

Decoding the Immuno-Oncology-Microbiome axis: Breakthroughs in mechanisms, technologies, and clinical applications

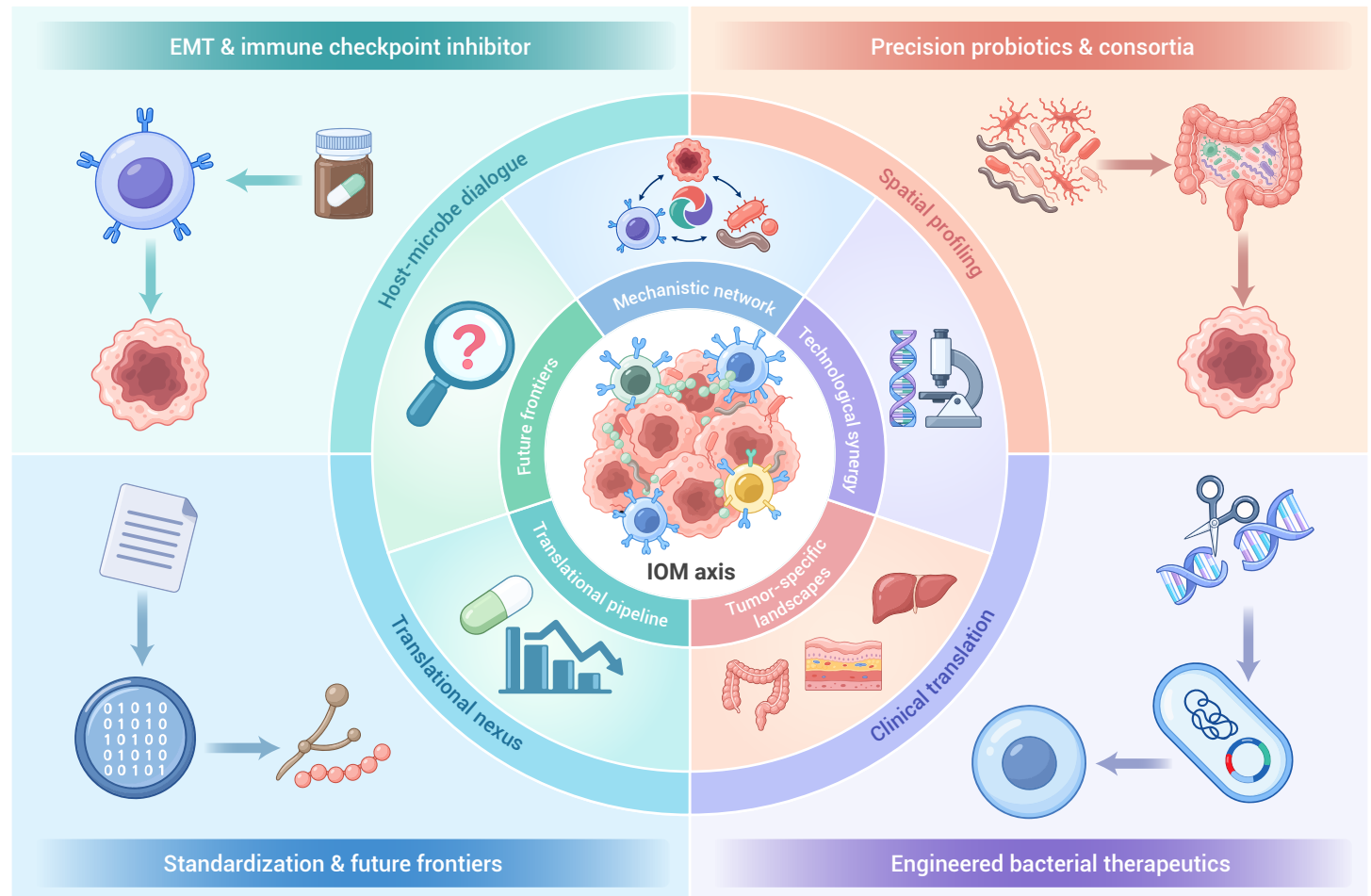
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- The IOM axis links microbes, immune cells, and tumors.
- Beneficial microbes boost immunotherapy, but *Fusobacterium nucleatum* can block immune cells.
- Single-cell and spatial sequencing reveal distinct microbial signatures in cancers.
- Clinical efforts now test fecal microbiota transplantation (FMT), probiotics, and engineered bacteria.
- Hurdles include high costs, technical variability, and strain-specific opposite effects.

Decoding the Immuno-Oncology-Microbiome axis: Breakthroughs in mechanisms, technologies, and clinical applications

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The Immuno-Oncology-Microbiome (IOM) axis is a tripartite regulatory system involving microbiota, immune cells, and tumors, which critically modulates cancer progression and therapy response. This systematic review synthesizes key advances from 2022 to 2025, illustrating the field's evolution from macro-associations to precise molecular mechanisms, driven by innovations in single-cell multi-omics, spatial biology, and synthetic biology. Mechanistically, the IOM axis functions *via* bidirectional pathways: immunostimulatory effects are mediated by microbial metabolites and components that sensitize tumors to immunotherapy, while immunosuppression is induced by pathobionts that remodel the tumor microenvironment to promote immune evasion and chemoresistance. Technologically, high-resolution tools have replaced traditional sequencing, enabling host-microbe transcriptomics, microbial microniche mapping, and the development of engineered bacterial therapeutics. Cancer-specific IOM signatures reveal organ-axis specialization, with distinct immune crosstalk patterns in colorectal cancer, melanoma, and hepatocellular carcinoma. Translational advances include standardized fecal microbiota transplantation, cancer-type specific probiotics/postbiotics, and engineered bacterial platforms, alongside microbial biomarkers for predicting therapy response. Despite progress, bottlenecks such as technical inconsistency, high costs, inter-individual heterogeneity, and understudied low-abundance taxa remain. Future priorities focus on low-cost spatial technologies, strain-level mechanistic resolution, and standardized clinical implementation, positioning microecological therapies as integral to precision oncology.

INTRODUCTION

Definition and scientific value of the Immuno-Oncology-Microbiome axis

The Immuno-Oncology-Microbiome (IOM) axis is defined as a tripartite dynamic interaction system encompassing microbiota, immune cells, and tumor cells within the tumor microenvironment (TME). This conceptual framework was first formally articulated by Sepich-Poore et al. to describe the dual influence of microbes on cancer pathogenesis through host immunity.¹ It integrates both the “remote regulation” mediated by gut microbiota through metabolites or immune cell modulation and the “in situ effects” exerted by intratumoral microbiota *via* direct contact or local metabolite secretion, thereby establishing a comprehensive regulatory axis from microbial communities to host antitumor immunity. Recent work by Ding, Liu, and Li further extended this framework to emphasize its clinical translational dimensions.² Notably, the tumor-associated microbiome extends beyond bacteria; fungal microbiota (mycobiome), although constituting only 0.1–1% of the total microbial biomass, represent critical immunologically active components that significantly influence tumor initiation, progression, and therapeutic responses.³ Clinically, the profound impact of microbiota on immunotherapy efficacy has been extensively validated. Both intestinal and intratumoral microbiomes have emerged as pivotal determinants of immune checkpoint inhibitor (ICI) responses, with methodological advances accelerating mechanistic understanding and translational applications.⁴ The gut microbiome has been established as a key determinant of immunotherapy efficacy across multiple solid tumor types, positioning the IOM axis as a critical target for overcoming immune resistance and enabling precision inter-

vention.^{4,5} Elucidating the definition and regulatory networks of the IOM axis thus holds substantial scientific and translational value for advancing personalized cancer immunotherapy.

Three landmark phases of the research trajectory

The research trajectory of the IOM axis can be delineated into three progressive phases. The foundational period (2015–2020) established the critical link between the gut microbiome and immune checkpoint inhibitor (ICI) efficacy. Three landmark 2018 Science publications demonstrated that antibiotic-induced gut microbiome disruption diminishes responses to PD-1 blockade in melanoma patients, with specific bacteria such as *Ruminococcaceae* significantly enriched in responders.^{5–7} The deepening period (2021–2023) extended this paradigm from gut microbiota to intratumoral microbes. A pivotal 2022 Nature study employing spatial transcriptomics and INVADE-seq revealed that *Fusobacterium nucleatum* colonizes immunosuppressive microniches within colorectal cancer tissues, directly modulating the local immune microenvironment.⁸ Concurrent research demonstrated that *F. nucleatum*-derived formate promotes colorectal cancer progression,⁹ and that the chemotherapeutic agent 5-fluorouracil directly inhibits this pathogen.¹⁰ The precision period (2024–2025) has begun to achieve single-cell and spatial resolution of IOM axis interactions. The scRandom-seq technology, published in Angewandte Chemie in 2024, enables simultaneous capture of host and microbial single-cell transcriptomes, revealing bacterial infection-induced host cell heterogeneity.¹¹ The Standards for Technical Reporting in Environmental and host-Associated Microbiome Studies (STREAMS) guidelines now provide standardized technical reporting frameworks for environmental and host-associated microbiome studies,¹² while international consensus statements establish standardized frameworks for clinical microbiome testing.¹³ This paradigm evolution from macro-association to precise resolution has established a robust foundation for mechanistic exploration and clinical translation of IOM axis research.

