Review

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The Gut Microbiome as a Biomarker and Therapeutic Target of Immune Checkpoint Inhibitors: A Review for Oncologists

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Abstract

Immune checkpoint inhibitors (ICIs) have transformed cancer therapy, yet their benefits remain limited to a subset of patients, underscoring the need for more reliable biomarkers and novel therapeutic strategies. The gut microbiome has emerged as a critical modulator of systemic immunity and a promising determinant of ICI response. Evidence links specific microbial features, taxa, and bioactive metabolites to enhanced antitumor immunity, while disruptions such as antibiotic exposure are associated with poorer outcomes. Advanced in sequencing and multi-omics technologies have provided deeper insights into microbiome-immune crosstalk, though methodological heterogeneity continues to challenge reproducibility. Translational studies demonstrate that microbiome-based intervention, including fecal microbiota transplantation (FMT), biotics supplementation, and engineered microbial strains, can enhance ICI efficacy or mitigate immune-related toxicities. Despite encouraging early clinical signals, broader implementation requires methodological rigor, standardized protocols, and innovative trial designs that account for host and environmental factors. For clinicians, the most immediate strategies involve prudent antibiotic stewardship and patient enrollment in microbiome-focused clinical trials. Overall, the gut microbiome represents both a predictive biomarker and a therapeutic target, offering a new frontier to refine immunotherapy and improve patient outcomes in oncology.

Keywords: immune checkpoint inhibitors; gut microbiome; cancer immunotherapy; biomarkers; multi-omics integration; microbial diversity; fecal microbiota transplantation; probiotics/prebiotics/postbiotics

1. Introduction

The approval of immune checkpoint inhibitors (ICIs) in the 2010s marked a paradigm shift in cancer therapy. As monotherapy or in combination, ICIs have produced unprecedented outcomes and are now the standard of care across several solid tumors. However, a substantial proportion of patients derive limited benefit, and efficacy can vary even among patients with the same cancer type and comparable biomarkers [1,2].

In parallel, the gut microbiome has emerged as a key regulator of host immunity. Accumulating evidence suggests that the microbiome may serve both as a predictive biomarker of ICI response and

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as a therapeutic target to enhance efficacy. Incorporating microbiota-based strategies into ICI therapy, therefore, represents a promising approach for improving clinical outcomes [3].

This review aims to provide practicing medical oncologists with a practical overview of the gut microbiome, from its fundamental crosstalk with antitumor immunity and methods of analysis to emerging therapeutic strategies to optimize immune checkpoint inhibition.

2. Current Biomarkers of ICIs and Their Limitations

ICIs have delivered long-term, durable responses in metastatic cancer and increased cure rates in early-stage disease. Despite this success, it is estimated that only ~13% of cancer patients are eligible and respond to ICI therapy [4]. Currently, several biomarkers are used in clinical practice to guide the use of ICI, including programmed death-ligand 1 (PD-L1) expression, mismatch repair protein (MMR), microsatellite instability (MSI) status, tumor mutational burden (TMB). Other promising markers include POLE/POLD1 mutation and tumor-infiltrating lymphocytes (TILs). Despite extensive validation across tumor types, no single biomarker reliably predicts response to ICIs in all settings. Their performance is context-dependent and limited by assay heterogeneity, tumor evolution, and sampling bias. A summary of their methods, advantages and limitations is presented in Table 1.

Table 1. Current biomarkers for immune checkpoint inhibitors.

Biomarker	Methods	Advantages	Limitations
Programmed death-ligand 1 (PD-L1) [5]	Immunohistochemistry (IHC)	widely available; quick turnaround; validated in several cancer types	Multiple FDA-approved companion assays (22C3, 28-8, SP142, SP263) with different score cut-off for ICIs and cancer types; subject to tumor heterogeneity and sampling bias
Mismatch repair (MMR) [6–8]	PCR or next generation sequencies (NGS) for MSI status; IHC for MMR proteins (MLH1, PMS2, MSH2, MSH6)	FDA approved MSI-high (MSI-H) or dMMR status as a tissue-agnostic biomarker; strong predictive value	Rare in solid tumors (~3-16%); limited availability of validated MSI assays in some centers
Tumor mutational burden (TMB) [9,10]	NGS	FDA approved TMB ≥10 mut/Mb by FoundationOne CDx as a tissue-agnostic biomarker for pembrolizumab; reflects overall neo-antigen landscape	Expensive and longer turnaround time; optimal cut-off may vary across cancer types; lack of standardized assessment methods
POLE/POLD1 Mutations [11,12]	NGS	Associated with an ultra-hypermutated phenotype and exceptionally high TMB	Not FDA approved; rare in solid tumors (~4%)
Tumor infiltrating lymphocytes (TILs) [13]	H&E pathology slide evaluation	Reflects actual immune response within tumor; assessable on routine pathology slides	Not FDA-approved; lack standardized scoring; subject to spatial and temporal heterogeneity

The limitations of these current ICI biomarkers justify the search for integrative models that incorporate host factors, including the gut microbiome, to better predict treatment outcomes.

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3. The Microbiome: A Key Environmental Factor in Immunity

The human immune responses vary considerably across individuals, shaped by a combination of intrinsic host factors and external environmental influences. Host factors such as genetic background, age and sex are known to affect immune cell composition and antitumor surveillance [14–16]. However, evidence from twin studies suggests that environmental factors, including the gut microbiome, infections, medications (notably antibiotics and immunomodulatory drugs), are the dominant force, accounting for up to 24–77% of variability in human immune traits [17,18].

3.1. Acquisition and Distribution

The gut microbiome is highly individualized down to the strain level [19,20], with environmental factors dominating over genetics in shaping its composition in adults [21–23]. Microbial colonization begins at birth via maternal transmission and evolves throughout life based on interactions with other humans, animals, diet, antibiotic exposure, and the surrounding environment [24]. Microbial communities are found throughout the gastrointestinal tract, but their composition varies significantly by location due to differences in oxygen levels, pH, and transit time [25,26]. The large intestine hosts the most abundant and diverse community, making it the primary focus for biomarker discovery and therapeutic modulation.

