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The intratumoral microbiome in solid cancers: Spatial ecology, functional crosstalk and translational horizons

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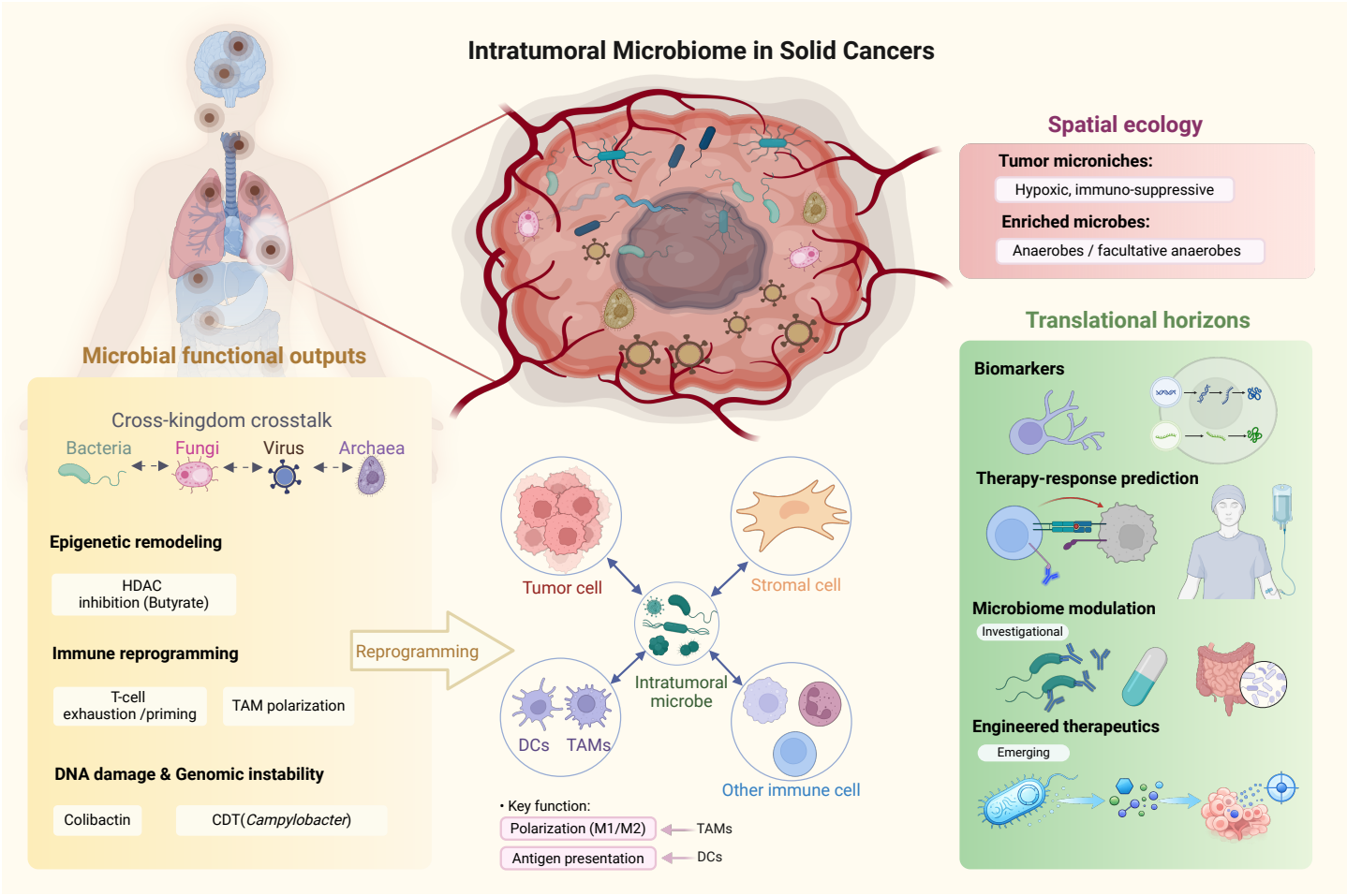
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Intratumoral microbiota (ITM) form spatial ecosystems interacting with cancer, immune, and stromal cells.
- ITM affect tumor onset and progression via genotoxic effects, immune and epigenetic regulation.
- ITM may alter therapeutic response via metabolic and signaling regulation.

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The intratumoral microbiome in solid cancers: Spatial ecology, functional crosstalk and translational horizons

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The long-held paradigm of solid tumors as sterile entities has been revised by the advent of advanced molecular profiling technologies, including high-throughput sequencing, spatial transcriptomics, and integrated multi-omics. These innovations have revealed tumors as dynamic, polymicrobial ecosystems, colonized by diverse communities of bacteria, fungi, viruses, and archaea. Intratumoral microbial communities display cancer-type specificity, reside in specialized microniches, and are frequently detected inside malignant and immune cells. Such close interactions enable microbes to regulate tumor development via multiple mechanisms. Microbial metabolites, such as butyrate and indole-3-aldehyde, modify epigenetic patterns, DNA damage control and cellular metabolism, affecting tumor proliferation, immune escape and metastatic capacity. Polymicrobial crosstalk among bacteria, fungi, and viruses further remodels immune cell infiltration and polarization, which modulates tumor responsiveness to chemotherapy, radiotherapy and immune checkpoint blockade. Tissue-resident and circulating microbial nucleic acid signatures act as minimally invasive biomarkers for tumor early diagnosis, prognostic assessment and patient stratification. Translational studies cover antimicrobials, engineered probiotics, bacteriophage treatments, metabolite analogs and intratumoral microbiota transplantation to improve therapeutic outcomes. Nevertheless, the field faces pressing challenges—including standardizing low-biomass sampling and sequencing workflows, robustly decontaminating data, establishing causal microbe–tumor relationships, integrating host and microbial multi-omics, and navigating regulatory and ethical frameworks. Addressing these hurdles through longitudinal cohort studies, rigorous mechanistic modeling, and cross-disciplinary collaboration will be essential to harness the tumor microbiome's full potential and usher in a new epoch of microbiome-informed precision oncology.

INTRODUCTION

For much of the twentieth century, solid tumors were conventionally viewed as sterile compartments, an assumption grounded in early culture-based studies that reported minimal recovery of bacterial species from excised neoplasms under stringent aseptic conditions.[1]

This paradigm held sway despite sporadic observations of opportunistic infections in immunocompromised cancer patients. The dawn of the 21st century, marked by the advent of culture-independent, high-sensitivity methods irrevocably challenged this long-standing notion. Employing amplicon sequencing of bacterial 16S rRNA genes revealed trace bacterial DNA within colorectal tumors and breast cancers, at levels previously undetectable by traditional culture techniques.[2,3] Subsequent lipopolysaccharide (LPS) and lipoteichoic acid (LTA) staining combined with electron microscopy revealed intratumoral bacteria are frequently detected intracellularly in both malignant and immune cells.[4] Advancements in the field derive from unplanned clinical discoveries and gradual technical improvements that span Coley's toxin trials in the 1890s to contemporary next-generation sequencing and multi-omics approaches (Figure 1A).

Over the past decade, the understanding of intratumoral microbial ecosystems has rapidly expanded beyond bacteria. Fungal communities, collectively termed the intratumoral mycobiome, have been identified in diverse malignancies.[5,6] They frequently colocalize with malignant epithelial cells in pancreatic, breast, and ovarian cancers, while localizing to macrophages in melanoma and lung cancer.[5,6] While less abundant, a bioinformatic study suggested that archaea may engage in unique metabolic interactions with host cells, and their dysregulated genes/pathways are potential targets for lung adenocarcinoma and squamous cell carcinoma.[7] Furthermore, the intratumoral virome is enriched by both well-characterized oncoviruses (e.g., HPV in cervical and head-neck carcinomas) and less characterized latent viruses.[8] The steep rise in publications addressing intratumoral bacteria, fungi, viruses, and their interactions over the past decade further illustrates this paradigm shift, underscoring the transition from anecdotal reports to systematic, multi-omics–driven investigation (Figure 1B).

Against this rapidly evolving backdrop, this comprehensive review aims to address four pivotal dimensions of the tumor microbiome: (1) Establishing Methodological Foundations: We critically review best practices for sampling low-biomass tissues, contamination control, and multi-omics integration—covering amplicon sequencing (16S, ITS), shotgun metagenomics, metatranscriptomics, metaproteomics, and spatially resolved methods (RNAscope-FISH, INVADE-seq, spatial transcriptomics). Computational pipelines and decontamination strategies are essential for robust microbial detection. (2) Comprehensive Taxonomic Mapping: We will synthesize current knowledge of the intratumoral microbiome's bacterial, fungal, viral, and archaeal constituents, delineating cancer-type specificity and spatial niches. We integrate findings from landmark multi-cancer tissue-based surveys with targeted mycobiome and virome profiling to present a pan-cancer atlas of microbial diversity. (3) Elucidating Functional Mechanisms: We meticulously examine how various microbial metabolites—such as short-chain fatty acids (e.g., butyrate) and indole derivatives—mediate intricate epigenetic modifications and signaling pathways. These microbial-derived signals are crucial in regulating tumor cell proliferation, DNA damage response, metabolic reprogramming, and metastatic fitness. We also highlight immune-modulatory roles, from macrophage polarization to T-cell priming and checkpoint blockade responsiveness. (4) Translational Potential and Clinical Impact: We assess clinical applications of the tumor microbiome, from prognostic microbial signatures linked to survival and recurrence to minimally invasive diagnostics based on circulating microbial DNA. We further explore microbiome-informed interventions—antibiotic/rebiotic regimens, engineered probiotics, bacteriophage therapies, microbial metabolite modulators, and microbiota transplantation—that promise to augment conventional oncologic treatments.