Logical framework of this review

This review is structured along a closed-loop trajectory of “mechanism dissection → technological support → cancer-type validation → clinical translation → challenges and prospects,” emphasizing the progressive logic that “technological innovation deepens mechanistic understanding, and mechanistic insights guide clinical application.” By tracing the evolution of the IOM axis from macro-level associations to molecular resolution, the review integrates recent breakthroughs in single-cell and spatial multi-omics to illustrate how high-precision tools are reshaping our understanding of host-microbe interactions. In contrast to correspondence-style summaries with narrow focus, this review introduces three distinctive modules: (i) cancer-type-specific IOM mechanisms, highlighting how organ-specific microbial niches dictate unique immune landscapes; (ii) interdisciplinary technology convergence, showcasing the synergistic integration of synthetic biology, spatial omics, and computational modeling; and (iii) graded clinical translation, stratifying microbiota-based interventions into biomarker-driven, interventional, and engineered therapeutic levels.

While a recent iMeta article by Pi et al. provided a multi-level overview of the IOM axis with a focus on biotechnology platforms and therapeutic strate-

gies, the present review differs in several fundamental aspects.¹⁴ First, it adopts a closed-loop “mechanism-to-translation” trajectory rather than a technology-centered organization, systematically linking mechanistic dissection to graded clinical translation. Second, it introduces a rigorous evidence-grading framework that explicitly distinguishes preclinical, retrospective, and prospective clinical evidence throughout. Third, it dedicates independent modules to cancer-type-specific IOM signatures (Section Cancer-Specific IOM Axis Signatures) and cross-kingdom fungal–bacterial interactions (Section The Fungal Dimension: Cross-Kingdom Interactions and Immune Regulation)—topics that are not developed in the iMeta article. Fourth, its temporal scope (2022–2025) captures the most recent breakthroughs post-dating the earlier review. These features collectively position the present work as a complementary and updated synthesis of the rapidly evolving IOM field.

Together, this framework bridges the entire spectrum from fundamental microbiology to bedside application, offering a comprehensive and forward-looking reference for both basic researchers and clinicians navigating the rapidly evolving IOM field.

CORE MECHANISMS OF THE IOM AXIS: FROM “MACRO-ASSOCIATION” TO “MOLECULAR INTERPLAY”

Bidirectional pathways of microbiota in modulating tumor immunity

Immune-activating pathways: pro-therapeutic directions. Microbial components potentiate cancer immunotherapy through two complementary axes: direct remodeling of tumor cell immunogenicity and systemic enhancement of antigen presentation and effector cell function.

In the first axis, distinct microbial metabolites and proteins target multiple layers of immune checkpoint regulation, forming a cascading intervention. Short-chain fatty acids (SCFAs) upregulate major histocompatibility complex class I (MHC-I) expression on tumor cells *via* histone deacetylase (HDAC) inhibition, increasing tumor visibility and sensitizing immunologically “cold” chromosomal instability (CIN) colorectal cancer to checkpoint blockade.¹⁵ Building on this, *Akkermansia muciniphila* outer membrane proteins further suppress PD-L1: Amuc_1434 inhibits its transcription, while Amuc_1100 dampens JAK-STAT signaling or promotes RNF181-mediated PD-L1 degradation, collectively blocking immune evasion at both transcriptional and post-translational levels.^{16,17} Ursodeoxycholic acid (UDCA), a licensed hepatoprotective drug, reverses CD8⁺ T cell exhaustion by inhibiting farnesoid X receptor (FXR), synergizing with the above tumor-directed strategies to restore effector function.¹⁸

The second axis enhances antigen cross-presentation and effector cell fitness. Flagellin, a TLR5 agonist, activates dendritic cells to license T cell priming.¹⁹ Engineering chimeric antigen receptor (CAR) T cells to express flagellin transforms them into “adjuvant-delivery platforms,” concurrently killing tumor cells and activating intratumoral antigen-presenting cells. Thermoresistant flagellin (FlaB) serves as a vaccine adjuvant combined with photothermal therapy, inducing immunogenic cell death and releasing tumor antigens for cross-priming—a strategy complementary to CAR-T-mediated endogenous antigen presentation.²¹ In parallel, dietary mannose enriches butyrate/propionate-producing microbiota, expanding the progenitor-exhausted CD8⁺ T cell (Tpex) pool and bolstering the effector reservoir.²² Meanwhile, the monoamine oxidase (MAO) inhibitor phenelzine combined with *A. muciniphila* clears immunosuppressive metabolite 5-hydroxyindoleacetic acid (5-HIAA), relieving microenvironmental inhibition and synergizing with the above activating strategies.²²

These strategies, though all aimed at enhancing antitumor immunity, span a wide spectrum of evidential maturity and translational proximity. UDCA, mannose, and SCFAs leverage existing non-oncology clinical use and are supported by suggestive human evidence—gene expression correlations, ex vivo patient-derived tissue activity, and retrospective immunotherapy-associated signatures. However, dedicated prospective trials testing their ICI-sensitizing efficacy remain lacking, and the current human data are correlative or ex vivo rather than interventional. By contrast, flagellin-based strategies have shown antitumor activity exclusively in preclinical models and face additional manufacturing and delivery complexities that prolong translational timelines. No study has yet compared these strategies head-to-head, leaving unknown whether clinically accessible agents can match the immune activation achieved by sophisticated biologics, and in which contexts.

Immunosuppressive pathways (pro-chemoresistance direction). Host-microbe interactions establish immunosuppressive networks that facilitate tumor immune evasion and compromise the efficacy of immune checkpoint inhibitors (ICIs). Two distinct mechanisms exemplify how tumor-resident microbiota remodel the immune landscape toward a “pro-chemoresistance” phenotype.

Direct regulation by *Fusobacterium nucleatum*. The oral commensal turned oncobacterium *Fusobacterium nucleatum* directly orchestrates an immunosuppressive tumor microenvironment through its surface adhesin FadA. FadA binds to E-cadherin on tumor cells, triggering the activation of nuclear factor- κ B (NF- κ B) and Wnt/ β -catenin signaling pathways.²³ This engagement initiates a cascade of immune remodeling, leading to the recruitment of immunosuppressive populations, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs).²³ The accumulation of these cells effectively creates an “immune desert” phenotype within the tumor microenvironment, depriving effector T cells of essential support and promoting tumor progression. *F. nucleatum* has also been implicated in gastric carcinogenesis, underscoring its broad oncogenic potential across digestive tract malignancies.²⁴

Metabolic interference by *Escherichia coli* metabolites. Intratumoral bacteria also disrupt antitumor immunity through metabolic byproducts. *Escherichia coli* resident in tumors enhances lactate production, which mediates M2 macrophage polarization *via* a novel epigenetic mechanism. Specifically, lactate induces lactylation of retinoic acid-inducible gene I (RIG-I), suppressing NF- κ B binding to the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) promoter and subsequently reducing NLRP3 inflammasome activity.²⁵ This loss of NLRP3 impairs both the immunosuppressive functions of Tregs and the antitumor activity of CD8⁺ T cells, thereby dampening the efficacy of ICIs by reducing T cell infiltration efficiency. The resulting M2-skewed macrophage population further reinforces the immunosuppressive milieu, contributing to resistance against immunotherapies.

These two pathways operate through mechanistically distinct modes: FadA mediates direct receptor engagement and acute signaling, whereas *E. coli*-derived lactate drives epigenetic remodeling with more sustained effects. Therapeutically, disrupting FadA–E-cadherin binding risks compromising epithelial integrity given E-cadherin’s ubiquitous expression, whereas targeting RIG-I lactylation may offer greater tumor selectivity—an advantage that remains theoretical pending *in vivo* testing. Whether these pathways coexist in individual tumors and act synergistically is unknown, as each has been studied in isolation. Whether these pathways operate independently or cooperatively, and in which tumor types each predominates, will determine if therapeutic disruption should be sequential, combined, or pathway-specific.

Characteristics of intratumoral microbiota: colonization and function

The colonization of intratumoral microbiota is driven by physicochemical factors within the tumor microenvironment. Hypoxic and low-pH regions selectively enrich anaerobic bacteria, particularly *Fusobacterium nucleatum* (*Fn*). Spatially resolved multi-omics in breast cancer has localized *Fn* to hypoxia-associated tumor cell-dense areas overlapping with hypoxia-inducible factor 1- α (HIF-1 α) expression.²⁶ Similarly, in oral squamous cell carcinoma, *Fn*-enriched regions exhibit elevated HIF-1 α , confirming that hypoxic conditions facilitate its colonization.²⁷ Furthermore, bacterial abundance correlates positively with tumor stage and metastatic risk. In cervical cancer, the gram-negative bacteria *Escherichia coli*, *Prevotella bivia*, and *Fn* are significantly enriched in tissues with lymph node metastasis, where their lipopolysaccharide promotes lymphatic dissemination *via* the Toll-like receptor 4 (TLR4)/mitogen-activated protein kinase (MAPK) signaling pathway.²⁸ In colorectal cancer, *Campylobacter jejuni* is enriched in metastatic primary lesions, and its secreted cytolethal distending toxin drives metastasis by activating JAK2-STAT3-MMP9 signaling.²⁹ In bladder cancer, *Pseudomonas aeruginosa* abundance correlates with advanced stage and facilitates myeloid-derived suppressor cell recruitment through the TLR5-ERK1/2-CCL20 axis.³⁰

A common mechanistic thread emerges: tumor-enriched bacteria drive metastasis *via* TLR or TLR-coupled pathways, mobilizing immunosuppressive or tissue-remodeling cell populations. However, the specific TLR subtype

and downstream effector vary by bacterial species and tumor context—TLR4-driven lymphatic dissemination, JAK2-STAT3-mediated matrix remodeling, or TLR5-dependent MDSC recruitment—suggesting that therapeutic targeting must be tailored accordingly. Whether these bacterial species independently predict metastatic risk beyond established clinicopathological factors remains untested prospectively, and current evidence derives largely from retrospective analyses and murine models. The key translational question is whether to target the shared TLR hub—broad applicability at the cost of selectivity—or individual bacterial species and their virulence factors for greater precision.^{28,29}

