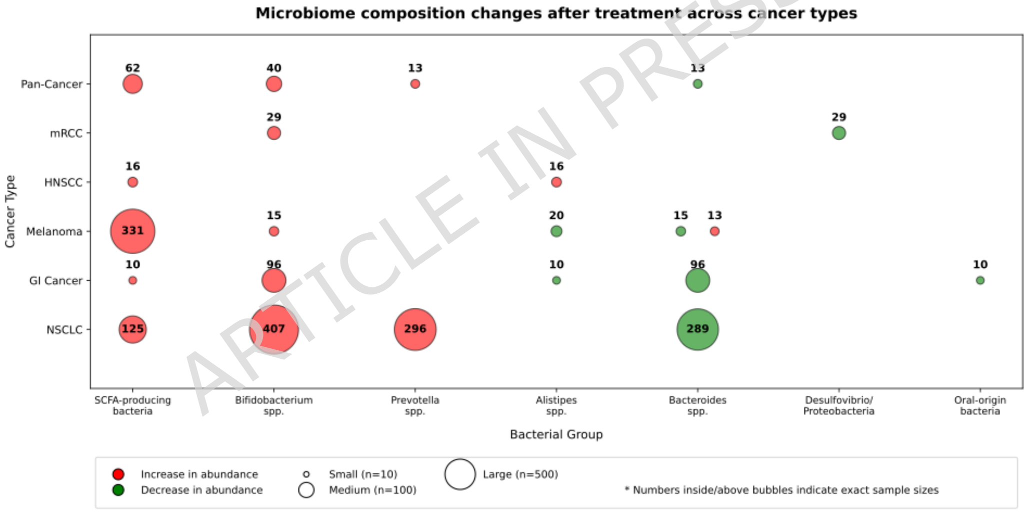


Figure 4. Synthesis of microbiome shifts across different microbial modulating strategies (MMS) and cancer types

The bubble plot displays changes in the relative abundance of specific microbiota (X-axis) across different cancer types (Y-axis) following MMS treatment. Circle size is proportional to the total aggregate sample size (n) across all contributing studies for that specific data point, with the exact numerical value provided adjacent to each bubble. Color hue represents the direction of the reported shift: red indicates an increase in relative abundance, while green indicates a decrease. Color intensity is uniform and represents the direction of change only. Bubbles are plotted only where data from clinical trials or cohorts were available.

Abbreviations: Pan-Cancer = Aggregated data across multiple cancer types; mRCC = Metastatic renal cell carcinoma; HNSCC = Head and neck squamous cell carcinoma; GI Cancer = Gastrointestinal cancers; NSCLC = Non-small cell lung cancer; SCFA = Short-chain fatty acid; spp. = Species (plural).

3.8 Microbiome diversity changes

Alpha diversity (a measure of within-sample microbial richness and evenness) and beta diversity (a measure of between-sample microbial community differences) have been variably reported across studies and cancer types. Overall, these data suggest a trend toward increased alpha diversity, especially among responders, following microbiome-modulating interventions and a convergence in beta diversity, indicating successful engraftment of the donor microbiota or responder-associated community structure, particularly in FMT studies (80-83).

3.8.1 Microbial diversity among responders

Differences by cancer type and intervention, as well as the effect size between responders and nonresponders, were noted. Patients who responded well, whether with lung, gastrointestinal, melanoma, or kidney cancers, often presented an increase in alpha diversity, indicating that their gut microbiome became richer and more balanced after the intervention (80-84). This suggests a restoration toward a healthier and more resilient microbial community. These changes were most noticeable in studies involving FMT, where clear shifts in microbial communities were observed.

In contrast, nonresponders generally showed little or no increase in alpha diversity and sometimes a decrease in alpha diversity, indicating a limited ability of their microbiome to change (81-84). This difference may be influenced by individual factors or the baseline microbiome composition. Beta diversity analyses supported these findings, showing that the gut microbiomes of responders tended to become more similar to those of healthy or responder donors, especially following FMT. This convergence suggests that microbiome modulation can successfully shift the microbial community toward profiles associated with better immunotherapy outcomes. This pattern was observed across different types of cancer and in heterogeneous patient groups.