In synthesizing these dimensions, this review underscores both the trans-

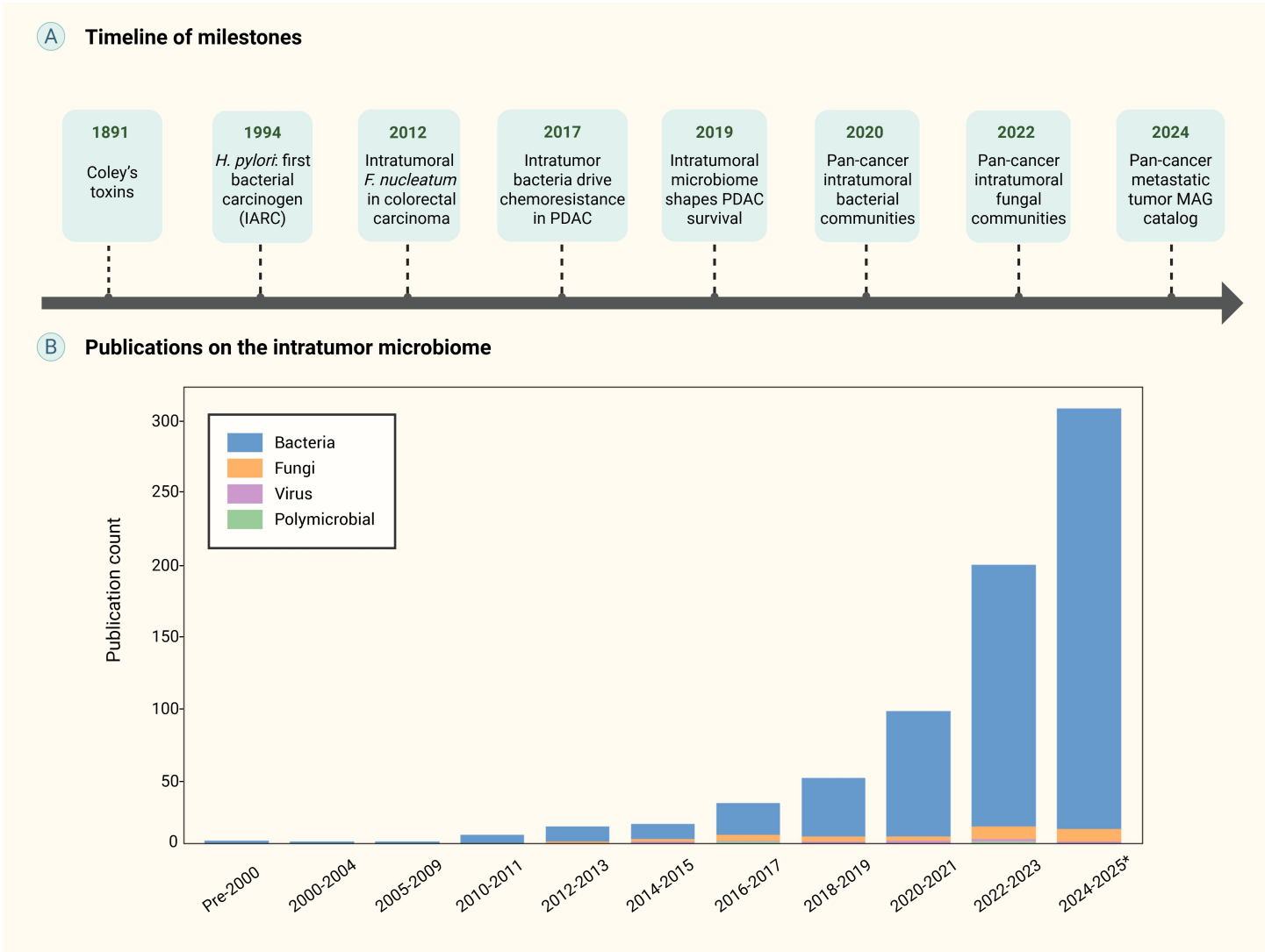

Figure 1. Historical milestones and bibliometric expansion of the intratumoral microbiome field Two complementary views chart how the discipline emerged over the past 130 years. (A) Eight landmark events trace the arc from Coley's bacterial-toxin injections (1891) through the IARC Group 1 classification of *Helicobacter pylori* as a bacterial carcinogen (1994), the metagenomic detection of intratumoral *Fusobacterium nucleatum* in colorectal carcinoma (2012), functional demonstrations that intratumoral bacteria drive chemoresistance (2017) and shape survival (2019) in pancreatic ductal adenocarcinoma, and three pan-cancer resources defining intratumoral bacterial (2020), fungal (2022) and metastatic metagenome-assembled-genome (2024) communities. (B) Curated PubMed records on the intratumoral microbiome (n = 725; search executed in Aug 2025) were retrieved by combining sixteen intratumoral-microbiome–specific title/abstract location terms with four mutually exclusive kingdom filters covering Bacteria, Fungi, Viruses and Polymicrobial studies, with pre-2010 hits manually screened to exclude bacterial-therapy, oncolytic-virus and gene-therapy reports; stacked bars span mixed intervals (Pre-2000, two 5-year bins and eight 2-year bins through Aug 2025*, the final bin covering Jan 2024–Aug 2025, 20 months). Together, the panels reveal a sharp post-2010 inflection that mirrors the maturation of next-generation sequencing and spatial profiling technologies. Abbreviations: IARC, International Agency for Research on Cancer; MAG, metagenome-assembled genome; PDAC, pancreatic ductal adenocarcinoma. Created with BioRender.com.

formative potential and remaining challenges—including the critical need for protocol standardization, distinguishing causality from correlation, ethical considerations, and regulatory pathways. Overcoming these hurdles will be paramount to realizing the full promise of microbiome-informed precision oncology.

METHODOLOGICAL FOUNDATIONS

We begin by establishing the methodological foundations of intratumoral microbiome research. Because most solid tumors harbor extremely low microbial biomass and are highly susceptible to environmental contamination, the reliability of any downstream taxonomic, mechanistic, or clinical inference critically depends on how the data were generated. We therefore introduce the experimental and computational toolkit first, so that the subsequent Landscape, Functional, and Clinical sections can be read with a shared standard for what constitutes credible evidence.

Sampling and contamination control in low-biomass tissues

Characterizing the intratumoral microbiome presents unique challenges due to the exceptionally low microbial biomass within most solid tumors and the high risk of exogenous contamination.[9,10] Rigorous sampling and contamination control protocols are therefore essential to generate reliable data. First, aseptic collection of tumor specimens—ideally under sterile field conditions with bacterial- and fungal-free instruments—minimizes environmental contaminants at the point of resection.[4] Secondly, mechanical tissue disruption (bead beating) combined with DNA extraction kits ensure comprehensive lysis of intracellular organisms.

Negative controls must accompany every step:[5,9,10] 1) Paraffin-only controls for formalin-fixed paraffin-embedded (FFPE) workflows (processing empty cassettes through cutting, deparaffinization, and extraction) reveal background contaminants introduced during block preparation. 2) DNA extraction blanks (no tissue added) detect kit- and reagent-derived microbial DNA. 3) PCR no-template controls flag contamination arising during library amplification.

Including positive spike-in controls—such as a defined number of non-native bacterial or fungal cells—permits estimation of absolute microbial loads and assessment of extraction efficiency.[9,10] Finally, sample randomiza-

Table 1. Methodological toolbox for tumor microbiome profiling.