Molecular regulatory networks of host-microbiota interplay

Host-microbe interactions exhibit multi-layered regulatory characteristics in disease progression, with microbial metabolites orchestrating host immune status through signal pathway crosstalk and epigenetic remodeling. At the signaling level, microbial metabolites demonstrate dual activation capabilities on key inflammatory pathways. Recent evidence reveals that deoxycholic acid (DCA), a secondary bile acid, and lipoteichoic acid (LTA) derived from Gram-positive bacteria synergistically activate STAT3 and NF- κ B phosphorylation cascades by engaging Takeda G protein-coupled receptor 5 (TGR5) receptor and TLR2 on macrophages, respectively. This mechanism not only drives macrophage polarization toward a mixed M2/M1 phenotype but also fuels obesity-related hepatocellular carcinoma progression by reshaping the tumor immune microenvironment.³¹ TGR5, as a membrane receptor for bile acids, has been documented to couple both NF- κ B and STAT3 signaling hubs downstream, providing a molecular basis for understanding the pleiotropic effects of microbial metabolites in the tumor microenvironment.³²

Beyond immediate signal transduction, the persistent epigenetic modifications induced by microbial metabolites serve as critical drivers of immune homeostasis disruption. In fibrosing cholangiopathies, cholestatic bile acids engage the sphingosine-1-phosphate (S1P) receptor-STAT3 signaling axis, subsequently mediating DNA methyltransferase (DNMT)-dependent methylation of the forkhead box P3 (Foxp3) gene promoter. This epigenetic alteration compromises the suppressive function of regulatory T cells (Tregs) and promotes their transdifferentiation toward a Th17-like phenotype, thereby exacerbating bile duct epithelial injury and liver fibrosis.³³ This finding not only uncovers a novel mechanism by which bile acids, as microbial metabolites, regulate immune cell lineage commitment but also provides critical insights into the role of microbiome-host crosstalk in chronic inflammatory diseases.

Parallel to bile acid-mediated signaling, microbial metabolism of dietary amino acids constitutes a second regulatory axis, with tryptophan and arginine as the most characterized nodes. Tryptophan-derived indole derivatives act as aryl hydrocarbon receptor (AhR) ligands: in melanoma, *Lactobacillus reuteri*-derived indole-3-aldehyde activates AhR in CD8⁺ T cells, enhancing anti-PD-1 responses and correlating with improved patient survival;³⁴ in colorectal cancer, *Lactobacillus gallinarum*-derived indole-3-carboxylic acid suppresses Treg differentiation through the indoleamine 2,3-dioxygenase 1 (IDO1)/kynurenine/aryl hydrocarbon receptor (AhR) axis.³⁵ However, whether AhR activation by tryptophan metabolites also exerts immunosuppressive effects in colorectal cancer (CRC) or hepatocellular carcinoma (HCC)—as reported in pancreatic cancer—remains unknown. In contrast, microbial arginine depletion operates unidirectionally: gut Proteobacteria consume arginine via the arginine deiminase pathway, restoring Treg suppressive capacity and impairing CD8⁺ T cell-mediated tumor clearance.³⁶ Comparing these pathways, tryptophan metabolites exert context-dependent effects via receptor signaling, whereas arginine depletion acts through predictable nutrient limitation. AhR-targeted interventions face cell type-specific delivery challenges, while arginine-restoration strategies offer a more straightforward translational path. Whether these pathways intersect within tumors remains unexplored.

Collectively, microbial metabolites—spanning bile acid signaling, amino acid catabolism, and beyond—orchestrate a multi-layered regulatory network through acute signal activation, chronic epigenetic remodeling, and nutrient limitation, with each axis presenting distinct therapeutic opportunities and challenges.

The fungal dimension: cross-kingdom interactions and immune regulation

While the mechanisms discussed above derive predominantly from bacterial studies, fungi represent an immunologically active yet underexplored component of the IOM axis. Gut and intratumoral fungi modulate host immunity through pattern recognition receptors including dendritic cell-associated C-type lectin-1 (Dectin-1) and Toll-like receptor 2 (TLR2), and fungal dysbiosis has been associated with multiple cancer types including CRC and melanoma.³⁷ However, the functional significance of most tumor-associated fungi remains uncharacterized.

A critical advance is the discovery of direct fungal-bacterial interactions that influence tumor progression. In CRC, *Candida albicans* hyphal adhesin Flo9 binds *Fusobacterium nucleatum* outer membrane protein RadD, facilitating *F. nucleatum* mucosal colonization and synergistically promoting tumorigenesis; L-arginine disrupts this Flo9-RadD interaction, reducing their combined pro-tumorigenic activity.³⁸ In melanoma, machine learning models integrating bacterial and fungal metagenomic features outperformed single-kingdom models in predicting immunotherapy response (AUC 0.71 versus 0.64–0.65), with *Akkermansia-Rutstroemia* co-occurrence identified as a favorable-response feature.³⁹ This finding, while correlative, suggests that fungal-bacterial co-abundance patterns may influence ICI outcomes.

Cross-kingdom interaction research remains constrained by incomplete fungal reference databases, limited functional annotation tools, and a reliance on a few model fungi—*Candida*, *Saccharomyces*, *Malassezia*—leaving the vast majority of tumor-associated fungi functionally uncharacterized. Whether fungal-bacterial interactions operate across tumor types beyond CRC and melanoma, and whether they can be therapeutically targeted, represents a critical knowledge gap. Elucidating the full functional landscape of tumor-associated fungi and their cross-kingdom interactions thus represents a critical frontier for IOM axis research.

THE TECHNOLOGICAL LANDSCAPE OF IOM AXIS RESEARCH: FROM BROAD DETECTION TO PRECISE RESOLUTION

Evolution and limitations of microbial community analysis technologies

The study of the tumor microbiome is undergoing a profound transformation driven by technological innovation, with a trajectory that clearly delineates the inevitable progression from correlative description toward causal mechanistic understanding. Early investigations primarily relied on conventional techniques such as 16S rRNA sequencing and immunohistochemistry. Leveraging established clinical workflows and low costs, these methods successfully established associations between specific microbial abundances and patient staging, treatment response, and prognosis in cohorts including oral⁴⁰ and cervical cancers,³⁹ thereby laying a critical foundation for the field. However, constrained by low resolution and indirect functional evidence, these technologies struggle to address the core question of what functional roles bacteria actually play within tumors.^{40,41} Standard culture-based approaches face inherent limitations: even optimized media poorly approximate in situ conditions—a problem recognized even in comparatively simple niches like the urinary tract⁴²—leaving many tumor-resident microbes unculturable, while those that grow may diverge functionally from their in vivo state. Culture-independent tools bypass these constraints: smRandom-seq captures single-bacterium transcriptomes directly from tissue without cultivation,¹¹ and SRS-FISH links metabolic activity to identity at single-cell resolution via isotope probing.⁴³ Spatial technologies further preserve tissue architecture, reconstructing in situ microbe-host cell interactions lost upon homogenization or culture.⁴⁴ These spatial tools, however, remain research-stage; their reproducibility and clinical utility await systematic validation.

To overcome this bottleneck, emerging technologies represented by smRandom-seq and SRS-FISH have emerged. These approaches advance analytical precision to the single-bacterium and even single-cell level. For the first time, these tools provide the means to delineate the “functional portrait” of tumor-associated bacteria.

However, both conventional and emerging omics techniques inevitably lose the spatial coordinates of microorganisms within tissues. Against this backdrop, spatial multi-omics technologies have rapidly advanced, serving as a bridge connecting microbial localization with host function. Studies integrating high-resolution spatial transcriptomics with imaging have demonstrated

that extracellular tumor-infiltrating bacteria are not randomly distributed but are enriched within specific microniches characterized by reduced cellular density and proliferative activity; this physical contact directly induces cell-cycle arrest in tumor cells and confers chemotherapy resistance.⁴⁴ Concurrently, a large-scale, decade-long prospective study of gastric carcinogenesis, integrating single-cell, spatial, and DNA sequencing, not only mapped the clonal evolution of precancerous lesions but also unexpectedly revealed associations between intestinal metaplasia molecular subtypes and oral microbiota, providing key variables for optimizing clinical risk prediction models.⁴⁵

Rather than a linear succession, these technology classes serve complementary roles: conventional sequencing excels in large-cohort associations at low cost; single-bacterium tools enable functional interrogation at unprecedented resolution; and spatial technologies reconstruct the in situ cellular context of microbial activity. Each, however, carries trade-offs: conventional methods lack functional and spatial resolution; single-bacterium tools remain constrained in throughput and clinical validation; and spatial technologies incur high costs and perform poorly in low-biomass samples. Integrating all three in a single study remains logistically difficult and rare—a bottleneck that must be resolved before the multi-dimensional picture can be fully realized. Resolving this bottleneck will be essential for translating the IOM axis from association to intervention.

In summary, conventional sequencing, single-bacterium tools, and spatial technologies serve complementary roles—the former enabling large-cohort associations, the latter two providing functional and spatial resolution—yet their integration into a unified analytical pipeline remains a logistical bottleneck that the field must overcome.