However, not all interventions led to meaningful changes; for example, some studies using standard probiotics or involving nonresponders reported minimal shifts in diversity (69, 85, 86). This reflects variability in the flexibility of the cancer-associated microbiome among individuals. In summary, the ability to increase alpha diversity and shift beta diversity toward donor-like communities may be an important microbial signature linked to a successful immune response in cancer treatment.

3.9 Safety profile

Across the included studies, a total of 10 studies (24, 55, 58, 64, 66, 68, 71, 74, 75, 87) involving 143 patients reported extractable data on MMS-AEs (Figure 5A). The incidence of grade 1-2 MMS-AEs varied widely, ranging from 0% to 100%. Among the ten studies, nine reported low-grade

MMS-AEs, which were predominantly mild to moderate in severity, whereas one study reported no MMS-AEs of any grade. The pooled random-effects estimate indicated that 42% of patients experienced grade 1–2 MMS-AEs (95% CI: 14%–77%, $I^2 = 53.8\%$, $p = 0.021$; 95% PI: 1%–99%). Additionally, severe MMS-AEs (grade ≥3) were less common across the studies, with most studies reporting no such events. The pooled random effects estimate revealed an overall incidence of 1% (95% CI: 0%–31%, $I^2 = 0\%$, $p = 0.8169$; 95% PI: 0%–95%).

A review of 10 studies involving 206 patients provided valuable information on irAEs, with a relatively high burden of low-grade toxicities, grade 1-2 irAEs, observed. Ten studies reported the incidence of grade 1–2 irAEs, with a pooled estimate of 57% (95% CI: 33%–78%, $I^2 = 81.0\%$, $p < 0.0001$; 95% PI: 6%–97%). For grade ≥ 3 irAEs, twelve studies provided data, with a pooled incidence of 17% (95% CI: 7%–34%, $I^2 = 70.1\%$, $p < 0.0001$; 95% PI: 1%–80%). Importantly, no cases of treatment-related mortality were reported.

The significant heterogeneity observed across these safety outcomes, evidenced by the broad prediction intervals, likely reflects differences in patient populations, tumor types, ICI backbones, and the lack of standardized AE ascertainment methods for microbiome interventions.