Technique or platform	Lowest biomass reliably detected	Taxonomic resolution (typical)	Functional-gene / metabolic resolution	Spatial information	Typical input	Key contamination risks/mitigations	Key references
16S rRNA / ITS2 amplicon sequencing	5 ng total DNA	Genus-level	Limited – inferred by PICRUSt2	No	More than 12.5 ng total DNA; fresh-frozen or FFPE (short amplicons)	PCR carry-over – use dual-indexing, extraction blanks	Johnson 2019 (Nat Commun);[14] Nejman 2020 (Science);[4] Abellan-Schneyder (BMC Microbiol) 2021[17]
Shotgun metagenomics (short-read WGS)	1 ng total DNA	Species/strain level	MAGs-based physiological and molecular profiling	No	More than 50 ng total DNA; fresh/FFPE	Human read bleed-through; reagent DNA – include host depletion, in-silico decontam (Decontam, Kraken-Uniq)	Battaglia 2024 (Cell);[15] Sun 2022 (Genome Biol)[18]
Metaproteomics	-	Species (peptide spectra matching)	Direct enzyme / functional pathway inference	No	Fresh tissue	Host protein overabundance, reagent contaminants	Van 2021 (Microbiome);[12] Jiang 2022 (Research)[19]
Spatial RNA-FISH / RNAscope®	Single bacterium	Species (probe-dependent)	None (identity only)	Yes	FFPE sections	Autofluorescence; probe cross-reactivity – include scrambled probes	Nejman 2020 (Science);[4] Galeano-Niño 2022 (Nature)[13]
INVADE-seq (10x scRNA-seq + microbial UMIs)	Single cell	Genus	Cell-type-specific host metabolic rewiring; microbial metabolism inferred from taxonomy	No (cellular)	Fresh single-cell suspension	Ambient RNA, multiplets – cell hashing, computational filtering	Galeano-Niño 2022 (Nature)[13]
Spatial transcriptomics (10x Visium + PathSeq)	Single spot	Genus–species (low depth)	Spatial regional host metabolic gradient	Yes	FFPE/fresh section	Spot bleed-over; ambient RNA – MAX-barcoding, strict QC	Galeano-Niño 2022 (Nature);[13] Narunsky-Haziza 2022 (Cell)[5]
Culture-enhanced 'culturomics' (anaerobic & microaerophilic plates + MALDI/NGS)	Single colony	Strain	Live isolate: Full functional assays; metabolomics	No (bulk)	Fresh tissue homogenate	Strict sterile operation, negative controls for each culture condition	Riquelme 2019 (Cell)[16]
Bacterial whole-genome assembly from tumor WGS (MAGs)	~0.01× genome coverage / sample	Strain	Gene catalogue, pangenome	No	Bulk DNA libraries	Chimeric bins – stringent bin-quality metrics (MAGpurify, CheckM2)	Battaglia 2024 (Cell)[15]

Abbreviations: ITS2, internal transcribed spacer 2; WGS, whole-genome sequencing; RNA-FISH, RNA fluorescence in situ hybridization; UMIs, unique molecular identifiers; MALDI/NGS, matrix-assisted laser desorption/ionization/next-generation sequencing; MAGs, metagenome-assembled genomes; ASV, amplicon sequence variant; AMR, antimicrobial resistance; FFPE, formalin-fixed paraffin-embedded; PICRUSt, phylogenetic investigation of communities by reconstruction of unobserved states; PCR, polymerase chain reaction.

tion across extraction and sequencing batches, coupled with careful metadata capture (e.g., operator, kit lot, instrument), enables downstream in silico identification and removal of batch-associated contaminants using algorithms such as Decontam, SNM, SCRuB, and SourceTracker.[11] These combined measures form the cornerstone of any robust study of low-biomass tumor microbiomes.

Multi-omics approaches

Here we provide a comprehensive overview of the methodological toolbox essential for tumor microbiome profiling, contrasting the key performance metrics of major techniques, including 16S sequencing, shotgun metagenomics, and spatial transcriptomics (Table 1).[4,5,12-19] These techniques are evaluated based on their lowest reliably detectable biomass, taxonomic and functional resolution, and capacity for providing spatial information.

Amplicon sequencing (16S/ITS). Targeted amplicon sequencing remains the most widely adopted method for profiling bacterial and fungal communities in tumor tissues. Conventional bacterial 16S rRNA gene surveys typically amplify one or more hypervariable regions (e.g., V3–V4) using universal primers, enabling taxonomic resolution down to the genus level, albeit with limited species - level discrimination.[14] To overcome this limitation, Nejman et al. developed a multiplexed 5R 16S rDNA sequencing protocol that amplifies five short regions along the 16S rRNA gene.[4] By amplifying approximately 68% of the bacterial 16S rRNA gene using short amplicons, this method significantly increases the coverage and resolution for species-level detection compared to the widely used V4 or V3-V4 amplification, while remaining compatible with degraded DNA from FFPE tumor samples.[4] For fungi, the internal transcribed spacer 2 (ITS2) region serves as the gold - standard marker, providing higher phylogenetic resolution across Ascomycota and Basidiomycota.[5] Amplicon workflows are inherently sensitive and cost-effective for low-biomass samples but are susceptible to PCR bias, primer mismatches, and contamination; hence, stringent negative controls and mock - community spikes are essential to validate results.[11]

Shotgun metagenomics, metatranscriptomics, and metaproteomics. Shotgun metagenomic sequencing bypasses PCR amplification by randomly sequencing all DNA in the sample, offering comprehensive, species-level taxonomic profiling and access to functional gene repertoires.[20] This approach has reconstructed near-complete microbial genomes from tumors, illuminating unique strains adapted to the intratumoral milieu. However, standard protocols are biased toward bacteria; fungal DNA is typically underrepresented, requiring deeper sequencing or targeted enrichment for reliable detection.[20,21] Metatranscriptomics can extend this paradigm by sequencing total RNA, thereby capturing active microbial transcripts and revealing in situ metabolic and virulence pathways.[22,23] For fungi, poly(A) selection or specialized rRNA depletion is often necessary to enrich eukaryotic transcripts, as standard methods are dominated by bacterial and host RNAs.[24,25] Metaproteomics—mass spectrometry–based profiling of microbial proteins—further validates the expression of key enzymes and effectors but requires substantial biomass and advanced bioinformatic pipelines to deconvolute host–microbe proteomes.[12,26]

Single-cell approaches for mapping host–microbe interactions. Single-cell technologies enable the high-resolution dissection of host–microbe interactions, directly linking microbial signals to the transcriptional states of individual host cells. Recent advancements leverage single-cell RNA sequencing (scRNA-seq) to simultaneously profile host gene expression and microbial communities. Ghaddar et al. developed SAHMI (Single-cell Analysis of Host-Microbiome Interactions), a computational pipeline designed to recover, denoise, and acquire microbial signals directly from host tissue scRNA-seq data.[27] SAHMI classifies sequences from scRNA-seq FASTQ files using Kraken2, followed by microbial read extraction. Denoising and contaminant removal are achieved by examining the relationship between total and unique k-mer counts for each taxon and by utilizing negative control samples. SAHMI generates a barcode-metagenome counts matrix. It then removes barcodes not detected in the scRNA-seq matrix, retaining paired host cell and microbial barcodes. This pairing is critical for single-cell microbiome analysis as it directly associates identified microbes with specific host cells. Complementary pipelines, such as CSI-Microbes, extend these capabilities by detecting microbial reads from scRNA-seq data and performing differential abundance analyses across bacterial taxa, further broadening the analytical toolkit for single-cell microbiome research.[28] Moreover, Galeano Niño et al. developed INVADEseq (invasion-adhesion-directed expression sequencing), a new scRNA-seq method that introduces a primer targeting the conserved region of bacterial 16S rRNA into the 10x Genomics 5′ protocol, enabling simultaneous capture of bacterial and host transcripts to identify cell-associated bacteria and the host cells with which they interact.[13,29]

Tools for spatial profiling of tumor-resident microbes. Understanding the spatial organization of microbes within tumors is critical for deciphering microbe–host interactions. RNAscope-FISH employs fluorescence in situ hybridization with 16S or ITS probes to visualize bacterial and fungal cells directly within tissue sections, achieving single-cell resolution.[13] Spatial transcriptomics platforms, such as 10x Genomics Visium, couple barcoded slide capture with microbial sequence annotation to map microbial and host gene expression in situ, revealing distinct microbial microniches and their associated immunologic states.[13] Applying Visium in oral squamous cell carcinoma and colorectal cancer revealed that microbial communities are organized into heterogeneous microniches that coincide with immunosuppressive and low-proliferative tumor regions.[13] Complementing these methods, digital spatial profiling (DSP) platforms, such as NanoString GeoMx, enable high-plex quantification of RNA or protein expression within user-defined regions of interest (ROIs), facilitating integrated analysis of microbial presence with local host signaling pathways.[30] For example, GeoMx-guided spatial profiling of *Fusobacterium nucleatum* in breast cancer demonstrated that bacteria-rich regions exhibit altered expression of genes and proteins involved in multiple pathways, including MAPK signalling.[30] These spatially resolved multi-omics tools are revolutionizing our understanding of the intratumoral microbiome's ecology and function.

Computational pipelines and decontamination strategies

Robust computational workflows are indispensable for distinguishing authentic intratumoral microbial signals from pervasive low-biomass contaminants. A typical pipeline comprises: Removal of Host Reads & Quality Filtering. Raw FASTQ files are first aligned against the human reference genome (e.g., GRCh38) using BWA-MEM[31] or Bowtie2[32] to discard human-derived reads, thereby enriching for microbial sequences. Subsequent trimming of low-quality bases and adapter sequences (e.g., with Trimmomatic)[33] further refines read sets.

Taxonomic classification. High-speed k-mer–based classifiers such as Kraken2 or Centrifuge assign taxonomic labels to non-human reads by querying comprehensive microbial databases (including bacteria, archaea, fungi, viruses).[34] Confidence thresholds (e.g., >0.1 confidence) are applied to reduce spurious assignments.

Negative control–driven contaminant identification. Using extraction blanks, paraffin-only, and PCR NTCs, contaminant taxa are identified and removed either via simple prevalence filters or statistical methods like Decontam, which models taxa prevalence versus DNA concentration across real and negative samples to flag contaminants.[11] Taxa detected at greater relative abundance in controls than in true samples are typically excised.