Multi-omics integration strategies: technological synergy

Multi-omics integration strategies are systematically reshaping the research paradigm of the tumor microbiome by synergistically combining multidimensional data, including microbial profiling, metabolomics, transcriptomics, spatial imaging, and clinical phenotypes. From the perspective of typical combinatorial patterns, current approaches can be categorized into four main paradigms, with the first three focused primarily on bacterial communities.

The first is the vertical construction of causal chains linking microbes, metabolites, and host targets. In endometrial cancer research, investigators combined 16S sequencing with metabolomics to identify butyrate, and subsequently employed in vitro functional assays and CDGSH iron sulfur domain 1 (CISD1) overexpression/rescue models to establish the first causal “intratumoral microbiota-butyrate-ferroptosis” axis underlying progesterone sensitivity.⁴⁶ Similarly, in right-sided colon adenomas, integration of single-cell transcriptomics, spatial transcriptomics, and animal models revealed that the S100A11-receptor for advanced glycation end products (RAGE) axis mediates immune evasion, and therapeutic targeting with a RAGE antagonist was validated to enhance anti-PD1 efficacy.⁴⁷ The second paradigm involves multi-platform orthogonal validation coupled with spatial correlation. In brain tumor research, investigators employed FISH, immunohistochemistry, metagenomic sequencing, and spatial imaging across a large multi-institutional cohort to confirm the presence of intracellular bacteria and spatially correlate these signals with local antimicrobial and immunometabolic signatures, effectively addressing long-standing controversies regarding low-biomass samples.⁴⁸ The third paradigm is a closed-loop model integrating clinical cohorts, multi-omics profiling, and functional validation. In ovarian cancer immunotherapy, integrated molecular, immune, microbial, and metabolic profiling identified T and B cell enrichment and distinct metabolic pathways associated with exceptional clinical responses.⁴⁹ Colorectal cancer research advanced this further by combining machine learning with fecal microbiota transplantation experiments, directly demonstrating that patient-derived gut microbiota are sufficient to drive immunotherapy resistance while simultaneously developing a high-accuracy diagnostic model.⁵⁰

These four paradigms, while all advancing beyond descriptive association, differ markedly in their accessibility and evidence strength. The fourth paradigm—cross-kingdom integration—extends multi-omics strategies to incorporate fungal internal transcribed spacer (ITS) or metagenomic data alongside bacterial and host profiles. In CRC, integrated bacterial metage-

nomics and fungal ITS sequencing uncovered a direct Flo9-RadD interaction between *Candida albicans* and *Fusobacterium nucleatum* that synergistically drives tumor progression.³⁸ This paradigm, however, faces the additional burden of incomplete fungal reference databases and limited functional annotation tools. This creates a practical dilemma: the most rigorous evidential framework is also the least accessible, and the field has yet to establish whether findings from one paradigm generalize to another. Addressing this accessibility gap—through low-input multi-omics protocols or standardized cross-cohort data harmonization frameworks—will be critical for extending these integrative strategies beyond a handful of specialized centers. The convergence of these integration paradigms, once democratized, will form the analytical backbone of mechanism-driven IOM axis research.

The democratization of these integrative paradigms will be essential to extend multi-omics-driven IOM axis research beyond specialized centers and into routine translational application.

Interdisciplinary technologies: from observation to manipulation

The convergence of synthetic biology and bioinformatics is propelling tumor microbiome research from observation and correlation toward intervention and remodeling. Synthetic biology engineers bacteria as precision therapeutic platforms, with strategies falling into three main categories. The first is effector molecule secretion systems, utilizing bacteria as “living drug factories” to deliver therapeutic proteins, exemplified by radiation-responsive *Escherichia coli* expressing anti-triggering receptor expressed on myeloid cells 2 (TREM2) single-chain antibodies to modulate tumor-associated macrophages.⁵¹ The second encompasses responsive control systems, including near-infrared optogenetic modules that enable remote, non-invasive spatiotemporal precision in gene expression, such as light-sensitive *E. coli* activating immune pathways upon illumination to achieve tumor suppression.⁵² The third comprises multifunctional integration platforms, where bacteria are conjugated with nanomaterials bestowing multiple therapeutic capabilities, including polydopamine coating combined with immune checkpoint blockade,⁵³ cytochrome modification mediating photothermal therapy,⁵⁴ drug-loaded liposomes enabling acoustically controlled uniform drug distribution,⁵⁵ and orally administered engineered bacteria modulating microbial metabolism.⁵⁶ An emerging direction extends synthetic biology beyond direct tumor or immune cell targeting toward remodeling the resident microbiota. A recent proof-of-concept engineered *Lactococcus lactis* to secrete guided antimicrobial peptides that selectively inhibit *Fusobacterium nucleatum* while preserving overall microbial diversity in a simulated gut microbiome.⁵⁷ Orally administered probiotics that modulate gut microbial metabolism represent a complementary strategy.⁵⁶ These approaches, however, remain in their infancy, with delivery efficiency and colonization stability representing key unresolved challenges.

These strategies collectively evolve from single-function to multifunctional integration, and from constitutive expression to responsive regulation.

Bioinformatics provides a powerful computational engine for mining microbial mechanisms from vast multi-omics datasets, with applications primarily grouped into four categories. The first is microbial signal identification from single-cell data, where pipelines like CSI-Microbes⁵⁸ and SAHMI⁵⁹ enable mining of microbial reads from public datasets, increasing detection rates in few samples by 40% and revealing myeloid cells as major sources of bacterial RNA in the tumor microenvironment. The second involves machine learning-driven diagnostic model construction, exemplified by Random Forest models selecting key genera for non-invasive gastric cancer diagnosis (AUC 0.86)⁶⁰ and MAS scores quantifying microbiota-driven transcriptional activity to identify prognostic genes.⁶¹ The third category is computational discovery of mechanisms, where re-analysis of single-cell data alone uncovered that *Fusobacterium nucleatum* impairs IgA plasma cell development, revealing a previously unrecognized immune evasion mechanism.⁶¹ The fourth encompasses large-scale cross-cohort integrative analyses, such as deep learning on nearly ten thousand global samples identifying gut microbiome functional archetypes that emerge as potential confounders in disease association studies.⁶² The convergence of these two fields forms a closed loop: bioinformatics pinpoints intervention targets from complex data, while synthetic biology provides the tools for precise targeting, collectively advancing tumor microbiome research from mechanistic dissection toward therapeutic translation.

CANCER-SPECIFIC IOM AXIS SIGNATURES

Criteria for cancer type selection

This review establishes criteria for cancer type inclusion: (i) association between microbiome and immunotherapy efficacy supported by clinical evidence, with Phase II or higher data available for at least one of the three core cancer types; (ii) existence of a defined organ-specific microbial axis framework; and (iii) microecological interventions having entered registered clinical trials at any phase. Based on these criteria, colorectal cancer, melanoma, and hepatocellular carcinoma are selected as core analytical subjects. It should be noted that the evidence maturity varies considerably across cancer types and intervention tiers; accordingly, the level of supporting evidence is explicitly graded throughout this review, with preclinical, early-phase clinical, and randomized controlled data clearly distinguished.

Breast cancer is currently excluded from core analysis, yet recent advances precisely delineate the progressive trajectory of IOM axis research from “correlative discovery” toward “interventional validation.” At the foundation of this evidence hierarchy, a secondary analysis of the randomized controlled trial ALICE first demonstrated in metastatic triple-negative breast cancer that gut microbiota alpha diversity predicts atezolizumab survival benefit—patients with high diversity exhibited a progression-free survival (PFS) hazard ratio of 0.34, whereas those with low diversity derived minimal benefit, providing RCT-level associative evidence linking gut microbiota to immunotherapy benefit in breast cancer.⁶³ Building on this, investigations extended from “gut microbiota” to the “intratumoral microbiota” compartment: intratumoral microbial load was found to positively correlate with CD4⁺CXCL13⁺ T cell infiltration, and a microbiome-integrated radiomics fusion model achieved an AUC of 0.945 for predicting pathological complete response, indicating the potential of such integrative tools, though independent validation is needed.⁶⁴ At the apex of this progressive chain, the first phase I/II interventional trial of oral probiotics combined with pembrolizumab in advanced breast cancer has completed, representing the first clinical exploration of microecological intervention in breast cancer.⁶⁵

Nevertheless, fundamental limitations persist: predictive findings have yet to translate into interventional efficacy confirmation; the completed interventional trial demonstrated limited efficacy (median PFS of only 1.8 months); and breast cancer lacks a definitive organ-microbial axis framework comparable to the gut–liver or gut–lung axes. Therefore, breast cancer currently remains in the “clinical translation exploration phase” of IOM axis research, awaiting subsequent phase II or higher interventional trials to provide conclusive efficacy evidence before inclusion in this framework for expanded discussion.

IOM axis characteristics in three major cancer types

Colorectal cancer: the best-characterized IOM axis model. Colorectal cancer (CRC) represents the most extensively characterized model of the IOM axis. The defining feature of the CRC IOM axis is the dual role of *Fusobacterium nucleatum*. This oral pathobiont promotes colorectal tumorigenesis through FcγA-mediated E-cadherin engagement, triggering β-catenin and NF-κB signaling, and drives immunosuppressive myeloid cell recruitment. Yet *F. nucleatum* paradoxically sensitizes microsatellite-stable CRC to anti-PD-1 therapy through butyrate-mediated HDAC3/8 inhibition and TBX21-dependent CD8⁺ T cell reinvigoration.⁶⁶ This context-dependent duality—in which the same bacterium can drive or suppress tumor immunity depending on its metabolic output—illustrates a core principle of the IOM axis. Clinically, CRC benefits from the broadest evidence base among the three cancer types, spanning RCT-level evidence for probiotic reduction of chemotherapy toxicity (Section “Optimization of combination therapy strategies: enhancing efficacy and reducing toxicity”), the first demonstration of oral engineered bacterial colonization in human colorectal tumors (Section “Level 3 interventions: engineered bacteria-based therapies”), and ongoing randomized FMT–ICI trials (Section “Level 1 interventions: fecal microbiota transplantation”). Together, these features position CRC as the reference model for IOM axis translation. This combination of mechanistic depth and clinical breadth makes CRC the most advanced IOM axis model. Though the translation of microbial insights into immunotherapy-specific benefits—beyond supportive care—remains an ongoing challenge.