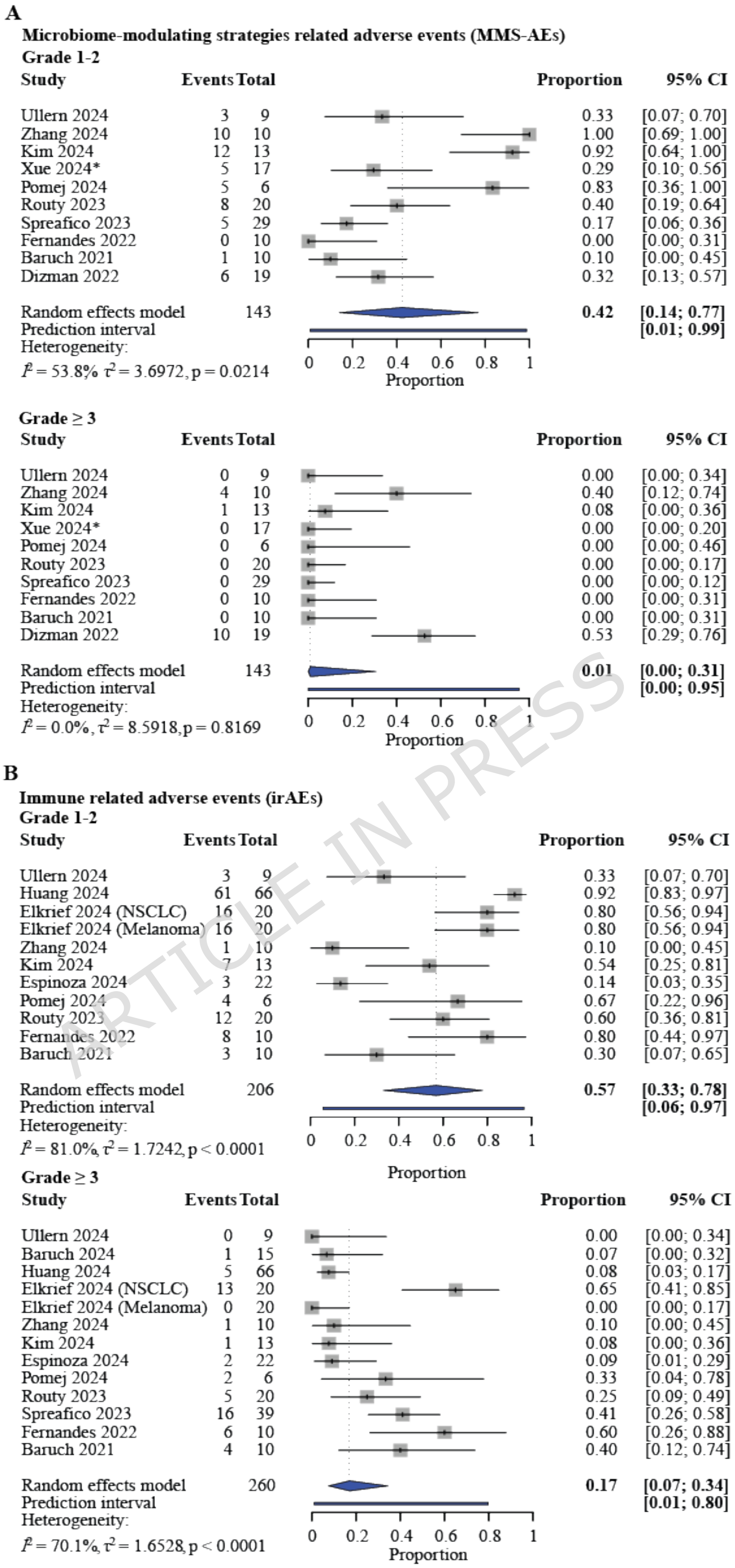


Figure 5. Proportion of patients experiencing any adverse events (reported) across various studies examining the safety of combining FMT

with ICIs in terms of A) Microbiome-modulating strategies related adverse events (MMS-AEs), and B) Immune related adverse events (irAEs).

4 Discussion

This systematic review and meta-analysis provide comprehensive evidence that microbiome-modulating strategies (MMSs) influence immune checkpoint inhibitor (ICI), the clinical efficacy and safety of immune checkpoint inhibitors (ICIs) across cancer types. Inclusion of both published and abstract-level data minimized a comprehensive synthesis of available evidence while minimizing publication bias, but it also introduced substantial heterogeneity across study contexts and designs. To address potential bias, we evaluated publication bias via Egger's test ($p = 0.201$), which indicated no significant asymmetry, whereas the Doi plot suggested major asymmetry (LFK = 2.58), likely reflecting small-study effects. Leave-one-out sensitivity analysis confirmed the robustness of the pooled ORR (range: 29%–32%), supporting the consistency of the primary findings despite these limitations. Nevertheless, the high heterogeneity and reliance on single-study evidence for some subgroups restrict generalizability, and these findings should be considered interpreted as hypothesis-generating rather than definitive evidence.

Overall, MMS was associated with a pooled objective response rate (ORR) of 40% (95% CI: 31%–49%; $I^2 = 63.4\%$; $p = 0.0003$) (Figure 3). Subgroup analyses (Table 2 and Supplementary File 3, Figures S4–8) revealed distinct patterns: FMT achieved an ORR of 39% (95% CI: 15%–69%; $I^2 = 72.5\%$; $p = 0.0262$) for melanoma and 54% (95% CI: 33%–74%) for a single study of metastatic renal cell carcinoma (mRCC, with a disease control rate of 85%), whereas probiotics were most effective for lung cancer, with an ORR of 55% (45%–64%; $I^2 = 72.5\%$; $p = 0.0262$; $I^2 = 0\%$; $p = 0.3683$) and a low progressive disease rate of 7%. Single-study evidence of the effects of synbiotics in patients with melanoma reported an ORR of 75% (43%–95%). These variations likely reflect differences in the tumour microenvironment and immune responsiveness across cancer types (88), as well as distinct mechanisms by which MMS influences the gut microbiota and systemic immunity (26, 89). For comparison, the historical ORRs associated with ICIs alone are typically approximately 20% across similar cancer types (90). Collectively, these findings suggest that MMS could serve as a promising adjunct to ICIs, particularly in cancers with historically modest response rates, although variability in microbiome composition and treatment protocols may influence outcomes. Future research should prioritize large, randomized controlled trials with standardized MMS protocols and biomarker-driven patient selection to validate these observations and clarify the underlying mechanisms.