Batch effect correction and normalization. Residual technical variation—stemming from batch, sequencing center, or library preparation—can dominate low-biomass profiles. Counts are often transformed to log-counts per million (log-CPM) or variance-stabilized units prior to downstream analyses.[35]

Cross-validation & independent validation. To ensure robustness, pipelines should be benchmarked on public datasets (e.g., HMP2 stool metagenomes) and independent cohorts (e.g., paraffin-extracted samples), verifying recovery of expected microbial signatures and absence of known kit contaminants.[36]

Genome assembly and binning (optional). For shotgun data, metagenomic assembly followed by binning (MetaBAT2) can reconstruct high-quality microbial genomes directly from tumors, enabling strain-level analyses and functional annotation.[15]

Implementing these stringent computational decontamination steps, in concert with experimental controls, is critical for reliable identification and quantification of intratumoral microbial communities.

Culturomics and functional validation

Complementing sequencing-based surveys, culturomics employs high-throughput culture methods to isolate living bacteria and fungi from tumor specimens, enabling definitive identification, strain-level characterization, and in vitro functional assays. Cultivation experiments with freshly resected human breast tumor homogenates on a panel of 35 distinct aerobic and anaerobic agar media successfully recovered viable intratumoral bacterial isolates, confirming the in-tumor viability of core breast cancer-associated microbial taxa.[4]

Once isolates are obtained, whole-genome sequencing of cultured strains enables comparative genomics to reveal tumor-adapted features—such as unique adhesins, invasins, and metabolic pathways—distinct from commensal reference strains.[37] In vitro co-culture assays with tumor cell lines and immune cells assess the functional consequences of microbial colonization, including induction of DNA damage (via colibactin-producing *E. coli*), modulation of cytokine release, and chemoresistance (via long isoform of the bacterial enzyme cytidine deaminase (CDD_L)–producing *Gammaproteobacteria*).[37]

In vivo validation employing germ-free or antibiotic-treated mouse models further establishes causality: intravascular inoculation of *Fusobacterium nucleatum* colonises murine mammary tumors and accelerates tumor growth and metastatic progression in a Fap2-dependent manner.[38] For fungi, germ-free mice gavaged with *Malassezia globosa* accelerate pancreatic intraepithelial neoplasia progression, by driving the complement cascade via mannose-binding lectin (MBL) activation.[6] These functional validation studies are critical to move beyond correlative observations and firmly establish the mechanistic roles of intratumoral microbes in shaping tumor phenotypes and therapeutic responses.

Collectively, these methodological foundations can be conceptualized as a step-wise "ribbon" workflow for intratumoral microbiome discovery (Figure 2). Starting from rigorous contamination control during sampling, to multi-omics profiling, single-cell and spatial mapping, computational decontamina-

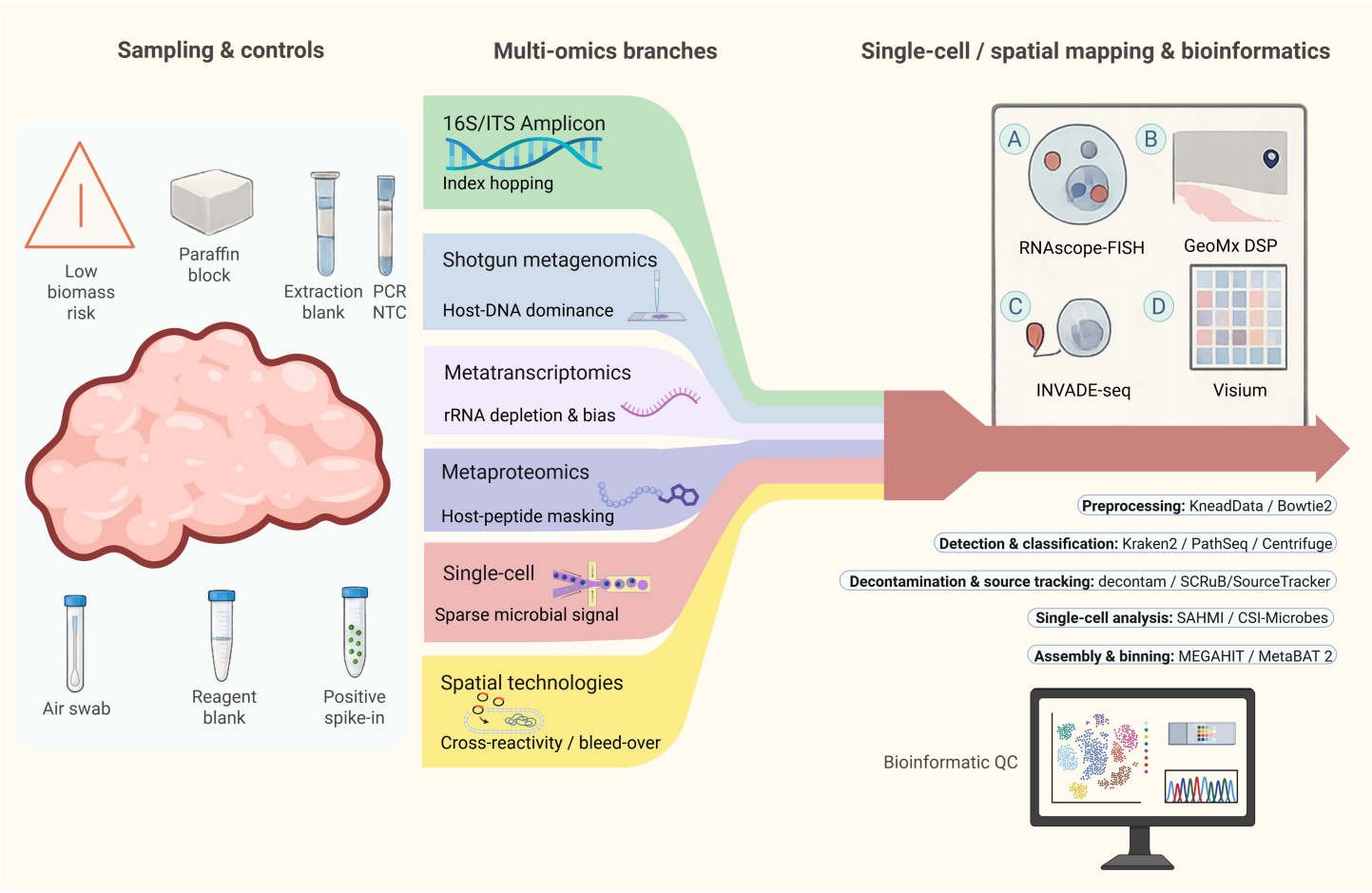

Figure 2. A ribbon workflow for low-biomass intratumoral microbiome discovery (1) Sampling and controls: aseptic tumor resection (low-biomass risk flagged in red) is paired with paraffin blocks, extraction blanks, PCR no-template controls, air swabs, reagent blanks and defined positive spike-ins to fingerprint kit-, environmental- and laboratory-derived contaminants. (2) Multi-omics branches, with six colour-coded lanes profiling complementary molecular layers — 16S/ITS amplicon (index hopping), shotgun metagenomics (host-DNA dominance), metatranscriptomics (host rRNA dominance), metaproteomics (host-peptide masking), single-cell (sparse microbial signal) and spatial technologies (cross-reactivity and spot bleed-over) — each annotated with its principal pitfall. (3) Single-cell and spatial mapping coupled to a bioinformatic stack: four orthogonal approaches resolve host–microbe localisation, namely (i) RNAscope-FISH, (ii) GeoMx digital spatial profiling, (iii) INVADE-seq and (iv) Visium spatial transcriptomics, feeding a five-layer pipeline spanning preprocessing (KneadData, Bowtie2), detection and classification (Kraken2, PathSeq, Centrifuge), decontamination and source tracking (decontam, SCRuB, SourceTracker), single-cell analysis (SAHMI, CSI-Microbes) and assembly and binning (MEGAHIT, MetaBAT 2) to yield a contamination-aware dataset. Abbreviations: DSP, digital spatial profiling; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence in situ hybridisation; INVADE-seq, invasion–adhesion-directed expression sequencing; NTC, no-template control; SCRuB, source-tracking for contamination removal in microbiomes. Created with BioRender.com.

tion, and culturomics-based functional validation—establish an integrated toolkit for dissecting the intratumoral microbiome (Figure 2). By combining experimental precision with computational rigor, they enable reliable detection of low-biomass microbial signals, resolution of spatial and cellular heterogeneity, and mechanistic validation of microbe–tumor interactions. Such a comprehensive approach is indispensable for moving the field beyond descriptive associations toward a causal and functional understanding of how tumor-resident microbes influence cancer biology and therapeutic outcomes.

Critical appraisal of microbial signal detection from host-derived omics data

The advent of large-scale cancer genomics consortia, such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), has inspired attempts to reconstruct tumor-associated microbiota by mining residual non-human reads from host-derived whole-genome and transcriptomic datasets. This retrospective strategy promised a cost-efficient means to survey microbial diversity across thousands of tumor samples, leveraging existing resources without the need for additional wet-lab work. Yet, the methodological validity of this approach has come under increasing scrutiny, following a series of reproducibility crises that have profoundly reshaped the field's understanding of what constitutes credible microbial evidence.