Melanoma: the strongest immune–microbiota association. Melanoma represents the paradigm for understanding how the gut microbiome shapes

immune checkpoint inhibitor (ICI) responses, supported by the most complete human cohort evidence linking microbial composition to anti-PD-1 efficacy. The melanoma IOM axis is distinguished by the convergence of diverse microbial mechanisms on a common endpoint of enhancing CD8⁺ T cell effector function. These include flagellin-mediated T cell cross-priming, metabolite-driven macrophage reprogramming, and Toll-like receptor activation by bacterial lipopolysaccharide. Clinically, melanoma has progressed furthest in microbiome-based interventions among the three cancer types. FMT combined with ICI has advanced to randomized Phase II evaluation (Section Level 1 interventions: fecal microbiota transplantation), defined microbial consortia have entered Phase I testing (Section Level 2 interventions: probiotics/postbiotics), and engineered bacterial therapeutics have completed first-in-human trials (Section Level 3 interventions: engineered bacteria-based therapies). This interventional maturity positions melanoma as the translational vanguard of the IOM axis. Whether this interventional lead will translate into regulatory-approved microbiome-based therapies, and whether the microbial mechanisms elucidated in melanoma extend to less immunogenic tumor types, remain key open questions.

Hepatocellular carcinoma: a definitive gut–liver axis model. Hepatocellular carcinoma (HCC) serves as the prototypical model for the gut–liver axis, where the liver receives approximately 70% of its blood supply from the portal vein and is directly exposed to gut-derived microbial metabolites. The HCC IOM axis is characterized by barrier disruption and metabolite-mediated immune remodeling. *Akkermansia muciniphila* strengthens intestinal barrier integrity and immunosuppression,⁶⁷ while *Phocaeicola vulgatus* suppresses indoleacetic acid production to impair CD8⁺ T cell cytotoxicity.⁶⁸ Despite the strong anatomical rationale, HCC lags behind CRC and melanoma in clinical interventional evidence. The most mature data derive from the surgical rather than immunotherapeutic context—a probiotic cocktail improved postoperative liver function recovery in an RCT (Section Level 2 interventions: probiotics/postbiotics)—while randomized FMT–ICI trials remain ongoing (Section Level 1 interventions: fecal microbiota transplantation). Bridging the gap between the compelling mechanistic rationale and clinical translation represents the central challenge for the HCC IOM axis. Closing this gap will require dedicated interventional trials that leverage the gut–liver axis for immunotherapy-specific endpoints, rather than relying on surgical adjuvant settings alone.

Comparative maturity across cancer types. Across the three cancer types, a clear maturity gradient of IOM axis research is evident. CRC benefits from the deepest mechanistic characterization and the broadest clinical evidence base, though this remains concentrated in supportive care rather than direct immunotherapy augmentation. Melanoma leads in ICI-focused interventional evidence, with FMT and defined consortia in active clinical evaluation. HCC, despite the strongest anatomical rationale *via* the gut–liver axis, remains at an earlier translational stage, with most clinical evidence derived from surgical rather than immunotherapeutic settings. This gradient reflects not only differential scientific investment but also fundamental differences in tumor immunobiology, anatomical microbial accessibility, and the availability of clinically relevant preclinical models.

IOM axis dynamics in metastatic tumors

In the metastatic setting, the intratumoral microbiome is shaped primarily by the local microenvironment of the metastatic site rather than the primary tumor of origin. An ancillary analysis of the SHIVA trial demonstrated that microbial richness, evenness, and β-diversity in metastatic biopsies were strongly associated with biopsy site ($P < 0.0001$) but not with primary tumor type ($P = 0.52–0.82$).⁶⁹ Consistent with this, a pan-cancer analysis of 4,160 metastatic tumor biopsies across 26 cancer types identified organ-specific microbial tropism, with anaerobic bacteria selectively enriched in hypoxic metastases.⁶¹ Together, these findings indicate that the metabolic conditions of the metastatic niche—particularly hypoxia—exert stronger selective pressure on the resident microbiota than does the primary tumor lineage.

Functionally, tumor-resident bacteria in metastases can actively promote disease progression through metabolite-driven immunosuppression. In colorectal cancer liver metastasis, *Escherichia coli*-derived lactate drives M2 macrophage polarization *via* RIG-I lacylation and NLRP3 inflammasome suppression, impairing CD8⁺ T cell function and Treg regulation.²⁵ In lung

Table 1. Key microbial taxa in the IOM axis across cancer types.

Cancer type	Key microbial taxa	Role in IOM axis	Clinical relevance	References
Colorectal cancer	<i>Fusobacterium nucleatum</i>	Paradoxically sensitizes microsatellite stable (MSS) CRC to anti-PD-1 via butyrate-mediated HDAC3/8 inhibition and CD8 ⁺ T cell reinvigoration; also promotes tumorigenesis through FadA–E-cadherin/β-catenin/NF-κB signaling and MDSC recruitment	High intratumoral Fn predicts favorable anti-PD-1 response in MSS CRC; FMT from Fn-high patients transfers ICI sensitivity in preclinical models	66
Hepatocellular carcinoma	<i>Akkermansia muciniphila</i>	Strengthens intestinal barrier integrity, reduces LPS-driven immunosuppression, and promotes T cell infiltration	Low abundance associated with anti-PD-1 resistance and poor PFS in metabolic dysfunction-associated fatty liver disease (MAFLD)-HCC; probiotic administration improves postoperative liver function recovery (RCT)	67
	<i>Phocaeicola vulgatus</i>	Suppresses indoleacetic acid production, impairing CD8 ⁺ T cell cytotoxicity	Enriched in anti-PD-1 non-responders; IAA supplementation reverses CD8 ⁺ T cell dysfunction in preclinical models	68
Metastatic tumors	<i>Escherichia coli</i> (CRC liver metastasis)	Drives M2 macrophage polarization via lactate-mediated RIG-I lactylation and NLRP3 inflammasome suppression	Enriched in CRC liver metastases; RIG-I lactylation inhibitor sensitizes to 5-FU in preclinical models	25
	<i>Roseburia</i> (lung cancer)	Promotes M2 macrophage polarization and metastasis via butyrate-mediated HDAC2 inhibition and H19 upregulation	Enriched in tumors with shorter recurrence-free survival; circulating microbial DNA predicts recurrence	73
	<i>Fusobacterium nucleatum</i> (lung metastases)	Associated with resistance to immune checkpoint blockade in metastatic lung lesions	Identified in pan-cancer analysis of 4,160 metastatic biopsies; potential predictive biomarker	71
	<i>Streptococcus</i> spp. (pan-cancer)	Hub genus in microbial networks of metastatic tumors across colorectal, breast, and lung cancers	Distinguishes metastatic from non-metastatic tumors; potential metastasis-specific microbial signature	72

cancer, butyrate-producing *Roseburia* similarly promotes M2 polarization through HDAC2 inhibition and H19 upregulation.⁷⁰ Despite the diversity of bacterial species and metabolites involved, the convergence on M2 polarization and CD8⁺ T cell impairment points to a common immunosuppressive module that may operate across metastatic sites and primary tumor types.

These microbiome-immune interactions carry clinical consequences. Lower intratumoral microbial richness in metastases has been independently associated with reduced tumor-infiltrating lymphocytes, lower PD-L1 expression, and shorter overall and progression-free survival.⁶⁹ *Fusobacterium nucleatum* in metastatic lung lesions was associated with resistance to immune checkpoint blockade,⁷¹ while distinct microbial network structures dominated by *Streptococcus* further distinguish metastatic from non-metastatic tumors across colorectal, breast, and lung cancers.⁷² Circulating microbial DNA has emerged as a candidate non-invasive biomarker for monitoring metastatic IOM axis dynamics,^{71,73} though whether it reflects microbial signals from metastases or from the primary tumor remains unclear. A fundamental unresolved question is whether the metastatic microbiota originates from the primary tumor via translocation or is independently assembled by the metastatic niche. Systematic paired primary-metastasis microbiome comparisons and spatial metagenomic approaches will be required to resolve this.

TRANSLATIONAL ADVANCES: FROM BENCH TO BEDSIDE

Microbiota-derived biomarkers: from association signals to candidate clinical tools

Predictive efficacy biomarkers: retrospective signals with emerging prospective validation. Microbial predictive biomarkers are progressing from statistical associations toward clinical validation, though the field remains at the retrospective level (Level C). No reported candidate has completed prospective multi-center validation in a randomized trial (Level A); accordingly, we use “candidate biomarker” throughout. In MAFLD-related HCC, a retrospective single-cohort analysis proposed a cut-off of 3.8×10^{-4} for *Akkermansia muciniphila*. Below this threshold, barrier integrity loss, chronic

inflammation, and poor anti-PD-1 response (7% vs. 41% response rate) were observed.⁶⁷ This threshold remains unvalidated in independent cohorts.