3.2. How the Gut Microbiome Modulates Anti-Tumor Immunity

The gastrointestinal tract houses the body's largest immune reservoir [27]. The gut microbiome modulates both innate and adaptive immune responses through two primary mechanisms: direct stimulation via microbial components (e.g., lipopolysaccharides activating Toll-like receptors) and indirect signaling via production of bioactive metabolites. (e.g., short-chain fatty acids and secondary bile acids). Table 2 provides examples of key mechanisms in microbiome-immune crosstalk and Figure 1 illustrates the microbiome's influence across the cancer-immunity cycle.

Table 2. Examples of Mechanisms of Microbiome-Immune Crosstalk.

Innate immunity	
- Maintenance of intestinal mucosal integrity and homeostasis through production of mucins and metabolites [28]. - Bacterial components (e.g., LPS and flagellin) activate pattern recognition receptors (e,g., TLRs), promoting maturation of dendritic cells (DCs), polarization of M1 macrophages, and activation of NK cells [29]. - Bacterial metabolites enhance the function of innate lymphoid cells [30].	
Examples of microbiome interactions across the cancer-immunity cycle	
Release of cancer cell antigens	- Microbial toxins can induce DNA damage, generating neoantigens [31] - The microbiota can influence tumor-associated antigens presentation and immunogenic cell death [32]
Antigen Presentation by Immune Cells & T-cell Activation and Priming	- Cross-reactivity between microbial and tumor antigens [33,34] - Promotes DC activation and maturation via TLR signaling [35] - Enhanced cytokine production (e.g., IL-1, IL-12) by dendritic cells [36] - Microbiota-derived SCFAs (butyrate and propionate) modulate DC function and T-cell differentiation by histone deacetylases (HDAC) inhibition [37–39]

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	- Microbiota-derived inosine boosts IFN-γ production in CD8+T cells via adenosine receptor (A2A) signaling [40]
T-cell Trafficking & T-cell Infiltration into the Tumor	- Regulation of chemokines and cytokines influencing T-cell trafficking (e.g., TNF-α, CXCL9, CXCL10) [41,42] - Modulation of immunosuppressive cells like Myeloid-Derived Suppressor Cells (MDSCs) [43,44]
Cancer Cell Recognition by T-cells & Tumor Cell Killing	- Influences the expression of immune checkpoint molecules (e.g., CTLA-4, PD-1/PD-L1) [45–47] - Can upregulate HLA class I on tumor cells, enhancing their recognition by T-cells[48,49]

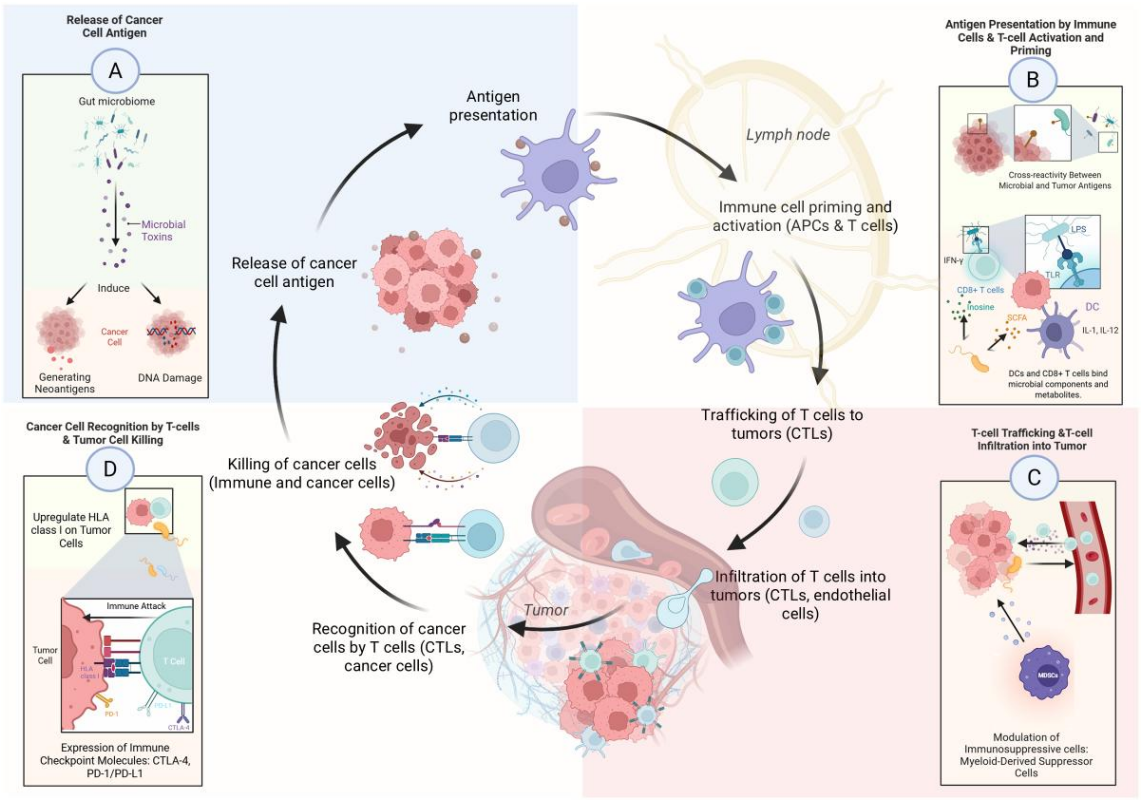

Figure 1. Examples of microbiome interaction across the cancer-immunity cycle.