The *Nature* 2020 study by Poore et al. reported distinct microbial signatures across 33 cancer types and linked them to tumor classification and patient survival.[39,40] However, the study was formally retracted in 2024 after multiple independent re-analyses demonstrated that the majority of detected taxa likely stemmed from technical artifacts—including barcode cross-talk, reagent and environmental contamination, and misclassified human reads—rather than authentic microbial sequences. Moreover, polyA selection in RNA-seq and targeted capture in exome workflows inherently deplete microbial nucleic acids, rendering such data unsuitable for quantitative inference of microbial communities.[40-42] Collectively, these findings revealed that the apparent microbial signal in TCGA datasets largely reflects sequencing noise rather than bona fide intratumoral microbiota.

A parallel, though less severe, controversy emerged around the 2022 Cell study by Narunsky-Haziza et al., which constructed a pan-cancer atlas of tumor-associated fungi and bacteria and proposed the existence of cancer-type-specific mycobiome ecologies.[5] A subsequent analysis published in *Science Translational Medicine* in 2025 re-evaluated the publicly available dataset from this work and noted higher-than-expected read counts for several fungal species, which may in part stem from contamination in fungal reference genomes, human DNA, and vector sequences.[43]

Nevertheless, these controversies do not imply that TCGA or similar host-derived datasets are intrinsically unreliable. Rather, they underscore the current lack of universally accepted decontamination pipelines and the need for independent biological validation. Preliminary estimates suggest that studies leveraging TCGA-derived microbial signals may indeed be affected by

substantial contamination, particularly when not supported by orthogonal assays. However, the core observations—such as the enrichment of specific microbial taxa in distinct cancer types—have been corroborated by subsequent investigations using direct tissue sampling, in situ detection, and culture-independent microbial profiling.[4,13,15] Thus, when interpreted cautiously and integrated with experimental corroboration, host-derived omics remain an invaluable exploratory resource.

Despite these setbacks, public cancer genomics datasets remain valuable when approached with methodological restraint and analytical rigor. When used judiciously, they can serve as hypothesis-generating frameworks that guide more focused, prospective investigations. Several best practices have emerged from recent methodological reflections: (1) Incorporating matched normal tissues, extraction blanks, and detailed metadata on sequencing batches to model background contaminants; (2) Applying stringent read-length and quality thresholds, alongside up-to-date taxonomic classifiers with conservative confidence settings; (3) Cross-validating putative taxa using independent evidence such as in situ hybridization, qPCR, or culture-based assays; (4) Avoiding overinterpretation of relative abundance metrics derived from workflows not optimized for microbial nucleic acid recovery.

In essence, retrospective mining of TCGA and similar resources can provide meaningful heuristic insights but cannot substitute for prospectively designed, contamination-controlled studies that directly target microbial DNA and RNA. Importantly, these methodological lessons do not weaken the field but rather chart a constructive path forward. Research adopting standardized sampling, multimodal validation, and open data sharing is now producing high-confidence discoveries, laying the groundwork for a more rigorous and predictive microbial oncology.

LANDSCAPE OF THE INTRATUMORAL MICROBIOME

With the methodological framework above in mind—particularly the constraints imposed by low microbial biomass, contamination control, and decontamination pipelines—we now turn to the composition of the intratumoral microbiome itself, describing the bacterial, fungal, viral, and archaeal constituents that have been detected across solid tumors.

Bacteria across solid tumors: Cancer-type specificity and intracellular niches

Early dogma held that solid tumors were sterile, yet landmark metagenomic surveys have revealed diverse bacterial communities within most tumor types studied. Nejman *et al.* conducted 16S rDNA and metagenomic analyses on 1,526 tumor and adjacent normal tissue samples across seven cancer types—including breast, lung, ovary, pancreatic, melanoma, bone, and brain—and demonstrated that each tumor type harbors a distinct bacterial signature, with breast cancers exhibiting the richest and most diverse intratumoral microbiome.[4] Subsequent pan-cancer analyses of TCGA whole-genome sequencing data by Dohlman *et al.* corroborated these findings, showing cancer-type–specific bacterial genera whose relative abundances discriminate tumor from normal tissue.[44] For example, *Fusobacterium nucleatum* is the species most significantly associated with colorectal cancer (CRC) compared with matched normal tissue.[44]

Intracellular localization of bacteria within tumors and immune cells is a recurring theme. Using fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) against bacterial LPS/LTA, Nejman *et al.* visualized bacterial bodies in the cytosol of both malignant epithelial cells and tumor-associated macrophages.[4] Complementary correlative light and electron microscopy (CLEM) studies further confirmed intracellular bacteria in patient melanoma samples.[45] These observations suggest that bacterial colonization is not merely extracellular biofilm formation but involves active invasion or endocytosis, potentially exploiting tumor cell immune evasion and nutrient-rich niches.

Functionally, intracellular bacteria may modulate tumor cell behavior directly. *Fusobacterium nucleatum* FadA adhesin interacts with E-cadherin to activate β-catenin signaling in colorectal cancer cells, driving proliferation and invasion in vitro and in murine xenografts.[46] Similarly, bacteria within tumor-associated macrophages can skew polarization toward a pro-tumor M2 phenotype.[47]

Taken together, a growing body of evidence establishes that solid tumors are colonized by cancer-type–specific bacterial communities that occupy unique spatial niches—often intracellular—and interact intimately with host cells to influence oncogenic signaling, immune modulation, and therapy response. Ongoing efforts to resolve the mechanisms of bacterial entry, intracellular survival, and host manipulation will be critical to harnessing these insights for diagnostic and therapeutic innovation.

Fungal and archaeal constituents: Emerging data and pan-cancer mycobiomes

While bacteria have dominated tumor microbiome research, recent pan-cancer studies have revealed that fungi, though lower in biomass, are pervasive across solid malignancies, forming a distinct mycobiome.[5] In a landmark analysis of 17,401 tissue, blood, and plasma samples spanning 35 cancer types, Narunsky-Haziza *et al.* used ITS2 amplicon sequencing and shotgun metagenomics to detect fungal DNA in nearly all tumor types. The study further revealed cancer-type-specific fungal communities that primarily engage in co-occurrence and permissive interactions with bacteria, although localized resource competition does occur in certain regions.[5]

Fungi were identified in nearly all cancer types examined, albeit at much lower relative abundance than bacteria. The dominant genera included *Saccharomyces*, *Malassezia*, *Candida*, and *Aspergillus*, with distinct cancer-type-specific enrichments: *Malassezia* in pancreatic and colorectal cancers, and *Candida* in head and neck and lung cancers, mirroring earlier observations in individual cancer studies.[6,48,49]

Histological validation using fungal-specific FISH and IHC against anti–β-glucan antibodies confirmed intracellular fungal cells within tumor epithelia and tumor-associated macrophages in breast, melanoma, and lung samples.[5] Intracellular fungi can promote pancreatic ductal adenocarcinoma (PDAC) by driving the complement cascade via MBL activation, a mechanism demonstrated in PDAC models where *Malassezia* specifically mediates complement activation and oncogenesis.[6]

Multi-domain analyses reveal that fungal and bacterial communities within tumors frequently form permissive rather than antagonistic ecologies.[5] For example, unsupervised clustering analyses based on fungal co-occurrence patterns identified three unique fungi–bacteria–immune axis clusters, including F1 (*Malassezia-Ramularia-Trichosporon*), F2 (*Aspergillus-Candida*), and F3 (multi-genera including *Yarrowia*).[5] These interkingdom networks likely arise from shared adaptation to hypoxic, immune-suppressed tumor microniches.

In addition to fungi, emerging research is beginning to reveal the presence of another class of microorganisms, archaea, in tumors. Though understudied, archaea have been detected in lung adenocarcinoma and squamous cell carcinoma, and their dysregulated genes and pathways are potential candidate molecular targets pending experimental validation.[7] Their presence may reflect extreme anaerobic microenvironments and participate in complex metabolic networks, such as hydrogen consumption and methanogenesis, potentially impacting tumor redox state and stromal interactions.

Collectively, these emerging data underscore the complexity of the pan-cancer mycobiome and nascent archaeome. These findings may provide potential clues for cancer diagnosis, prognostic assessment, and new treatment strategies, such as microbiome-based therapies. Future efforts employing shotgun metatranscriptomics, spatial transcriptomics, and single-cell profiling will be essential to unravel the functional roles of fungi and archaea in tumor progression and therapy resistance.