In contrast, the value of combinatorial models lies in their robustness and multi-dimensional information integration. The SPEED model (LARC, N=33, *Lachnospiraceae* and *Blautia wexlerae*) achieved training AUC 98.8% but validation AUC 77.78%, illustrating overfitting and inter-population variability.⁷⁰ Its functional readout—butyrate production and anti-inflammatory potential—may be more transferable than individual taxa. However, SPEED remains Level C: it has not been tested as a stratification tool in a prospective randomized trial. In melanoma, a multi-cohort meta-analysis identified an eight-taxa bacterial/fungal model combining *Akkermansia*, *Romboutsia*, *Rutstroemia* and *Zancudomyces* achieving an AUC of 0.71 for predicting immunotherapy response.³⁹ *Zancudomyces* enrichment in non-responders suggests antifungal strategies, but AUC 0.71 indicates that microbial composition alone is insufficient for clinical prediction; integration with tumor-intrinsic features is needed. In CRC, high *F. nucleatum* was retrospectively associated with better anti-PD-1 response via butyrate-mediated CD8⁺ T cell reinvigoration.⁶⁶ In a prospective observational study (NCT05635149), *Lactobacillus* abundance predicted DCR (AUC 0.886) and longer PFS (6.4 vs. 2.8 months); this analysis was post-hoc, thus Level C.⁷⁴ These candidates reveal plausible mechanistic links between microbiota and immunotherapy efficacy. Transition to clinical tools requires: prospective multi-center cohorts with pre-specified hypotheses, cross-population validation, and randomized trials demonstrating improved outcomes with biomarker-stratified treatment (Level A).

Diagnostic and prognostic biomarkers. The value of diagnostic biomarkers is evolving from mere “tumor presence identification” to “tumor source localization.” While *Fusobacterium nucleatum* is universally enriched in colorectal cancer and serves as a broad-spectrum screening indicator, it cannot distinguish tumor location. Recent studies have unveiled site-specific microbial fingerprints that fundamentally reflect the ecological characteristics of different intestinal segments: right-sided colon cancer enriched with *Veillonella parvula* (AUC 91.59%), left-sided colon cancer with *Streptococcus angio-*

Table 2. Microbial biomarkers of the Immuno-Oncology-Microbiome axis: type, performance and clinical significance in malignancies.

Biomarker type	Cancer type	Sample type	Biomarker	Cut-off / performance	Clinical significance	References
Predictive	Colorectal cancer (MSS)	Tumor tissue / Stool	<i>Fusobacterium nucleatum</i>	High abundance associated with improved PFS (P=0.002) and OS (P=0.01); Fn-high FMT mice showed significant tumor suppression (P<0.0001)	Predicts favorable response to anti-PD-1 therapy via butyrate-mediated immune remodeling	66
Predictive	Colorectal cancer (MSS mCRC)	Stool	<i>Lactobacillus</i> , <i>Bifidobacterium</i>	Lactobacillus AUC 0.886 for DCR prediction; high abundance group median PFS 6.4 vs 2.8 months	Predicts efficacy of fruquintinib plus sintilimab therapy	74
Predictive	Rectal cancer (locally advanced)	Stool	SPEED model (<i>Lachnospiraceae</i> bacterium + <i>Blautia wexlerae</i>)	AUC 98.80% (training), 77.78% (validation)	Predicts pathological complete response to neoadjuvant immunotherapy plus chemoradiotherapy	70
Predictive	Melanoma	Stool	Akkermansia, Romboutsia, <i>Rutstroemia</i> (8 bacterial/fungal taxa)	Combined model AUC 0.71	Predicts immunotherapy response (enriched in responders)	39
Predictive	Melanoma	Stool	<i>Zancudomyces</i> (fungus)	Combined model AUC 0.71	Predicts immunotherapy non-response (enriched in non-responders)	39
Predictive	MAFLD-related HCC	Stool / Gut	Akkermansia muciniphila	High abundance cutoff 3.8×10^{-4} ; high abundance group CR/PR rate 41% vs 7%; PFS P=0.022	Low Akk predicts PD-1 resistance and poorer PFS	67
Predictive	Hepatocellular carcinoma	Stool	18-species bacterial panel	AUC 75.63% (test set)	Predicts durable clinical benefit from immunotherapy	77
Diagnostic	Colorectal cancer (right-sided)	Stool	<i>Veillonella parvula</i>	Diagnostic AUC 91.59%	Aids tumor location-specific diagnosis	75
Diagnostic	Colorectal cancer (left-sided)	Stool	<i>Streptococcus anginosus</i>	Diagnostic AUC 91.69%	Aids tumor location-specific diagnosis	75
Diagnostic	Colorectal cancer (rectal)	Stool	<i>Peptostreptococcus anaerobius</i>	Diagnostic AUC 90.53%	Aids tumor location-specific diagnosis	75
Diagnostic	Colorectal cancer	Stool	<i>Fusobacterium nucleatum</i>	Enriched across all tumor locations	Associated with colorectal cancer presence	75
Prognostic	Colon cancer	Tumor tissue	High-risk taxa (17 species)	HR 3.3–7.9 (P<0.05), e.g., <i>Acinetobacter</i> sp. BMW17 HR=7.9	Positively associated with recurrence risk, indicating poorer disease-free survival	76
Prognostic	Hepatocellular carcinoma	Stool	<i>Actinomyces</i> sp. ICM47	High abundance group median PFS 7.45 vs 12.0 months (P=0.013)	Predicts poorer survival in ICI-treated HCC patients	77
Prognostic	Hepatocellular carcinoma	Stool	<i>Senegalimassilia anaerobia</i>	High abundance group median PFS 6.37 vs 12.0 months (P=0.025)	Predicts poorer survival in ICI-treated HCC patients	77

sus (AUC 91.69%), and rectal cancer with *Peptostreptococcus anaerobius* (AUC 90.53%). This enables non-invasive localization through stool testing—for patients with occult tumors or those unable to undergo colonoscopy, such information can guide imaging and endoscopic examinations toward specific intestinal segments, avoiding blind whole-colon screening.⁷⁵

Prognostic markers translate microbial features into survival signals but lack prospective pre-specified validation. In a retrospective colon cancer cohort, *Acinetobacter* sp. BMW17 carried a HR of 7.9 for recurrence.⁷⁶ However, this single-cohort finding with wide confidence intervals requires independent validation before clinical application. In HCC, *Actinomyces* sp. ICM47 and *Senegalimassilia anaerobia* were associated with shorter PFS (7.45 vs. 12.0 months) in a retrospective cohort.⁷⁷ Their co-occurrence as negative predictors in Section Predictive efficacy biomarkers: retrospective signals with emerging prospective validation suggests mechanistic convergence on immunosuppression; prospective validation is pending. Microbial biomarkers of the immune-tumor-microbiome axis: types, manifestations, and clinical significance in malignant tumors are shown in Table 2.

Microbiota-based interventions

It should be noted that the clinical evidence maturity varies considerably across these three intervention tiers. Randomized Phase II data are currently available only for select FMT and probiotic strategies, whereas the majority of evidence—particularly for engineered bacterial therapies—remains at the preclinical or Phase I stage.

Level 1 interventions: fecal microbiota transplantation. Fecal microbiota transplantation (FMT) combined with immune checkpoint inhibitors (ICIs) has entered early-phase clinical evaluation, though no completed randomized controlled trial has yet met its primary endpoint.

In the first-line setting, the multicenter Phase II FMT-LUMINate trial reported an objective response rate of 75% (15/20) in melanoma patients receiving FMT plus dual ICI.⁷⁸ Notably, responders exhibited depletion of baseline bacterial species following FMT rather than donor strain acquisition, challenging the prevailing engraftment-centric model of FMT efficacy.⁷⁸ Also in first-line melanoma, the Phase I MIMic trial (N=20) reported an overall response rate (ORR) of 65% with median overall survival of 52.8 months;

however, the authors explicitly note that the small sample size limits these findings to hypothesis generation.⁷⁹ The only randomized evidence to date comes from the Phase II TACITO trial in metastatic renal cell carcinoma (N=45), in which donor FMT combined with pembrolizumab plus axitinib did not meet its primary endpoint but significantly improved median PFS (24.0 versus 9.0 months, HR=0.50, P=0.035).⁸⁰ In the post-immunotherapy resistance setting, a Phase I trial in anti-PD-1-refractory gastric cancer (N=10) reported an ORR of 20%, though the evidence remains preliminary.⁸¹

For hepatocellular carcinoma, the FLORA trial (NCT05690048)—a multicenter, randomized, double-blind, placebo-controlled Phase II study—is currently evaluating FMT (INTESTIFIX 001, an encapsulated high- α -diversity donor preparation) combined with atezolizumab/bevacizumab.⁸²

Standardization efforts have advanced in parallel: donor selection has evolved toward high- α -diversity or ICI-responder donors, oral capsule formulations enable drug-like administration, and vancomycin pre-treatment is routinely employed to enhance donor microbiota engraftment.⁸² Collectively, FMT combined with ICI represents a promising but unvalidated strategy whose clinical role awaits definition by ongoing randomized trials.

Level 2 interventions: probiotics/postbiotics. Level 2 interventions exhibit distinct cancer-specific strategies, reflecting differential clinical maturity across tumor types.

In colorectal cancer, a randomized controlled trial demonstrated that probiotic combinations significantly reduce chemotherapy-induced diarrhea (P<0.01) and restore fecal short-chain fatty acid levels in the postoperative adjuvant setting.⁸³ This represents the most mature clinical evidence among the three cancer types, though it addresses supportive care rather than direct immunotherapy augmentation. Preclinically, specific strains—including *Bifidobacterium* breve and the postbiotic MS-20—have shown antitumor and immunomodulatory activity in CRC models, but these findings await clinical translation.