The gut microbiome is not a passive bystander but an active modulator of systemic immunity, with the potential to either enhance or suppress antitumor immune responses. Its ability to influence every step of the cancer-immunity cycle makes it a powerful biomarker and a promising therapeutic target in oncology.

4. Analysis of Gut Microbiome and Response to ICIs

4.1. Analysis Pipeline Overview

The investigation of gut microbiome and ICI response follows a structured pipeline: (1) prospective sample collection at key timepoints (2) identification of patients into ICI responder and non-responder groups; (3) microbiome profiling and bioinformatic processing; and (4) statistical analysis to integrate microbiome data with clinical outcomes (Figure 2).

Reproducibility is a major challenge in microbiome research. Discrepancies between studies often arise from variations at each step of the pipeline. Adherence to reporting standards, such as the Minimum Information about a Marker Gene Sequence (MIMARKS), and detailed documentation of protocols are crucial for enabling cross-study comparisons and validating findings [50].

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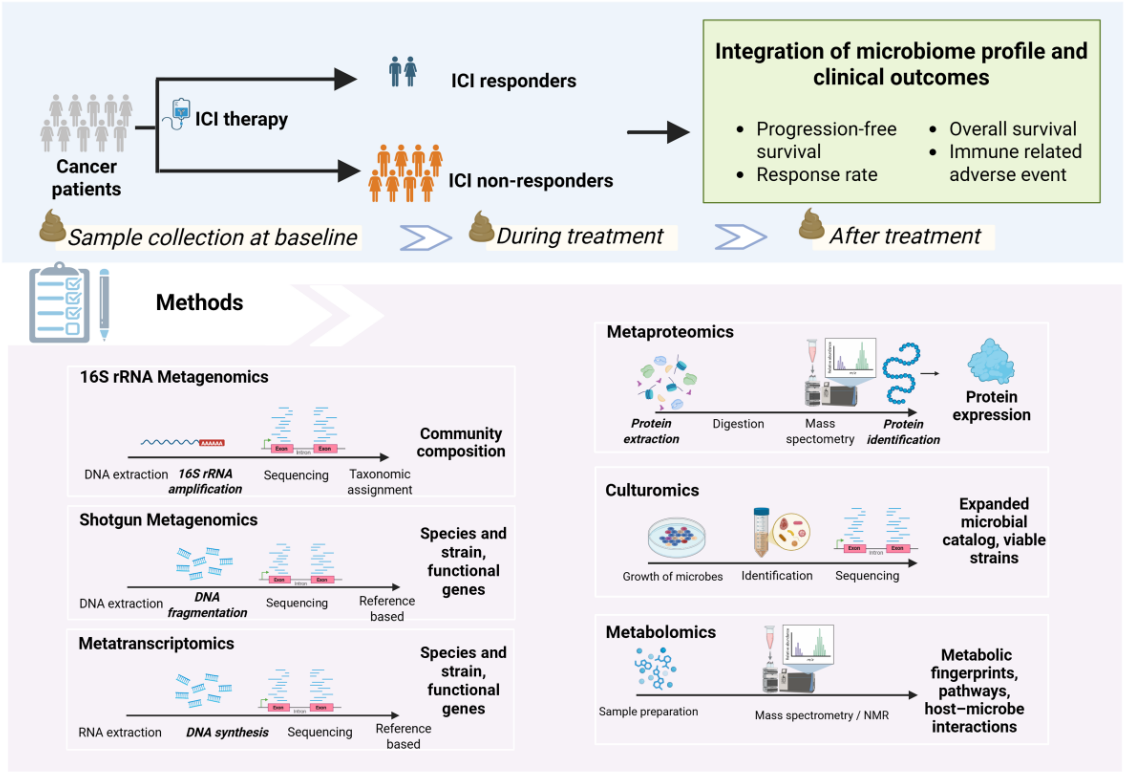

Figure 2. Analysis pipeline of gut microbiome and ICI response.

4.2. Sample Types for Microbiome Profiling

4.2.1. Fecal Samples

Fecal specimens are currently the gold standard for gut microbiome analysis, serving as a proxy for the distal colon's microbial community. Collection is non-invasive, repeatable, and provides sufficient biomass for analysis [51]. To preserve microbial integrity, samples should be immediately cryopreserved at −80°C or stored in commercial preservation buffers. Standardized protocols for collection, storage, and transport are essential, as variability can significantly alter results [52,53].

4.2.2. Oral Samples

Oral samples (e.g., saliva, swabs) are easily accessible but are less studied for systemic immune modulation [54]. These samples are also limited by low microbial biomass, high human DNA contamination, and significant variability between different oral sites [55,56].

4.2.3. Direct Gut Sampling (Swab, Biopsy and Swallowable Capsules)

Mucosal biopsy and luminal swab enable direct study of host tissue and microbiome interaction. Catheter aspiration facilitates sampling from difficult-to-reach sites. However, these methods are invasive, requiring special preparation and may not be suitable for routine use. Swallowable capsules can autonomously collect intestinal fluid at the targeted gut location with minimal invasiveness. Nevertheless, their adoption is limited by technical complexity and availability [51].

4.2.4. Tumor Samples

Historically considered sterile, tumor tissue is now recognized to harbor its own distinct microbial communities, referred to as the intratumoral microbiome. Early hints came from William B. Coley's work on bacterial toxin-mediated antitumor effects in the 1890s and successful microbe culture from tumor samples in the early 20th century [57,58]. Modern sequencing technologies have

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since confirmed their presence. Intratumoral microbes are now considered integral components of the tumor microenvironment and are linked to treatment outcomes, representing a promising new frontier of investigation [59–64].