Virome contributions and viral–bacterial–fungal interactions

Although viruses have long been recognized as oncogenic drivers—most notably human papillomavirus (HPV) in cervical and head and neck cancers and Epstein–Barr virus (EBV) in nasopharyngeal carcinoma—recent research has extended the scope to the broader tumor virome. Pan-cancer metagenomic surveys of TCGA WGS and RNA-seq data have detected viral reads in nearly every tumor type, revealing that the tumor virome encompasses not only classical oncoviruses but also a diverse community of commensal or 'passenger' viruses.[8]

Oncoviruses exhibit marked tumor tropism. HPV subtypes 16/18 exhibit high specificity for anogenital and oropharyngeal tumors, integrating into host genomes and driving E6/E7–mediated p53 and Rb inactivation.[50] EBV is

detected in essentially all undifferentiated nasopharyngeal carcinomas and defines a distinct molecular subtype of gastric cancer (about 8-10% of cases), where latent membrane protein 1 (LMP1) promotes NF-κB-mediated survival.[51] Additional oncoviruses include hepatitis B and C viruses in hepatocellular carcinoma and Merkel cell polyomavirus in Merkel cell carcinoma.[52-54]

Beyond the well-characterized oncogenic viruses, tumors harbor diverse non-oncogenic viral elements that can modulate tumor biology through poorly defined mechanisms. Distinct from exogenous tumor-colonizing viruses, human endogenous retroviruses (HERVs) are inherited viral genetic remnants embedded in the human genome, whose expression correlates with patient clinical prognosis. For instance, elevated HERV-derived ERV1 expression is significantly associated with poor overall survival in patients with renal cell carcinoma (P = 0.0081, log-rank test).[8] In addition, tumor-associated anelloviruses (torque teno viruses), a typical group of commensal viruses, exhibit higher titers in immunosuppressed cancer patients, although their direct contributions to tumorigenesis remain unclarified.[55]

Oncoviruses can remodel the bacterial microbiome. HPV-positive head and neck cancers exhibit enriched *Veillonella*, *Megasphaera*, *Anaerolineae* and decreased *Haemophilus* compared to HPV-negative tumors. These observations suggest that viral carcinogenesis and shifts in the bacterial microbiota may be interrelated through complex host–microbe interactions.[56,57] Similarly, EBV-associated gastric cancers harbor distinct *Lactobacillus* and *Streptococcus* signatures, possibly reflecting virus-induced mucosal inflammation that favors certain bacteria.[58]

Emerging data suggest that fungi and viruses may also interact within tumors. In hepatocellular carcinoma, aflatoxin B_1 produced by *Aspergillus flavus* and *Aspergillus parasiticus* induces characteristic p53 codon 249 mutations in hepatocytes, an effect that is strongly potentiated by concurrent hepatitis B virus (HBV) infection through impaired DNA repair and enhanced clonal expansion of mutated cells.[59] EBV+ nasopharyngeal carcinoma (NPC) patients exhibit significantly elevated oral *Candida albicans* loads compared to healthy controls.[60] Tumor-resident viruses can directly modulate host immunity. HPV E6/E7 expression leads to PD-L1 upregulation and T-cell exhaustion, which potentially synergize with bacterial LPS–TLR4 signaling to create an immunosuppressive microenvironment.[61,62]

These complex viral–bacterial–fungal networks underscore the need for integrated viromic, bacteriomic, and mycobiomic investigations using multi-omic and spatial platforms. Longitudinal profiling of oncoviral load, fungal colonization in pre- and post-treatment tumors will be crucial to disentangle causality from correlation and to exploit these interactions for prognostic biomarkers and combination therapies.

Spatial heterogeneity: microniches, single-cell colonization, and metastatic seeding

Intratumoral microbes are not uniformly distributed but instead localize to specialized microniches shaped by gradients of oxygen, nutrients, and immune infiltrates. These environments may offer protection from immune surveillance and provide unique metabolic substrates. Spatial transcriptomics and in situ imaging have revealed discrete bacterial and fungal hotspots that often correlate with hypoxic, necrotic, or immunosuppressed regions of the tumor. For example, Galeano Niño *et al.* used 10× Visium to show that *Fusobacterium nucleatum* localizes predominantly at immunosuppressive and low-proliferative microniches in colorectal cancer and oral squamous cell carcinoma.[13]

Beyond regional hotspots, it is critical to understand the direct physical interactions between microbes and individual host cells. Single-cell and microdissection approaches have begun to resolve microbe–host cell interactions at cellular resolution. In oral squamous cell carcinoma, INVADE-seq detected intratumoral *Fusobacterium* and *Treponema* predominantly colocalized with epithelial cells and monocyte-derived macrophage, and bacteria-enriched epithelial cluster displayed upregulation of signalling pathways involved in cancer progression.[13] In resectable PDAC, the unique tumor microbiome observed in long-term survivors may foster a pro-immune tumor microenvironment, characterized by enhanced CD8+ T cell recruitment and activation. Furthermore, this microbial signature could function as a prognostic biomarker for patient outcomes.[16]

Microniche colonization also impacts metastatic dissemination. In lung cancer models, Ma et al. found that intratumoral butyrate from *Roseburia* inhibits HDAC2 and increases H3K27 acetylation at the H19 promoter, upregulating MMP15 to facilitate invasion and inducing M2-like TAM polarization, thereby promoting lung cancer metastasis.[63] Battaglia *et al.* showed that the microbial composition of metastatic lesions is shaped more strongly by the anatomical site of metastasis than by the primary tumor of origin, supporting an organ-permissive niche model.[15]

These spatially resolved studies emphasize that mapping microbial colonization at microniche and single-cell levels is critical for understanding how tumor-resident microbes shape local immune suppression, extracellular matrix remodeling, and metastatic capacity. Future research should aim to go beyond static snapshots and enable dynamic tracking of these microbial microniches. Integrating imaging mass spectrometry with spatial metabolomics can reveal in real time how microbial metabolites shape the tumor microenvironment.

Collectively, these findings suggest that the intratumoral microbiome constitutes a structured, cancer-type specific ecosystem. Distinct microbial signatures are associated with particular tumor types and microenvironmental niches, with each cancer type exhibiting characteristic microbial consortia shaped by tissue-specific and local ecological pressures (Figure 3). This summary consolidates the recurrently identified microbial taxa across various human cancers, elucidating the mechanistic relationships between these microbes and cancer progression or biology (Table 2).[37,46,63-66] Evidence is stratified by symbols to denote clinical associations, in vivo functional validation, and in vitro mechanistic insights, providing a comprehensive framework for understanding these interactions.

DETERMINANTS OF MICROBIAL COMMUNITY STRUCTURE

Host tissue of origin versus metastatic organ tropism

One of the fundamental determinants of the intratumoral microbiome is the tissue of tumor origin, which largely reflects the unique resident microbial populations of each organ. Pan-cancer surveys of primary tumor samples have consistently demonstrated that each tumor type harbors a characteristic bacterial fingerprint. Notably, *Actinobacteria* (particularly *Corynebacteriaceae* and *Micrococcaceae* families) predominantly colonize non-gastrointestinal malignancies. In contrast, colorectal carcinomas exhibit microbial dominance of enteric anaerobes from *Firmicutes* and *Bacteroidetes* phyla. PDAC harbors a distinct microbial profile dominated by *Proteobacteria*, including *Gammaproteobacteria* taxa, closely resembling that of the duodenal microbiota.[4] These organotypic microbial signatures likely result from early cross-seeding events, wherein tumors are colonized by adjacent mucosal or epithelial microbiota during tumorigenesis, a process facilitated by local immune tolerance and metabolic compatibility.

However, metastases frequently colonize distant sites whose baseline microbiota differ from the primary tumor's niche. Recent pan-metastasis analyses reveal that while a core set of primary-tumor-associated bacteria often persists as so-called metastatic hitchhikers, the metastatic microenvironment further shapes microbial composition by recruiting organ-resident taxa.[15] This may suggest a permissiveness of bacteria to organ-specific niches. Notably, the composition of microbial communities in metastatic lesions appears to be more strongly influenced by the anatomical site of colonization. Among various cancer types, colorectal cancer metastases showed the most pronounced microbial divergence across metastatic sites. These findings suggest that organ-specific ecological conditions may create permissive or selective niches for distinct bacterial populations, thereby contributing to the establishment of site-specific microbial communities within metastases.[4,15]

Together, these findings support a tissue-plus-tropism model of intratumoral microbiome assembly, wherein microbial communities are established in two phases: (1) initial colonization by local microbiota at the primary tumor site and (2) ecological reshaping during dissemination and seeding of secondary niches. Paired primary-metastasis microbiome profiling and single-cell phylogenetic tracing are needed to quantify the relative contributions of each phase.[5] In clinical terms, recognition that metastatic tropism influences microbial ecology underscores the need to consider both primary and