In melanoma, the randomized, placebo-controlled, biomarker-stratified Phase Ib SER-401 trial (N=14) combined a *Firmicutes*-enriched spore formulation with nivolumab in the first-line setting A. Notably, the overall response rate was lower in the SER-401 arm (25%) than in the placebo arm (67%), and translational analyses revealed that vancomycin preconditioning disrupted the gut microbiota and impaired immune recovery—challenging the routine use of antibiotic preconditioning in microbiota modulation trials.⁸⁴ In parallel, MET4, a standardized 30-species synthetic microbial consortium, has entered Phase I evaluation as a donor-independent alternative to FMT, though efficacy data are pending.⁸⁵ These early-phase studies highlight both the potential and the unresolved challenges of defined microbial consortia in melanoma.

In hepatocellular carcinoma, a randomized clinical trial demonstrated that a probiotic cocktail containing *Bifidobacterium longum* reduced delayed liver function recovery rates, shortened hospital stays, and improved 1-year overall survival in postoperative patients.⁸⁶ This provides proof-of-concept that microbiota modulation can yield clinically meaningful endpoints in HCC, though it has not yet been tested in combination with immunotherapy. Preclinically, Akkermansia muciniphila has shown potential to downregulate tumor PD-L1 expression via bile acid modulation in HCC models, but clinical validation of this approach is absent.

Across these cancer types, a clear maturity gradient is evident: CRC benefits from RCT-level evidence, albeit in supportive care; melanoma has progressed to early-phase randomized evaluation with defined consortia; and HCC has demonstrated clinical proof-of-concept in the surgical setting but not yet in immunotherapy combination. In summary, Level 2 interventions are evolving from single strains toward defined consortia, with cancer-specific strategies reflecting differential clinical maturity. However, across all three cancer types, “precision microbiota therapy” remains aspirational rather than achieved.

Level 3 interventions: engineered bacteria-based therapies. Level 3 interventions remain at the preclinical proof-of-concept stage across all three cancer types, with clinical translation only beginning.

Preclinically, engineered bacteria have progressed from constitutive payload secretion to intelligent sense-and-respond platforms. In colorectal cancer, near-infrared light-inducible engineered bacteria (NETMAP) provide external control over the timing and location of therapeutic release,⁸⁷ while quorum-sensing probiotic consortia demonstrate the feasibility of

autonomous, multi-target payload release in response to TME cues.⁸⁸ In hepatocellular carcinoma, engineered Akkermansia muciniphila membrane vesicles modified with GalNac achieve HCC-specific enrichment, linking tumor tropism to pyroptosis induction and trained immunity.⁸⁹ These designs illustrate a broader trajectory from passive drug carriers toward programmable, context-aware therapeutic platforms—though all remain untested beyond murine models.

Clinical evaluation of engineered bacteria has only recently begun, and is limited to Phase I safety and feasibility studies. In colorectal cancer, a clinical trial demonstrated that orally administered *Escherichia coli* Nissle 1917 selectively colonizes human tumor tissue over adjacent normal tissue, establishing tumor-selective bacterial delivery as a feasible strategy in patients; however, the trial assessed colonization as its primary endpoint and did not evaluate therapeutic outcomes.⁹⁰ In advanced solid tumors, SYNBI891—an *E. coli* Nissle strain engineered to express a stimulator of interferon genes (STING) agonist—became the first engineered bacterial therapeutic to complete a first-in-human trial. Intratumoral injection of SYNBI891 was safe, activated the STING pathway in injected tumors as evidenced by upregulation of IFN-stimulated genes, and resulted in stable disease in 4 participants refractory to prior PD-1/L1 blockade; no objective responses were reported.⁹¹

Across the IOM axis intervention landscape, a clear translational maturity gradient is evident. Fecal microbiota transplantation has advanced to randomized Phase II evaluation with emerging efficacy data (Section Level 1 interventions: fecal microbiota transplantation); probiotics and postbiotics are supported by randomized controlled trials demonstrating clinical benefit in defined settings (Section Level 2 interventions: probiotics/postbiotics); engineered bacteria, by contrast, have yet to report objective responses or survival outcomes in any clinical trial. Engineered bacterial therapies thus represent the most technologically sophisticated but translationally least mature strategy within the IOM axis. Bridging this gap—from elegant preclinical designs to clinical evidence of antitumor activity—defines the central challenge for synthetic biology-driven IOM axis intervention. Cancer intervention measures based on microbiota: stratified schemes, mechanisms, efficacy data, and research stages are shown in Table 3.

Optimization of combination therapy strategies: enhancing efficacy and reducing toxicity

Combination therapy strategies that pair microbiota modulation with conventional anticancer treatments pursue two complementary goals: amplifying antitumor immune responses and mitigating treatment-related toxicity. Current clinical evidence spans four combination classes, though most data derive from early-phase or single-arm studies.

Microbiota modulation plus immune checkpoint inhibitors. The most extensively studied combination class pairs FMT with ICIs. In the Phase I MIMic trial, first-line FMT combined with anti-PD-1 in advanced melanoma achieved an objective response rate of 65%; however, the small sample size (N=20) and single-arm design limit these findings to hypothesis generation.⁷⁹ In a prospective observational study of MSS colorectal cancer, prior radiotherapy exposure was associated with enrichment of *Lactobacillus* and *Bifidobacterium* genera, and the subsequent addition of fruquintinib plus sintilimab yielded an ORR of 28.0% compared with 6.7% for historical immunotherapy controls; however, the contribution of radiotherapy-induced microbiota changes to the observed response remains correlative rather than causal.⁷⁴ Whether FMT-ICI or radiotherapy-microbiota-ICI combinations confer additive benefit beyond ICI alone awaits randomized evaluation.

Microbiota modulation plus chemotherapy. A randomized controlled trial in postoperative colorectal cancer demonstrated that probiotic combinations reduced the incidence of severe chemotherapy-induced diarrhea from 28.6% to 10.7% (P<0.01) while restoring fecal short-chain fatty acid levels.⁸³ This represents the most mature clinical evidence among the four combination classes, though it addresses supportive care rather than direct antitumor efficacy.

Microbiota modulation plus radiotherapy. A controlled trial in rectal cancer patients undergoing neoadjuvant short-course radiotherapy reported that a synbiotic intervention preserved a greater proportion of mucosal bacterial diversity (25.1% versus 55.4% reduction) and reduced histological evidence of radiation-induced inflammation and fibrosis compared with controls.⁹² These

Table 3. Microbiota-based interventions for cancer: stratified regimens, mechanisms, efficacy data and research phases.

Level	Cancer type	Intervention	Regimen	Mechanism / rationale	Key efficacy data	Phase	References
Level 1: FMT	Melanoma (advanced, first-line)	FMT + dual ICI	Single oral FMT prior to anti-PD-1 + anti-CTLA-4	FMT eliminates deleterious baseline bacterial species rather than relying on donor strain engraftment	ORR 75% (15/20)	Phase II (FMT-LUMINate)	78
Level 1: FMT	Melanoma (advanced, first-line)	FMT + anti-PD-1	Single oral FMT with healthy donor stool followed by nivolumab or pembrolizumab	Microbiome manipulation overcomes primary or acquired resistance to ICI	ORR 65%; mPFS 29.6 months; mOS 52.8 months; 1/2/3-year survival 95%/74%/53%	Phase I (MIMic)	79
Level 1: FMT	Metastatic RCC (first-line)	FMT + pembrolizumab + axitinib	Donor FMT from complete ICI responders combined with standard first-line therapy	Donor strain engraftment increases α -diversity and shifts β -diversity	12-month PFS 70% vs 41% (P=0.053); mPFS 24.0 vs 9.0 months (HR=0.50, P=0.035)	Phase II RCT (TACITO)	80
Level 1: FMT	Gastric cancer (ICI-refractory)	FMT + nivolumab	Initial FMT (60 capsules) followed by maintenance FMT + nivolumab Q2W for 6 cycles	Colonization of donor-derived immunogenic microbes activates peripheral immune cell populations	ORR 20%; DCR 40% (N=10)	Phase I	81
Level 1: FMT	HCC (advanced, first-line)	FMT + atezolizumab + bevacizumab	INTESTIFIX 001 (encapsulated high- α -diversity donor FMT) + A/B Q3W; vancomycin pretreatment	FMT may improve antitumor immunity <i>via</i> gut–liver axis	Primary endpoint: tumor CD8 ⁺ T cell infiltration; target N=48 (2:1 randomization)	Phase II RCT ongoing (FLORA)	82
Level 2: Probiotics	CRC (postoperative)	Probiotic combination	Postoperative oral probiotics from surgery through first chemotherapy course	Restores chemotherapy-induced gut dysbiosis; increases SCFA-producing bacteria	Reduced severe diarrhea from 28.6% to 10.7% (P<0.01); restored fecal butyrate levels	RCT	83
Level 2: Probiotics	Melanoma (advanced, first-line)	SER-401 + nivolumab	<i>Firmicutes</i> -enriched spore formulation + nivolumab; vancomycin preconditioning	Vancomycin preconditioning disrupted gut microbiota and impaired immune recovery	ORR 25% (SER-401) vs 67% (placebo) (N=14)	Phase Ib RCT	84
Level 2: Probiotics	Advanced solid tumors (incl. melanoma)	MET4 + ICIs	Oral 30-species synthetic microbial consortium co-administered with ICIs	Donor-independent alternative to FMT; engrafts ICI-responsive taxa	Primary safety endpoint achieved (N=28); efficacy data pending	Phase I	85
Level 2: Probiotics	HCC (postoperative)	Probiotic cocktail (incl. <i>B. longum</i>)	Oral probiotic cocktail prior to liver resection	Modulates gut microbiota to improve liver function recovery	Reduced delayed recovery rates; shortened hospital stay; improved 1-year OS	RCT	86
Level 3: Engineered	CRC (preclinical)	NETMAP (NIR-inducible bacteria)	NIR light-induced engineered bacteria with switchable payloads	NIR light activates PadC photoswitch → c-di-GMP → MrkH-dependent transcription of therapeutic payloads	Significant tumor inhibition in CT26, orthotopic breast cancer, and CRC PDX models	Preclinical	87
Level 3: Engineered	CRC (preclinical)	Quorum-sensing probiotic consortium	Engineered consortium sensing pH, hypoxia, lactate → releases LdhA and PD-L1 nanobody	Quorum-sensing enables coordinated multi-payload release; lactate depletion + PD-L1 blockade	Significant tumor suppression in humanized CRC mouse models	Preclinical	88
Level 3: Engineered	HCC (preclinical)	Engineered <i>A. muciniphila</i> vesicles (SMB)	GalNAc-modified <i>A. muciniphila</i> membrane vesicles with siRNA and sonodynamic MOFs	GalNAc-mediated HCC-specific enrichment; ultrasound triggers pyroptosis; induces trained immunity in TAMs	Superior tumor suppression vs clinical ICIs in orthotopic HCC, PDX, and organoid models	Preclinical	89
Level 3: Engineered	CRC (clinical)	Engineered <i>E. coli</i> Nissle 1917	Oral EcN for 2 weeks prior to CRC resection	Tumor-selective colonization demonstrated; no therapeutic payload tested	EcN enriched in tumor vs normal tissue (primary endpoint met); therapeutic outcomes not evaluated	Phase I	90
Level 3: Engineered	Advanced solid tumors (clinical)	SYNB1891 ± atezolizumab	Intratumoral injection of EcN expressing STING agonist (CDA) ± atezolizumab Q3W	STING pathway activation in phagocytic APCs; type I IFN induction; bacterial chassis activates TLR4	Safe and well tolerated; STING target engagement confirmed; 4/24 SD (monotherapy); no objective responses	Phase I (first-in-human)	91