Compared with PD-1 monotherapy, dual ICI regimens appeared to improve response rates (43%, 95% CI: 17%–73%; $I^2 = 68.5\%$; $p = 0.0747$ vs 36%, 95% CI: 26%–48%; $I^2 = 62.3\%$; $p = 0.0021$), but this benefit was accompanied by a greater risk of disease progression (33% vs 24%). These findings echo prior evidence in melanoma and NSCLC that efficacy gains from dual checkpoint blockade often come at the cost of greater toxicity and treatment discontinuation (91, 92). Mechanistically, intensified immune activation may enhance tumour clearance in responders but also accelerate progression in non-responders or lead to early discontinuation due to immune-related adverse events (93, 94). Clinically, this underscores the need for careful patient selection and toxicity monitoring when considering dual ICIs, particularly in combination with microbiome-modulating strategies, where additive immune effects could further amplify risks.

In second-level subgroup analyses, probiotics combined with PD-1 inhibitors achieved better response rates of 43% (95% CI: 17%–73%; I^2 = 68.5%; p = 0.0747) than probiotics plus dual ICIs did 38% (95% CI: 23%–57%; I^2 = 72.0%; p = 0.0586). Similarly, a single study revealed that FMT plus PD-L1 blockade (41%, 95% CI: 18%–67%) revealed a higher pooled ORR compared to PD-1 inhibitors (31%, 95% CI: 20%–46%; I^2 = 59.3%; p = 0.0118). These patterns indicate potential interactions between microbiome intervention and specific checkpoint backbones, possibly reflecting differences in immune activation pathways or microbiota-driven modulation of PD-1 versus PD-L1 signalling. However, given the reliance on single-study evidence and high heterogeneity, these findings should be considered exploratory. Mechanistically, microbiome-ICI combinations have been proposed to enhance efficacy and overcome resistance, as demonstrated by FMT rescue of anti-PD-1-refractory melanoma and targeted probiotic augmentation of dual ICIs in RCC (24, 25, 50, 87, 95, 96). Future trials should systematically evaluate these interactions to identify optimal pairings and clarify the underlying mechanisms involved.

A notable finding in our subgroup analysis was the coexistence of a relatively high PR rate (34%) and a high PD rate (40%) within the pancancer dataset. While seemingly contradictory in a clinical context, this 'all-or-nothing' response pattern is likely a reflection of the significant data instability inherent in small, heterogeneous cohorts (97). This suggests that while microbiome modulation may drive potent responses in some patients, others may derive no benefit, potentially due to the diverse tumor biologies and varied baseline microbial compositions aggregated within the 'pancancer' classification. This discrepancy underscores the limitations of pooling early-phase, small-scale trials and highlights the urgent need for more robust, tumor-specific data to clarify these response patterns.

Our findings align with and extend existing evidence on microbiome modulation in ICI-treated patients. In NSCLC, probiotics combined with PD-1/PD-L1 inhibitors showed a higher point estimate for response rates, which is consistent with the pooled analysis by Nakatsukura *et al.*, who reported longer OS (25.6 vs 10.9 months) and PFS (7.6 vs 3.2 months) with probiotics (62). Similarly, a recent meta-analysis of 1,123 patients confirmed that probiotics significantly improved OS (HR 0.53) and ORR (OR 2.83) in ICI-treated NSCLC patients (98). These observations reinforce the potential clinical relevance of our subgroup findings, where probiotics paired with PD-1 inhibitors achieved higher response rates than probiotics combined with dual ICIs did. Conversely, synbiotics showed no survival benefit in the trial by Tong et al. (70), which is consistent with our subgroup estimates, suggesting that not all microbiome interventions confer equal benefit. Mechanistically, probiotics may enhance gut microbial diversity and short-chain fatty acid production, promoting T-cell activation, whereas synbiotics may lack sufficient immunomodulatory potency because prebiotics do not always selectively enhance the administered probiotic strains, and competitive microbial interactions can dilute immune signalling pathways critical for ICI synergy (99). Collectively, these data underscore the need for mechanistic studies and biomarker-driven trials to identify which microbiome strategies offer the greatest synergy with specific ICI regimens.