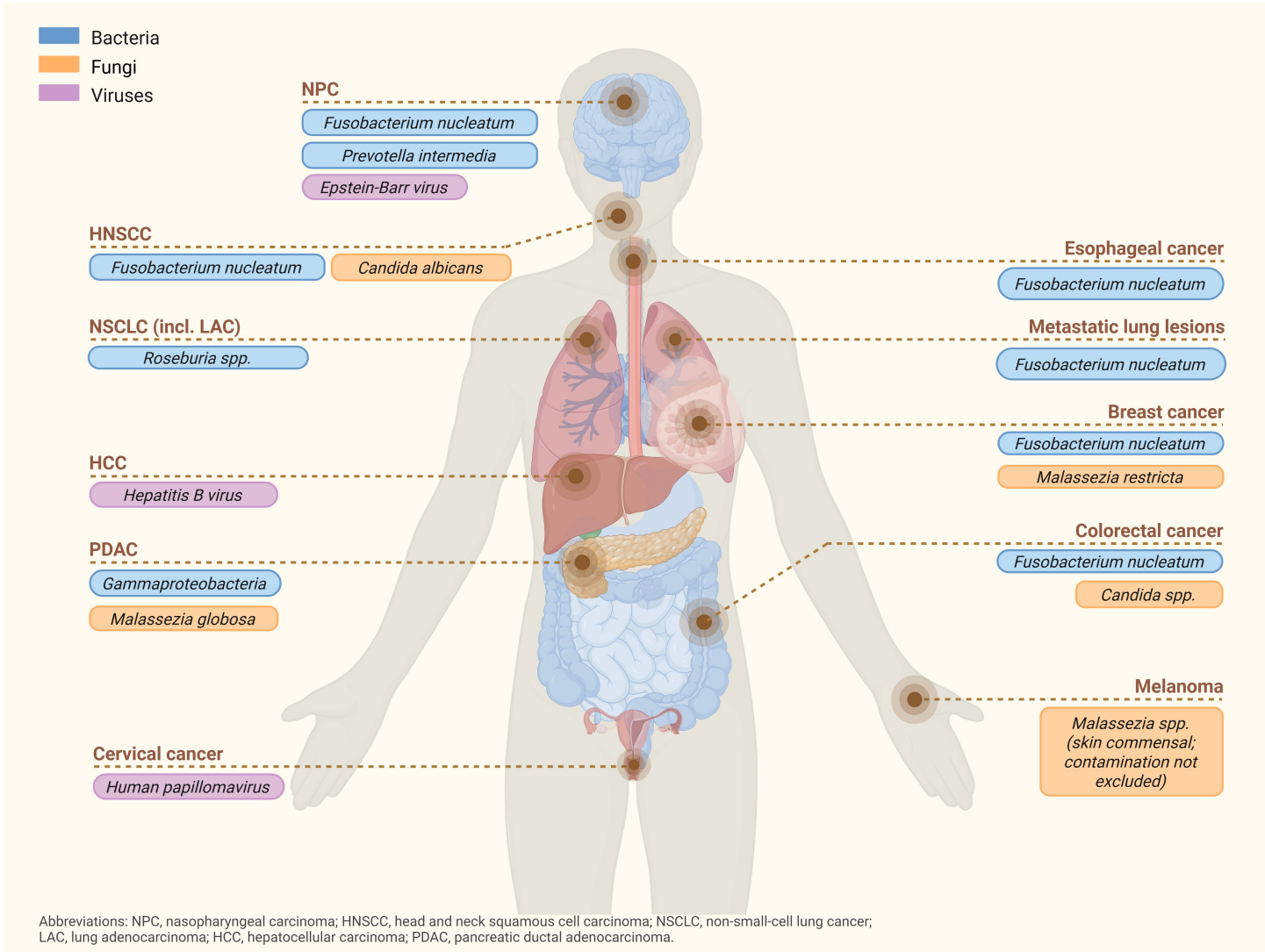

Figure 3. Pan-cancer anatomical atlas of intratumoral microbes Recurrent bacterial (blue), fungal (orange) and viral (purple) taxa identified by direct intratumoral sampling across eleven solid tumors are mapped to their anatomical sites. (i) NPC co-harbours EBV with the oral-translocated *Fusobacterium nucleatum* and Prevotella intermedia; HNSCC carries *Fusobacterium nucleatum* and *Candida albicans*. (ii) Oesophageal carcinoma harbours intracellular *Fusobacterium nucleatum*; NSCLC/LAC carries intratumoral *Roseburia* spp. distinct from gut commensal pools; lung metastases are enriched in *Fusobacterium nucleatum* across primary origins. (iii) Breast carcinoma is colonised by Fusobacterium nucleatum and *Malassezia globosa*. (iv) HCC is driven by HBV; PDAC harbours *Gammaproteobacteria* and *Malassezia globosa*, whose enrichment tracks tumor progression; colorectal carcinoma harbours *Fusobacterium nucleatum* and *Candida* spp. (v) Cervical carcinoma is driven by oncogenic HPV. (vi) Melanoma carries *Malassezia* spp., though trans-cutaneous sampling against a skin-commensal background cannot definitively exclude contamination. The pan-cancer recurrence of *Fusobacterium nucleatum* is consistent with an oral reservoir reaching tumors through site-specific routes — mucosal translocation (NPC, HNSCC), haematogenous spread (breast), and intracellular passenger transit in metastasising cells (CRC liver/lung metastases) — while Fap2–Gal/GalNAc binding supports local adhesion once the bacterium arrives; strain-level lineage tracing is still required for definitive route attribution. Abbreviations: EBV, Epstein–Barr virus; HBV, hepatitis B virus; HPV, human papillomavirus; NPC, nasopharyngeal carcinoma; HNSCC, head-and-neck squamous cell carcinoma; NSCLC, non-small-cell lung cancer; LAC, lung adenocarcinoma; HCC, hepatocellular carcinoma; PDAC, pancreatic ductal adenocarcinoma; CRC, colorectal carcinoma. Created with BioRender.com.

metastatic microbiome signatures when designing microbiome-targeted interventions or prognostic biomarkers.

Tumor microenvironmental pressures: Hypoxia, nutrient gradients, and immune contexture

The tumor microenvironment (TME) imposes diverse ecological pressures that further refine microbial community composition. Hypoxia, a hallmark of solid tumors, creates a formidable biogeographic filter that selectively enriches anaerobes and facultative anaerobes. Large-scale pan-cancer analyses (26 cancer types, 3,526 samples) reveal that 68% (112/165 genera) of tumor-associated bacteria exhibit anaerobic/facultative-anaerobic adaptations.[15] This selection pressure is particularly evident in hypoxic HPV-negative head and neck squamous cell carcinomas (n=31), where hypoxia-inducible factor-1 alpha (HIF-1α) expression positively correlates with anaerobic bacterial enrichment.[15] Similar hypoxia-driven colonization patterns occur in colorectal tumors, where *Fusobacterium nucleatum* promotes its own colonization by inducing ANGPTL4-mediated glycolytic reprogramming of host cells.[67]

Beyond oxygen limitation, nutrient partitioning within the TME—including glucose availability, amino acid scarcity, and lactate accumulation—imposes metabolic constraints that further structure the microbial community. In colorectal tumors exhibiting the Warburg effect, *Fusobacterium nucleatum* exemplifies how bacteria can actively reprogram host cell metabolism to create favorable colonization niches.[68] Specifically, *Fusobacterium nucleatum* infection upregulates the lncRNA ENO1-IT1, which directs the histone acetyltransferase KAT7 to increase H3K27 acetylation at the ENO1 promoter, thereby enhancing glycolytic enzyme expression and accelerating aerobic glycolysis in cancer cells.[68] This metabolic shift not only promotes tumor cell proliferation but also sustains an acidic, hypoxic microenvironment that favors anaerobic bacterial persistence.

Equally critical is the influence of the immune contexture. Spatial immune heterogeneity within the TME—from immunologically cold regions enriched in M2-polarized macrophages to inflamed hot zones populated by cytotoxic T cells—correlates with distinct microbial signatures. For example, in long-term

Table 2. Recurrently reported intratumoral taxa and their putative functional contributions to cancer biology.

Taxon (genus/species)	Predominant cancer-type(s) reported	Cellular niche	Signature metabolite / antigenic feature	Functional evidence	Key references
Fusobacterium nucleatum	CRC	Intracellular in tumor	FadA adhesin; LPS; outer-membrane vesicles	Mouse xenograft acceleration of CRC##; E-cadherin indicated β-catenin activation, autophagy-mediated chemoresistance⋆	Rubinstein 2013 (Cell Host Microbe);[46] Yu 2017 (Cell)[65]
Gammaproteobacteria (incl. Pseudomonas, Escherichia)	PDAC	Intra-epithelial and ductal lumina	CDD_L gemcitabine-inactivating enzyme	Mouse model, chemoresistance##	Geller 2017 (Science)[37]
Roseburia	Lung adenocarcinoma (early relapse)	Intra-tumor, mucus rich microniches	Butyrate (SCFA)	Butyrate promotes H19-driven EMT & M2 TAM polarization##	Ma 2024 (Cell Rep. Med.)[63]
Candida albicans	CRC	Extracellular / surface	Glycyl-L-phenylalanine, lysophosphatidylethanolamine, sphingosine 1-phosphate.	Reduction of CRC cell viability⋆	Zhang 2025 (World Journal of Clinical Oncology)[64]
Fusobacterium nucleatum	Glioma	Intra-tumor	N-acetylneuraminic acid; pro-inflammatory chemokine induction	Glioma proliferation promotion in mouse## and patient-derived glioma organoid models#; CCL2/CXCL1/CXCL2 chemokines upregulation⋆	Li 2025 (mSystems)[66]

Symbols: ## = in vivo functional proof; # = human clinical association; ⋆ = mechanistic pathway elucidated in vitro or ex vivo.

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; CRC, colorectal cancer; ICB, immune checkpoint blockade; SCFA, short-chain fatty acid; CDD_L, cytidine deaminase. LPS, lipopolysaccharide; PD-1, programmed cell death protein 1; DCs, dendritic cells; TAM, tumor-associated macrophage; EMT, epithelial-mesenchymal transition.

survivors of PDAC, intratumoral enrichment of *Pseudoxanthomonas*, *Streptomyces*, *Saccharopolyspora*, and *Bacillus clausii* correlates with higher CD8+ T-cell infiltration density and prolonged survival.[16]

Collectively, these microenvironmental pressures—hypoxia, nutrient supply, and immune architecture—act as selective filters that refine the intratumoral microbiome, driving local adaptation and shaping tumor–microbe co-evolution.