4.3. Relative vs. Absolute Quantification of Microbiome

Microbiome data can be interpreted in two ways:

- **Relative abundance**: This is the default output of standard sequencing that measures the proportion of each microbe within a sample (e.g., *Bacteroides* make up 20% of the community). While widely used, this approach is prone to compositionality bias–an increase in one taxon will automatically appear as a decrease in others, even if their absolute numbers remain unchanged [65,66].
- **Absolute abundance**: This measures the actual number or concentration of microbes (e.g., 10^9 CFU/g *of Lactobacillus*). This method avoids compositional bias by integrating sequencing data with other techniques, such as quantitative PCR (qPCR), flow cytometry, or the addition of synthetic spike-in standards (reference DNA or microbes added in known quantities for calibration). This provides a true measure of microbial load, which is critical for accurate biological interpretation [67,68].

Relying on relative abundance alone can lead to false correlations [69,70]. For example, after probiotic administration, an increase in the relative abundance of Lactobacillus may reflect a decline in other commensals rather than true colonization. In contrast, detecting Lactobacillus at levels of 10^9 CFU/g via absolute quantification confirms successful engraftment and intervention efficacy. Absolute quantification is crucial for developing robust and reliable biomarkers.

4.4. Methods for Microbiome Analysis

4.4.1. Sequencing-Based Methods

- **16S rRNA gene sequencing**: This cost-effective method targets the 16s rRNA gene, a universal "barcode" present in all bacteria. The 16S rRNA gene contains conserved regions that serve as universal primer binding sites, as well as hypervariable regions that are species-specific and allow for taxonomic classification [71]. It provides a broad overview of community composition, typically at the genus level. While excellent for assessing overall diversity, its lower resolution makes species- or strain-level identification challenging [72,73]. Recent advances in full-length 16S rRNA sequencing have improved the taxonomic resolution of this technique [74,75].
- **Shotgun metagenomics:** This technique sequences all genomic DNA in a sample, providing a high-resolution view of the community at the species and strain level. It can also identify fungal, viral, archaeal, and protozoan communities [76]. Additionally, this approach enables the inference of the functional and metabolic potential of microbial communities at the gene level. However, precise identification of novel functional genes may be limited by the availability and comprehensiveness of reference databases [77,78].
- **Metatranscriptomics (RNA-Seq):** This method analyzes RNA to reveal which microbial genes are actively expressed. It offers a dynamic snapshot of the microbiome's true functional activity, but it is technically challenging due to RNA's instability and complex data analysis [79,80]

4.4.2. Culture- and Metabolic-Based Methods

- **Culturomics:** While sequencing identifies microbes by their genetic code, culturomics aims to grow them in the laboratory. By using diverse culture conditions, this technique allows for the isolation of live strains, including rare or novel bacteria that may be missed by traditional methods [81]. Culturing microbes enables functional experiments and developing next-generation probiotics [82,83]. However, microbial culturing is labor-intensive, costly, requires advanced infrastructure, and carries a risk of contamination [84].

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- **Metabolomics:** This approach identifies and quantifies the small-molecule metabolites produced by the host and microbiome using mass spectrometry (MS) or nuclear magnetic resonance (NMR) [85]. Linking metagenomic data (the community's genetic potential) with metabolomic data (its actual chemical output) can provide deep mechanistic insights [86]. However, untargeted metabolomics has some limitations, including difficulty in accurately identifying many metabolites and interference from matrix effects such as ion suppression, which can affect measurement accuracy and make comparisons between studies challenging [87,88].

4.4.3. Multi-Omics Integration: A Holistic view

The most robust insights now emerge from multi-omics approaches that integrate metagenomics, metatranscriptomics, proteomics, and metabolomics. This holistic strategy can unravel complex host-microbe interactions and identify powerful, multifaceted biomarkers. While challenges in standardization and data complexity remain, multi-omics integration is essential for translating microbiome discoveries into reliable clinical applications.

5. Microbial Features Associated with ICI Response

Although reproducibility can be a challenge in microbiome research, several key microbial features have been consistently identified as predictors of ICI efficacy. These signatures represent promising biomarkers and therapeutic targets.

5.1. Microbial Diversity

Microbial diversity is assessed in two main ways:

- **Alpha diversity:** The richness (number of different organisms present) and evenness (their relative abundance) within a single sample.
- **Beta diversity:** The degree of compositional difference between samples.

A consistent finding across multiple cancer types is that higher alpha diversity at baseline is associated with improved response to ICI therapy [89–92]. Conversely, reduced diversity that is often observed in patients exposed to antibiotics correlates with poorer clinical outcomes [89,93].

5.2. Beneficial Bacterial Taxa

Most studies implemented 16S rRNA sequencing or shotgun metagenomics to measure relative abundance. Several bacterial taxa have been linked to enhanced ICI response:

5.2.1. *Akkermansia muciniphila*

A. muciniphila is a common human gut microbiota, specializing in degrading mucin and maintenance intestinal integrity [94]. In preclinical models, oral administration of *A. muciniphila* can reinvigorate exhausted T cells and restore anti-PD1 efficacy [95]. Higher relative abundance of *A. muciniphila* in stool at baseline is associated with improved progression-free survival in non-small cell lung cancer (NSCLC) and renal cell carcinoma (RCC) treated with PD-1 blockade [96–98]. In a prospective study of 338 advanced NSCLC patients, baseline stool *A. muciniphila* was associated with increased objective response rates and overall survival, independent of PD-L1 status [99].

5.2.2. *Faecalibacterium prausnitzii*

F. prausnitzii, a major butyrate producer, is one of the most abundant bacteria in gut microbiota, representing more than 5% of gut microbiota in healthy adults [100,101]. In patients diagnosed with cancer, the relative abundance of *F. prausnitzii* was significantly reduced compared to non-cancer subjects [102,103]. In vitro, *F. prausnitzii* strain EXL01 enhances dendritic cell and T cell activity and oral administration of this strain restores anti-PD-L1 efficacy in mouse models with antibiotic-

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induced microbiota disruption [104] Enrichment of *F. prausnitzii* at baseline has been observed in ICI responders across multiple cancer types [90,105,106].