Building on our subgroup findings, the observed benefit of FMT in melanoma aligns with preliminary data from the PERFORM trial (ORR 44%, PR 11%) (55) and is further supported by the TACITO phase II trial (50), which demonstrated superior 1-year PFS (66.7% vs 35.0%) and ORR (54% vs 28%) with FMT plus axitinib-pembrolizumab compared with placebo. These results suggest that microbiome modulation may enhance ICI efficacy in patients with melanoma, potentially by restoring gut microbial diversity and promoting T-cell activation. Interestingly, observational reports that over-the-counter probiotic use might reduce alpha diversity and blunt ICI efficacy (100) were not borne out in our synthesis, where controlled probiotic supplementation correlated with improved outcomes. This discrepancy underscores the importance of strain specificity and dosing in achieving immunomodulatory effects. Together, these results support the potential of MMS as a promising adjunct to ICIs, with FMT appearing particularly promising in melanoma and mRCC and probiotics in NSCLC.

In addition to clinical outcomes, our findings are biologically plausible given the consistent microbiome shifts observed across the included studies. Both probiotics and FMT enriched beneficial taxa such as

Bifidobacterium, *Faecalibacterium*, and other short-chain fatty acid (SCFA) producers while reducing potentially unfavourable groups, including *Bacteroides* spp. and oral-origin taxa (*Streptococcus mitis*) (Figure 4). These changes are relevant because SCFA production has been linked to enhanced $CD8^+$ T-cell metabolism, improved dendritic cell priming, and more efficient antigen presentation (101-104), all of which support ICI responsiveness. Conversely, depletion of oral-origin taxa may reduce systemic inflammation and restore gut barrier integrity, further promoting immune homeostasis (105).

In addition to taxonomic shifts, diversity metrics provide insight into the biological plausibility of MMS in enhancing ICI efficacy. Increased alpha diversity was consistently observed among responders, whereas beta diversity patterns converged toward donor or responder-like communities following FMT (24, 25, 58, 66, 75, 87). These findings suggest that restoring microbial richness and resilience may be a prerequisite for sustained immunotherapy benefit, as diverse communities are better equipped to maintain metabolic and immunomodulatory functions under treatment pressure (106). Mechanistically, *Akkermansia* and other health-associated commensals may promote mucin production and barrier integrity, reducing systemic inflammation, whereas SCFA-producing *Clostridia* support the metabolic fitness of effector T cells (107, 108). Together, these observations reinforce the concept that both compositional and functional restoration of the microbiome may underpin the clinical benefits of MMS, highlighting microbial diversity as a potential biomarker for patient stratification in future trials.

Finally, safety considerations are critical when the MMS is integrated into immuno-oncology regimens. Across the included studies, MMS combined with ICIs appeared generally safe and well tolerated. Low-grade MMS-AEs were reported in 42% of patients (95% CI: 14%–77%; I^2 = 53.8%; p = 0.0214), whereas severe events were rare in only approximately 1% (95% CI: 0%–31%; I^2 = 0%; p = 0.8169). Notably, no increase in treatment-related mortality attributable to MMS was observed, even among immunocompromised oncology patients. Furthermore, the addition of MMS did not increase ICI-related toxicities beyond expected levels, with grade ≥3 irAEs reported in 17% of patients (95% CI: 7%–34%; I^2 = 70.1%; $p < 0.0001$), which is consistent with historical benchmarks for ICIs (109). This favourable safety profile is particularly noteworthy given the immunocompromised status of the target population and suggests that MMS can be safely integrated into clinical protocols. However, these findings remain preliminary, and larger trials with standardized AE reporting and long-term follow-up are essential to confirm safety and guide best practices.