findings suggest that microbiota modulation may protect against radiotherapy-induced local tissue injury, though dedicated randomized trials in this setting remain limited.

Microbiota modulation plus surgery. In hepatocellular carcinoma, a randomized controlled trial investigated probiotic administration for 14 days prior to liver resection. Contrary to the hypothesized reduction in circulating endotoxins, no significant differences in endotoxin levels, post-operative liver function, or overall complication rates were observed between the probiotic and placebo arms; pro-inflammatory cytokine levels were unexpectedly higher in the probiotic group.⁹³ This negative trial underscores that the clinical effects of microbiota modulation may vary substantially by tumor type, intervention timing, and strain selection.

In summary, combination strategies pairing microbiota modulation with ICIs, chemotherapy, radiotherapy, or surgery have generated preliminary clinical signals, yet the evidence base remains fragmented and heterogeneous. The most robust data support probiotic use for chemotherapy-induced diarrhea (RCT-level evidence), while FMT-ICI and radiotherapy-microbiota strategies rely predominantly on single-arm or observational data. The negative probiotic-surgery trial in HCC further cautions against assuming that microbiota modulation will uniformly confer benefit across clinical contexts. Future trials will need to determine which combinations yield clinically meaningful improvements over standard-of-care alone, and whether baseline microbiota characteristics can identify patients most likely to benefit from specific combination regimens.

CURRENT RESEARCH LIMITATIONS AND FUTURE CHALLENGES

Current core bottlenecks (three key dimensions)

Despite remarkable progress in understanding the IOM axis, several interconnected bottlenecks impede its clinical translation. At the technological level, the lack of standardized protocols undermines data reproducibility: DNA extraction methods exert a greater impact on gut microbiota composition than any host factor, leading to inter-laboratory variations in microbial abundance of up to 30–50%.⁹⁴ Moreover, nearly half of published microbiome studies fail to meet minimum standards for sequence data availability, and poor metadata harmonization precludes robust cross-cohort comparisons.⁹⁵ Spatial omics technologies, while powerful, remain prohibitively expensive—platforms such as 10X Visium cost over \$10,000 per sample, limiting their routine application,⁹⁶ and methods like LCM-Smart-seq2 are hampered by high operational complexity.^{97,98} Clinically, pronounced inter-individual heterogeneity in gut microbiota drives variable responses to immunotherapy: α -diversity differs significantly between responders and non-responders, and antibiotic exposure modulates key taxa.⁹⁹ This heterogeneity extends to the strain level, where genomic structural variants within species such as *Akkermansia muciniphila* harbor functional genes that critically influence therapeutic outcomes.¹⁰⁰ Mechanistically, the functional role of rare taxa—typically defined as those with relative abundance below 0.1% remains largely unexplored. Even minor genetic variations between closely related strains can produce profound phenotypic differences, underscoring the need for single-cell and strain-level functional validation to decipher host-microbe regulatory networks.¹⁰⁰ Addressing these technological, clinical, and mechanistic challenges is essential to translate IOM axis discoveries into robust, personalized therapies.

Future research priorities

Future research on the IOM axis will focus on the synergistic advancement of technological breakthroughs, mechanistic dissection, and clinical translation. At the technological frontier, there is an urgent need to develop low-cost spatial microbiome sequencing technologies. Drawing on the paradigm of environmental micro-compartment genomics (EMCG), which efficiently acquires single-particle genomic sequences from nanolitre-volume samples,¹⁰¹ the goal is to reduce the cost per assay to below \$1,000. Concurrently, integrating single-cell multi-omics with artificial intelligence is essential to construct high-resolution “microbiome-immune-tumor” interaction databases for systematically deciphering the dynamic networks among these three elements.¹⁰² Mechanistic investigations must progress from the species level to strain-specific resolution. For instance, it is crucial to delineate the

differences among members of the *Fusobacterium nucleatum* complex (e.g., *F. animalis*, *F. polymorphum*) in adhesin expression and immune evasion capabilities.¹⁰³ Furthermore, the long-term epigenetic effects mediated by microbiota-derived metabolites through the “microbiota-derived metabolite–epigenetic” (microbiota-derived metabolite–epigenetic (MDME)) axis, in which microbial metabolites regulate host DNA methylation and histone modification, require elucidation.^{102,104} The core of clinical translation lies in establishing a closed-loop system encompassing “microbiota typing, precision intervention, and efficacy monitoring.” This involves stratifying patients based on microbial signatures to design personalized fecal microbiota transplantation or probiotic regimens, with therapeutic responses dynamically tracked using machine learning models.¹⁰⁵ Breakthroughs in these areas will propel IOM axis research from correlational description toward causal intervention, ultimately facilitating the routine clinical application of microecological therapies.

Key pathways for clinical implementation

The clinical implementation of the IOM axis depends on the dual drivers of standardization and interdisciplinary collaboration. At the standardization level, an international multidisciplinary consensus has established best practice frameworks for microbiome testing in clinical settings, providing recommendations on general principles, indications, analytical methods, and result reporting to regulate direct-to-consumer tests and pave the way for evidence-based microbiome diagnostics.¹³ Addressing technical reporting standardization, the STREAMS guidelines expand upon Strengthening The Organization and Reporting of Microbiome Studies (STORMS) with 67 items, offering machine-actionable reporting standards for environmental and host-associated microbiome studies.¹² Regarding contamination control—a critical concern in low-biomass tumor samples—international consensus guidelines now provide protocols for prevention and reporting throughout the entire workflow, from sample collection to data analysis.¹⁰⁶ The American Society for Microbiology’s evidence-based laboratory medicine practice guidelines for rapid diagnostic testing of bloodstream infections further offer a reference paradigm for standardization.¹⁰⁷ At the collaboration level, operationalizing team science within academic cancer center networks has proven effective for elucidating gut microbiome structure and function. The City of Hope Comprehensive Cancer Center exemplifies this approach, integrating expertise in microbiology, tumor immunology, and systems biology to accelerate the translation from mechanistic discovery to clinical application.¹⁰⁸ The synergistic advancement of these standardization frameworks and collaborative models will establish a solid foundation for the routine clinical application of IOM axis therapies.

CONCLUSION

Research on the IOM axis has shifted from macro-association to precision regulation. Single-cell and spatial omics technologies have revealed intratumoral microbiota as active modulators of the tumor immune microenvironment, while cancer-type specific signatures have transformed microbial features into actionable therapeutic targets. Maturation of intervention modalities, from standardized fecal microbiota transplantation to synthetic consortia and engineered bacterial therapeutics, reflects a shift toward mechanism-based IOM axis manipulation. However, technological bottlenecks, inter-individual heterogeneity, and understudied low-abundance taxa hinder clinical adoption. Future progress relies on low-cost, high-throughput multi-omics, multicenter trials for safety and efficacy, and interdisciplinary collaboration to build a closed-loop system. Overcoming these challenges will move IOM-targeted therapies into mainstream clinical oncology, delivering personalized treatments to improve cancer outcomes.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS.

During the preparation of this work the author(s) used [ChatGPT / other tool] in order to refine language and format headings according to journal guidelines. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Not Applicable.

DATA AND CODE AVAILABILITY

Not Applicable.

SUPPLEMENTAL INFORMATION

None.

LEAD CONTACT WEBSITE

None.