5.2.3. Bifidobacterium Species

Bifidobacteria are predominant in infants but decrease to less than 10% of the microbiota in adults [107]. In mouse models, oral administration of *Bifidobacterium* enhances ICI effects by augmenting dendritic cell function and producing the metabolite inosine [40,108–110]. Enrichment of *B. longum* and *B. breve* has been associated with improved outcomes in melanoma and NSCLC patients receiving ICIs, respectively [111,112].

5.2.4. Ruminococcaceae Family

Members of the Ruminococcaceae family are highly prevalent in the human gut microbiome with certain species having been linked to ICI response [113]. For example, *R. gnavus* enhanced anti–PD-1 efficacy in mouse models by promoting $CD4^+$ T cell migration into tumors and activating pro-inflammatory macrophages. [114]. In clinical cohorts of advanced NSCLC and melanoma patients, the *Ruminococcaceae* family was enriched in ICI responding patients [89,115]

5.3. Key Microbial Metabolites

5.3.1. Short-Chain Fatty Acids (SCFAs)

SCFAs—notably butyrate, acetate, and propionate—are produced by the gut microbiota through fermentation of dietary fiber. They play critical roles in linking the microbiome to both local and systemic immune regulation. These functions are mediated by several key mechanisms, including histone deacetylase (HDAC) inhibition, G-protein-coupled receptor (GPCR) signaling, and modulation of cellular energy and signaling pathways [116]. However, clinical data are mixed: some studies link high fecal and plasma SCFA levels to better ICI outcomes [117–119], while others find no significant correlation [120–122].

5.3.2. Inosine

Inosine, a purine nucleoside, is involved in purine metabolism, RNA function, and immune modulation [123]. Produced by bacteria like *Bifidobacterium pseudolongum,* inosine activates anti-tumor T-cells through adenosine A2A receptor signaling in preclinical models [40]. In RCC patients, higher plasma inosine levels were associated with response to nivolumab [124].

5.3.3. Tryptophan Metabolites

The essential amino acid tryptophan can be metabolized through multiple pathways that influence both cancer progression and anti-tumor immunity, including the kynurenine, indole-3-pyruvate, and serotonin pathways. Tumor cells frequently channel tryptophan into the immunosuppressive kynurenine pathway, whereas commensal gut microbes can redirect it toward alternative routes. For example, gut bacteria can convert tryptophan into a ligand for the aryl hydrocarbon receptor (AHR), a key regulator of intestinal homeostasis [125]. Certain metabolites such as indole-3-aldehyde (I3A) produced by *Lactobacillus reuteri* enhance the activity of CD8+ T cells in mouse models [126]. In cancer patients, plasma biomarkers such as the kynurenine-to-tryptophan (Kyn/Trp) ratio and metabolites like I3A and 3-hydroxyanthranilic acid (3-HAA) correlate with ICI efficacy [126–128].

5.3.4. Secondary Bile Acids

In addition to their role in lipid absorption, bile acids function as systemic signaling molecules regulating host metabolism and immune function [129]. Primary bile acids are synthesized in the liver and subsequently converted by microbial enzymes, mainly from *Clostridium* species such as

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Clostridium butyricum, into secondary bile acids. These secondary bile acids display stronger activation of host nuclear receptors than their primary counterparts [130]. In hepatocellular carcinoma (HCC) models, accumulation of conjugated bile acids in tumors impairs anti-PD-1 efficacy, whereas dietary supplementation of ursodeoxycholic acid (UDCA) suppresses tumor growth and promotes T cell responses [131]. Clinically, HCC patients receiving ICIs who had fecal enrichment of UDCA and ursocholic acid demonstrated improved treatment outcomes [132].

5.4. The Importance of Temporal Dynamics

The gut microbiome is not static; its composition shifts dynamically following ICI treatment, with distinct patterns emerging between responders and non-responders. Notably, certain microbial alterations emerge only after treatment initiation, rather than at baseline.

In melanoma and NSCLC patients receiving ICI therapy, responders exhibit stable microbial taxa and functional profiles over time compared to non-responders [133,134]. Other studies have shown that both responders and non-responders develop unique microbial abundance patterns after ICI initiation, even when baseline differences are minimal or absent [91,135,136].

5.5. Tools Incorporating Microbial Signature to Predict Prognosis and ICI Response

Emerging tools are being developed to integrate microbial signatures into prognostic models and ICI response prediction. Although still in early stages and requiring further validation, these approaches demonstrate the translational potential of microbiome-informed biomarkers.

- **TOPOSCORE:** Developed from metagenomic data of 245 NSCLC patient feces combined with *Akkermansia* quantification, TOPOSCORE is a qPCR-based assay targeting 21 bacteria to evaluate personal intestinal dysbiosis. Validated in NSCLC, colorectal cancer, genitourinary cancer and melanoma patients, TOPOSCORE was able to stratify patients with improved ICI outcomes. The test can be performed within 48 hours, making it potentially suitable for routine clinical practice [137].
- **miCRoScore:** miCRoScore is a composite muti-omics biomarker developed from microbiome and immune gene signature of 348 colon cancer patients. It outperforms conventional prognostic biomarkers in colon cancer, including Consensus Molecular Subtypes (CMS) and microsatellite instability, in predicting survival probability. Patients classified with high mICRoScore showed an excellent 97% 5-year overall survival in the training cohort, with no colon cancer-related deaths observed in the external validation cohort. [138]

6. Therapeutic Applications of the Gut Microbiome

The early evidence supporting the therapeutic potential of the gut microbiome in oncology came from preclinical studies in which fecal microbiota transplantation (FMT) from ICI-responsive patients into germ-free mice enhanced antitumor immune responses, whereas FMT from non-responders did not [96,112,115]. These findings were later translated into early-phase clinical trials. Subsequent proof-of-concept clinical studies in ICI-refractory melanoma patients confirmed that FMT in combination with re-induction anti-PD1 could overcome resistance to ICI in a subgroup of patients through the change in gut microbiome and tumor microenvironment [139,140]. Building on these findings, several strategies are now under investigation to modulate the microbiota with the aim of enhancing immunotherapy efficacy and mitigating treatment-related toxicity. These include FMT, supplementation of pre-, pro- and postbiotics (collectively referred to as biotics), antibiotic modulation, and engineered microbial strains (Figure 3).