Limitations

Despite encouraging evidence, several limitations must be acknowledged: 1) We observed substantial heterogeneity across primary outcomes (I^2 = 63.4–72.5%), which stems from variations in study designs, cancer types, and MMS protocols complicates, complicating direct comparisons and pooled interpretation; 2) Many included studies were early-phase, single-arm, or small cohorts, and cohort designs. Our assessment using MINORS scores indicated moderate methodological quality with considerable variability, limiting generalizability and the confirmatory strength of the pooled estimates; 3) Sensitivity analyses, specifically the leave-one-out approach, revealed an ORR range of 0.29 to 0.32, demonstrating a level of instability that necessitates a conservative interpretation of the pooled ORR as a preliminary signal rather than a definitive clinical benchmark; 4) Safety data were limited by inconsistent reporting because the wide confidence intervals (e.g., 14–77%) for reported toxicities highlighted significant uncertainty in immunocompromised populations; 5) the geographic concentration of studies, particularly in Asia and North America, raises concerns about ethnogeographic bias, as baseline microbiome composition varies widely across populations; 6) microbiome analyses were heterogeneous, with most relying on *16S rRNA* gene sequencing rather than shotgun metagenomics, which primarily provides taxonomic identification rather than direct functional data. Consequently, mechanistic inferences regarding metabolic outputs, such as SCFA production, remain associative and should be interpreted with caution until confirmed by shotgun metagenomic or metabolomic analyses; and 7) the lack of standardized protocols for microbiome analysis across the included studies, including differences in DNA extraction, sequencing platforms, and bioinformatic pipelines, precludes a quantitative meta-analysis of alpha and beta diversity, necessitating a narrative synthesis for these metrics. Despite these limitations, sensitivity analyses confirmed the robustness of our primary efficacy findings, and the overall trends were directionally consistent across cancer types and intervention modalities.

5 Conclusion

This systematic review and meta-analysis synthesized preliminary evidence that microbiome-modulating strategies (MMSs) can influence the clinical efficacy of immune checkpoint inhibitor (ICI) efficacy inhibitors (ICIs) across multiple cancer types. In exploratory subgroup analyses, signals of activity were observed for probiotics in NSCLC and FMT in melanoma and renal cell carcinoma; however, these findings are descriptive and do not represent formal comparative effectiveness between interventions. While the pooled analysis suggests a potential for tailored microbiome strategies, these results are hypothesis-generating and require validation in statistically powered cohorts. Mechanistic plausibility is supported by observed shifts in microbial diversity and the enrichment of beneficial taxa, such as short-chain fatty acid producers, though these associations remain correlative.

Importantly, MMSs appeared generally well-tolerated in this study, though the wide confidence intervals and limited sample size (n=143) for safety outcomes necessitate a cautious interpretation of their long-term viability. The substantial heterogeneity ($I^2 > 60\%$) and reliance on early-phase, single-arm trials and conference abstracts represent a critical limitation, indicating that current pooled estimates are sensitive to study-specific variability. To confirm these preliminary findings, future research must prioritize large, multicentre randomized controlled trials (RCTs) incorporating standardized donor criteria, rational strain selection, and baseline microbiome profiling. If safety and efficacy are validated through rigorous clinical evaluation, microbiome modulation may represent a biologically driven adjunct to optimize immunotherapy and expand patient benefit.

List of abbreviations

CENTRAL: Cochrane Central Register of Controlled Trials; CIs: confidence intervals; PRISMA-P: Preferred Reporting Items for Systematic review and Meta-analysis Protocols; RCTs: randomized controlled trials; US: United States.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data are available as supplementary files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MT and NH contributed to conceptualization, supervision, and critical revision of the manuscript. The MT took a role in project administration and introduced the required software and resources. MT, HL, VM, and ND contributed to the data curation, formal analysis, and methodology. All the authors contributed to the writing and editing of the manuscript. All the authors read and approved the final manuscript. NH is the guarantor of the review for the integrity of the work as a whole.

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