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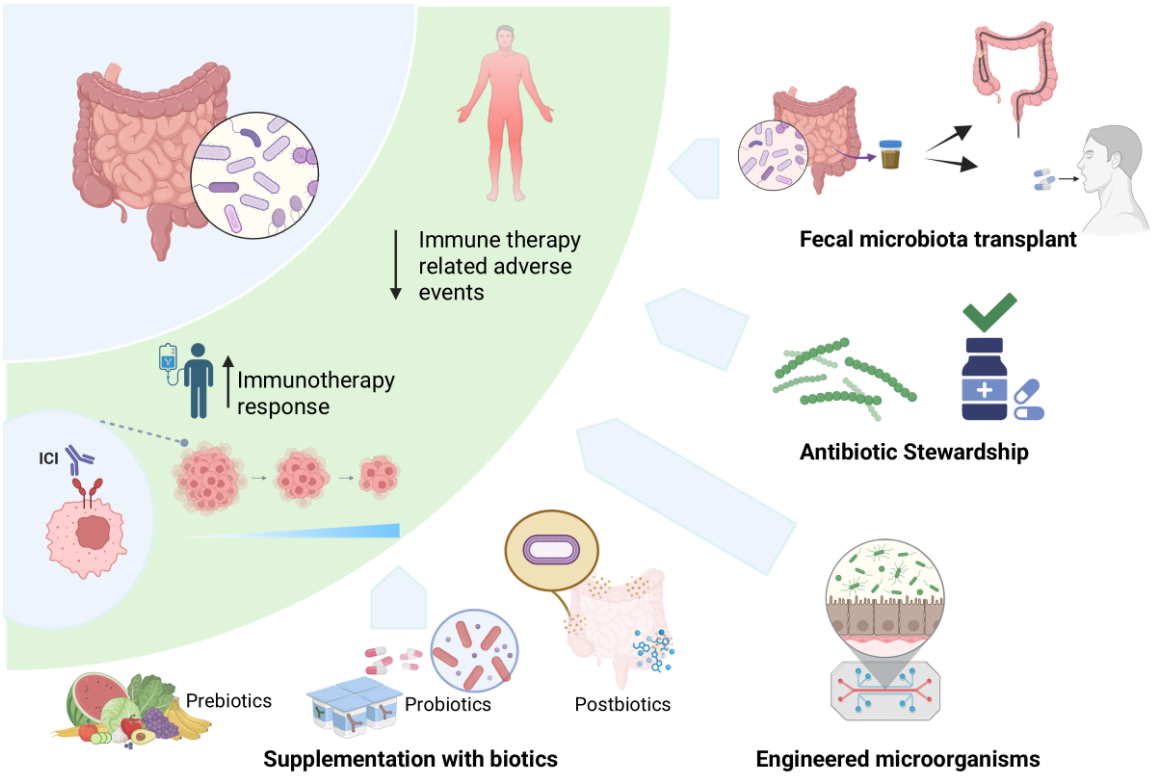

Figure 3. Therapeutic applications of the gut microbiome to enhance ICI therapy.

6.1. Fecal Microbiota Transplant (FMT)

6.1.1. FMT to Enhance ICI Efficacy

Early phase studies of FMT in combination with anti-PD1 re-challenge in ICI-refractory melanoma showed encouraging signals, with overall response rates (ORR) of 20-30% and durable disease control exceeding 6-12 months in responders. Responders of FMT typically demonstrated greater donor microbiome engraftment and enrichment of specific bacterial taxa. More recent studies have expanded FMT evaluation to earlier-line settings and across multiple tumor types (Table 3).

Table 3. Selected clinical trials of FMT combined with immune checkpoint inhibitors.

Study	N	Phase	Population	Intervention	Key outcomes	Grade ≥3 irAEs
Baruch et al. (2021) [139]	10	I	ICI-refractory melanoma	Responder-derived FMT + Nivolumab	ORR 30%; all responders with >6 mo PFS	0%
Davar et al. (2021) [140]	15	I	ICI-refractory melanoma	Responder-derived FMT + Pembrolizumab	ORR 20%; 3 patients with >12 mo stable disease	0%
MiMic (2023) [141,142]	20	II	Untreated meta-static melanoma	Healthy Donor FMT + pembrolizumab or nivolumab	ORR 65%; median PFS 29.6 mo; median OS 52.8 mo	25%
Kim et al. (2024) [143]	13	I	ICI-refractory solid cancer Gastric (n = 4), esophageal (n =5), HCC (n = 4)	Responder-derived FMT + nivolumab	ORR 7.7%	7.7%

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RENMIN-215 (2023) [144,145]	20	II	Refractory meta-static MSS colo-rectal cacer, >3 lines of treatment	Responder-derived FMT + Tislelizumab + Fruquintinib	ORR 20%; median PFS 9.6 mo; median OS 13.7 mo	10%
FMT-LUMINate (2024) NSCLC cohort [146]	20	II	Untreated meta-static cutaneous melanoma	Healthy Donor FMT + anti-PD1	ORR 80%	0%
FMT-LUMINate (2024) Melanoma Cohort [146]	20	II	Untreated meta-static cutaneous melanoma	Healthy Donor FMT + anti-PD1 + anti-CTLA4	ORR 75%	65% - Myocarditis 15%
TACITO (2024) [147]	50	II	Untreated meta-static renal cell carcinoma	Intervention Responder-derived FMT + pembrolizumab + axitinib Control Placebo + pembrolizumab + axitinib	ORR 54% vs. 28% Median PFS 14.2 vs. 9.2 mo; 1-year PFS rate 66.7% vs. 35%; median OS NR vs. 25.3 mo	10%

FMT was generally safe and feasible across studies, with most adverse events related to mild gastrointestinal symptoms (grade 1-2). Immune-related adverse events (irAEs) were overall comparable to historical anti-PD1 data. The high incidence of grade ≥3 irAEs in the FMT-LUMINate melanoma cohort likely reflects the addition of dual ICI therapy and may be donor-related factors (43% of participants received FMT from the same donor).

Despite promising efficacy signals, FMT protocols have shown considerable heterogeneity, including the selection of donors (responder-derived versus healthy individuals), the use of different preconditioning regimens (antibiotics and bowel preparation), and the route of administration (oral and colonoscopy), with or without maintenance dosing.

Higher microbial engraftment has consistently been associated with improved clinical outcomes. Identification of species-specific engraftment patterns and integrating metagenomic analyses may enable personalized FMT strategies [148–150]. Ongoing efforts aim to define the optimal donor characteristics, preparation, and delivery methods to maximize therapeutic benefit.

6.1.2. FMT to Mitigate ICI-Induced Colitis

Colitis is the most extensively studied irAE in relation to gut microbiome composition. Reduced microbiome diversity and prior antibiotic use have been linked to the development of ICI-related colitis [151–154]. Shifts in microbial composition, particularly with strain-specific enrichment, have also been observed; however, the results are inconsistent [155–158]. Administration of *F. prausnitzii* and *Bifidobacterium* helps mitigate ICI-related colitis in animal models [159,160]. Several case reports have shown improvement of steroid-refractory immune-related colitis following FMT administration [161,162]. Ongoing clinical trials are exploring the benefit of FMT in preventing (Clinical Trials.gov identifier: NCT04163289 and NCT06508034) and treating immune-related colitis in patient receiving ICIs (Clinical Trials.gov identifier: NCT04038619, NCT06206707, and NCT06499896). Preliminary

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data from a prospective study suggest that front-line FMT is safe and can serve as an effective treatment while reducing the need for corticosteroids [163].

6.2. Supplementation with Biotics: A Targeted Approach

Unlike FMT that introduces the entire microbial community and environment, biotics intervention such as prebiotics, probiotics, and postbiotics provides a more selective targeted approach to transform the gut microbiome environment and the host immune system. According to the International Scientific Association for Probiotics and Prebiotics (ISAPP) the definitions of prebiotics, probiotics and postbiotics are summarized in Table 4.

Table 4. Definition of biotics according to ISAPP [164–166].

Biotic	Definition	Function	Examples
Prebiotics	substrates that are selectively utilized by host microorganisms conferring a health benefit	nourish beneficial microbes, promoting their growth and metabolite production	Galactooligosaccharides (GOS), Fructooligosaccharides (FOS), Inulin, lactulose; naturally present in whole grains, onions, garlic, asparagus, bananas
Probiotics	live microorganisms that, when administered in adequate amounts, confer a health benefit.	directly introduce beneficial microbes to shape the gut environment	Fermented foods such as yogurt, kefir, miso, natto, kimchi, and some cheeses containing specific live microbes (e.g., *Lactobacillus acidophilus*, *Bifidobacterium longum*)
Postbiotics	preparation of inanimate microorganisms and/or their components that confer a health benefit.	deliver beneficial effects without living organisms, using inactivated microbial cells, components, or metabolites	Heat-inactivated *Bifidobacterium* or *Lactobacillus*, bacterial lysates

Per the ISAPP definition, purified metabolites alone, such as isolated butyric acid, do not meet the definition of postbiotic because they are not part of an inactivated microbial preparation.

Traditional probiotics mainly include a limited number of species such as *Lactobacillus* and *Bifidobacterium*, which have a long history of safe use as food ingredients or dietary supplements. Advances in microbiology techniques and bioinformatics have enabled the precise identification and culture of novel gut microbes with therapeutic potential. These next-generation probiotics (NGPs) include *Prevotella copri*, *Christensenella minuta*, *Parabacteroides goldsteinii*, *Akkermansia muciniphila*, *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*, *Bacteroides fragilis*, and *Eubacterium hallii [167,168]*. Notably, *A. muciniphila* and *F. prausnitzii* have shown potential to enhance immune checkpoint inhibitor (ICI) efficacy in preclinical and translational studies as mentioned above.

Several phase 1 studies have explored the benefits of adding probiotics in combination with ICIs in advanced RCC patients (Table 5)

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Table 5. Selected phase 1 clinical trials of probiotics combination with ICIs in advanced RCC patients.

Study	N	Phase	Population	Primary endpoint	Results
Dizman et al. (2022) [169]	30	I	Nivolumab + ipilimumab ± CBM588 (*Clostridium butyricum*)	Change in *Bifidobacterium* spp. Abundance at 12 weeks	No difference in *Bifidobacterium* spp. abundance. CBM588 arm had significantly improved PFS (12.7 vs 2.5 mo) and ORR (58% vs 20%). No increase in toxicity.
Ebrahimi et al. (2024) [170]	30	I	Cabozantinib + nivolumab ± CBM588	Change in *Bifidobacterium* spp. Abundance at 13 weeks	No difference in *Bifidobacterium* spp. abundance. CBM588 arm had significant higher ORR (74% vs 20%, P=0.01). 6-mo PFS: 84% vs 60%. No increase in toxicity.
Derosa et al. (2025) [171]	9	I	Nivolumab + ipilimumab + Oncobax®-AK (*Akkermansia massiliensis* strain p2261, SGB9228) in patients lacking stool Akkermansia	ORR, pharmacodynamics, safety	ORR 50% with evidence of immune and metabolic modulation. No increase in toxicity.

Despite promising mechanistic insights, clinical data on the use of biotics to enhance immunotherapy in cancer patients remain in the early stage. Current challenges include heterogeneity and a lack of standardized quality control of biotic products, interference from patients' background diet, and limited trial design accounting for microbiome variability. Therefore, no firm recommendations on routine use of prebiotics, probiotics, or postbiotics in ICI-treated patients can be made at this time. Several ongoing trials are now investigating dietary interventions and biotic supplementation as adjuncts to ICIs across multiple cancer types, aiming to establish whether microbiome-targeted strategies can improve response rates and reduce immune-related toxicities [172].

6.3. Engineered Microorganisms

Advances in genetic and synthetic biology have enabled the development of engineered microorganisms that can augment immunotherapy against cancer in multiple aspects. By modifying microbial chassis, researchers can add features that promote immune activation while minimizing systemic side effects associated with naturally derived microbes (Table 6).

Table 6. Examples of early-phase clinical trials exploring engineered microorganisms in combination with ICIs.

Mechanisms	Examples
Presentation of tumor antigens and cancer vaccine carriers	• Phase 2: ADXS-503, an engineered *Listeria monocytogenes* expressing 22 common NSCLC antigens, combined with pembrolizumab after progression. Induced antigen-specific T-cell responses with 15% ORR (2/13) [173]. • Phase 2a: VXM01, an oral VEGFR2 DNA vaccine delivered via engineered *Salmonella Typhi* Ty21a,

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	combined with avelumab in recurrent glioblastoma. Showed 12% ORR (3/25) [174].
Cytokine and chemokine release to enhance immune function	• Phase 1: SYNB1891, engineered *E. coli* Nissle expressing a STING agonist, given intratumorally ± atezolizumab. Activated IFN pathways and immune gene signatures in refractory cancers[175].

Beyond antigen delivery and cytokine release, engineered strains can also remodel the tumor microenvironment and deliver immunomodulatory payloads directly into the microenvironment. However, challenges include limited tumor colonization in humans, competition with endogenous microbiota, potential systemic inflammation, and reproducibility [176,177].

7. Conclusions and Outlook

The clinical translation of gut microbiome research in immuno-oncology depends on overcoming key challenges of standardization and validation. Future progress will require:

- **Methodological rigor:** implementation of absolute quantification, multi-omics integration, and standardized protocols for sample collection, processing and analysis
- **Innovative trial designs**: prospective studies that incorporate dietary profile, antibiotic exposure, and dynamic microbial signatures, with interval stool sampling and predefined microbiome-specific endpoint
- **Refined interventions:** development of optimized microbial consortia, inclusion of next-generation biotics, standardized reporting of biotic composition and FMT protocols, and rational FMT donor selection within a robust safety framework to enable scalability

The relationship between gut microbiome and cancer immunotherapy has evolved from a compelling association to an emerging field with growing clinical relevance and real clinical potential. For the practicing medical oncologist, the most immediate and impactful strategies remain prudent antibiotic stewardship and encouraging patient enrollment in microbiome-focused clinical trials.

In summary, the gut microbiome represents both powerful biomarker and a therapeutic target capable of reshaping immunotherapy outcomes. Continued methodological refinement, innovative clinical approaches, and translational research will be critical to realize its full potential and bring microbiome-informed strategies into routine oncology practice.

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Abbreviations

The following abbreviations are used in this manuscript:

16S rRNA	16S ribosomal RNA
3-HAA	3-hydroxyanthranilic acid
A2A	Adenosine A2A receptor
AHR	Aryl hydrocarbon receptor
CFU	Colony-forming units (e.g., CFU/g)

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CMS	Consensus Molecular Subtypes (colon cancer)
CTLA-4	Cytotoxic T-lymphocyte–associated protein 4
CXCL9 / CXCL10	C-X-C motif chemokine ligand 9 / 10
DC / DCs	Dendritic cell(s)
dMMR	Deficient mismatch repair
DNA	Deoxyribonucleic acid
E. coli	Escherichia coli
FDA	U.S. Food and Drug Administration
FMT	Fecal microbiota transplantation
GPCR	G-protein–coupled receptor
H&E	Hematoxylin and eosin (stain)
HCC	Hepatocellular carcinoma
HDAC	Histone deacetylase
HLA	Human leukocyte antigen
ICI / ICIs	Immune checkpoint inhibitor(s)
IFN-γ	Interferon-gamma
IL-1 / IL-12	Interleukin-1 / Interleukin-12
IHC	Immunohistochemistry
I3A	Indole-3-aldehyde
irAE(s)	Immune-related adverse event(s)
Kyn/Trp	Kynurenine-to-tryptophan ratio
LPS	Lipopolysaccharide
MDSC(s)	Myeloid-derived suppressor cell(s)
MLH1, PMS2, MSH2, MSH6	Mismatch repair proteins/genes
MMR	Mismatch repair
MS	Mass spectrometry
MSI	Microsatellite instability
MSI-H	Microsatellite instability–high
NGP(s)	Next-generation probiotic(s)
NGS	Next-generation sequencing
NK (cells)	Natural killer (cells)
NMR	Nuclear magnetic resonance
NR	Not reached (survival endpoint)
NSCLC	Non-small-cell lung cancer
ORR	Objective response rate
OS	Overall survival
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PFS	Progression-free survival
POLE / POLD1	DNA polymerase epsilon / delta 1 (genes)
qPCR	Quantitative PCR
RCC	Renal cell carcinoma
RNA-Seq	RNA sequencing (metatranscriptomics)
SCFA(s)	Short-chain fatty acid(s)
STING	Stimulator of interferon genes
TIL(s)	Tumor-infiltrating lymphocyte(s)

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TLR(s)	Toll-like receptor(s)
TMB	Tumor mutational burden
TNF-α	Tumor necrosis factor-alpha
UDCA	Ursodeoxycholic acid
VEGFR2	Vascular endothelial growth factor receptor 2

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