Oncogenic pathways and genomic instability: MSI vs. MSS tumors

Mismatch repair–deficient (MMR-D) tumors, characterized by high microsatellite instability (MSI) and elevated mutational burden, display distinct microbial profiles compared to microsatellite-stable (MSS) counterparts.[69–71] In a pan-cancer analysis of MSI and MSS tumors (n = 191), unsupervised clustering of microbial communities revealed two distinct clusters that significantly segregated according to MSI status. Interestingly, a subset of MSS tumors with a Cluster A-type microbial composition showed enriched interferon-γ (IFN-γ) signaling, resembling the immune phenotype of MSI tumors. This finding suggests that specific microbiome configurations may be associated with shared immunogenic features across genetically distinct tumor types.[15]

Mechanistically, MSI tumors harbor a high neoantigen load and constitutive activation of the cGAS–STING pathway, creating a pro-inflammatory milieu that may exert selective pressure favoring microbes capable of immune modulation. For instance, Fusobacterium nucleatum—enriched in some MSI contexts—can bind the immune checkpoint receptor TIGIT, thereby suppressing NK cell activity and facilitating immune evasion.[72,73] Additionally, fungal communities within tumors also reflect host genomic alterations. In CRC, increased intratumoral fungal heterogeneity and elevated fungal risk indices have been observed in tumors harboring KRAS mutations and MSI status.[74]

These correlations underscore a bidirectional interplay: oncogenic signaling and DNA repair deficiencies sculpt a selective microbial habitat, and microbial metabolites in turn can exacerbate genomic instability (e.g., via colibactin-mediated DNA double-strand breaks).[75] Integrative analyses combining host transcriptomic immune profiles with microbial genomic data will be essential to unravel causality and identify vulnerabilities in these co-evolved tumor–microbe ecosystems.

Therapy-induced microbial ecological shifts

Cancer treatments impose selective pressures that remodel the intratumoral microbiome, with important implications for efficacy and toxicity. Chemotherapy regimens can directly eliminate susceptible microbial taxa or, paradoxically, promote expansion of resistant species. A landmark study demonstrated that *Gammaproteobacteria* expressing CDD_L in pancreatic tumors inactivate gemcitabine, leading to drug resistance; antibiotic co-treatment restored chemosensitivity, illustrating how therapy alters—and is altered by—tumor microbes.[37] Similarly, in colorectal cancer, the efficacy of oxaliplatin is compromised in the presence of *Fusobacterium nucleatum*, which promotes chemoresistance by modulating host immune and autophagy pathways. Mechanistic studies have shown that *Fusobacterium nucleatum* activates TLR4–MYD88 signaling and regulates specific microRNAs (e.g., miR-18a⋆, miR-4802) to enhance autophagy, thereby reducing cancer cell susceptibility to chemotherapy.[65]

Radiotherapy also reconfigures microbial communities by inducing local inflammation and altering mucosal barriers. In a cohort study involving patients with oral or oropharyngeal cancer, those receiving radiotherapy or chemoradiotherapy exhibited a persistent increase in oral opportunistic pathogens (including enteric rods, *staphylococci* and *Candida* species) at one-year post-treatment, compared to patients treated with surgery alone.[76] While direct evidence of radiotherapy-induced changes to intratumoral microbiota remains limited, extrapolation from mucosal microbiomes suggests that irradiation likely reconfigures microbial communities within the tumor bed, potentially influencing both on-target tumor control and off-target tissue toxicity.[77]

The advent of immune checkpoint blockade (ICB) has further illuminated the role of therapy-driven microbial dynamics. ICB non-responders exhibit higher abundance of *Fusobacterium* or *Proteobacteria* that foster immunosuppressive myeloid populations.[78,79]

These therapy-induced shifts underscore a dynamic treatment-microbiome-tumor axis, in which conventional and immune-based treatments co-

Table 3. Mechanistic links between microbe-derived metabolites and cancer hallmarks.

Metabolite (chemical class)	Predominant intratumoral or gut-to-tumor–translocated producers	Primary host molecular targets	Cancer hallmarks impacted	Evidence tier	Key references
Butyrate	*Roseburia* (lung cancer)	H19 (lncRNA); M2 Macrophage (via HDAC/cytokines)	Epigenetic re-programming, immune evasion, metastatic fitness	Cell; Mouse; Patient	Ma 2024 (Cell Rep Med)[63]
β-Glucan fragments (fungal cell wall)	Intratumoral *Malassezia spp.* (PDAC)	MBL; Complement C3	Myeloid recruitment, immune suppression	Cell; Mouse; Patient	Aykut 2019 (Nature)[6]
Desaminotyrosine (flavonoid derivative)	*Clostridium orbiscindens* (gut microbiota, systemic circulation)	Type-I IFN potentiation	Enhanced T-cell priming, ICB synergy	Mouse	Joachim 2023 (EBioMedicine)[82]
Colibactin	*pks+ E. coli* (polyketide synthase island) CRC	Host DNA (Double-strand breaks / Alkylation)	Genomic instability and mutation	Cell; Mouse; Patient	Nougayrède 2006 (Science);[75] Pleguezuelos-Manzano 2020 (Nature)[83]
Indole-3-aldehyde	*Lactobacillus reuteri* (melanoma)	Aryl hydrocarbon receptor (AhR) in $CD8^+$ T cells	Enhanced anti-tumor immunity, immune checkpoint blockade synergy	Cell; Mouse	Bender 2023 (Cell)[84]

Evidence tier symbols: Cell = in-vitro mechanistic; Mouse = in-vivo pre-clinical; Patient = clinical association.

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; CRC, colorectal cancer; HDAC, histone deacetylase; ICB, immune checkpoint blockade; MBL, mannose-binding lectin; IFN, interferon.

evolve with, and are influenced by, intratumoral microbial ecologies. Strategically integrating microbiome monitoring into clinical protocols may enable anticipatory microbiome modulation, via antibiotics, probiotics, or diet, to enhance treatment response and mitigate resistance.

FUNCTIONAL INTERPLAY BETWEEN MICROBIOTA AND CANCER HALLMARKS

Microbial metabolites modulating epigenetics and signaling

Microbial-derived small molecules influence tumor cell behavior by rewiring epigenetic landscapes and signal transduction pathways. Among the best-studied metabolites, short-chain fatty acids (SCFAs)—especially butyrate—accumulate within tumor microenvironments colonized by butyrogenic bacteria such as *Roseburia*.[63,80] Butyrate acts as a histone deacetylase (HDAC) inhibitor, enhancing acetylation of histone H3K27 and promoting expression of pro-metastatic noncoding RNAs such as H19, which in turn upregulate MMP15 to facilitate invasion.[63]

In the context of histone-lactylation, L-lactate drives histone lysine lactylation (Kla) and, in late-phase M1-polarized macrophages, activates a homeostatic gene program (e.g., Arg1) via histone lactylation.[81] Although discovered in bacterially challenged macrophages, emerging evidence suggests tumor-resident lactate can similarly reprogram epigenetics in both stromal and tumor cells, creating a feedback loop between Warburg-driven lactate production and Kla-mediated gene activation.

Table 3 further clarified the chemical communication between the microbiome and the host by listing specific metabolites, their primary producers, the definitive host molecular targets (e.g., H19, MBL, IFN), and the resulting impact on cancer hallmarks.[6,63,75,82-84] Together, these examples highlight a metabolite-centric axis through which intratumoral microbes remodel chromatin and cellular signaling, thereby influencing proliferation, invasion, and immune evasion. Future efforts employing single-cell and spatially resolved multi-omics analyses, primarily at the level of individual host tumor and immune cells, will be critical to map the precise epigenetic "imprints" of microbial metabolites.

Immune modulation by intratumoral microbiota

Tumor-resident microbes exert powerful effects on both innate and adaptive arms of antitumor immunity, often reshaping the balance between immunosurveillance and immunosuppression.

Intratumoral bacteria and their metabolites can drive macrophage polarization toward either tumor-promoting M2 or tumor-restraining M1 phenotypes. For instance, D-lactate produced by *Lactobacillus* strains reprograms TAMs from an M2-like immunosuppressive state to M1-like effector cells through inhibition of the PI3K/Akt pathway and activation of NF-κB signaling.[85] Conversely, tumor cell-derived L-lactate from Warburg glycolysis induces histone lactylation in M1 macrophages, triggering late-phase expression of homeostatic genes including ARG1, thereby dampening inflammatory responses and facilitating wound healing–like, pro-tumor functions.[81,86] Neutrophil recruitment dynamics further exemplify microbial-immune interplay. Intratumoral *Fusobacterium nucleatum* secretes Fap2 lectin, which binds tumor cell Gal–GalNAc, triggering local CXCL1 and IL-8 production and consequent neutrophil influx that can create neutrophil extracellular traps (NETs) that may shield metastatic cells from immune-mediated clearance.[73,87-89] These contrasting macrophage and neutrophil dynamics underscore how microbial cues sculpt an innate immune landscape that can either curb or foster tumor progression. Intratumoral microbes influence T-cell–mediated immunity at multiple levels. Bacterial components such as LPS and peptidoglycan activate dendritic cells (DCs) via toll-like receptors, enhancing cross-presentation of tumor and microbial antigens to $CD8^+$ T cells.[90]

Collectively, these studies highlight a bidirectional dialogue wherein microbes modulate innate recruitment, adaptive priming, and checkpoint responsiveness, while the evolving immune milieu co-selects for microbial consortia that either bolster immunosurveillance or subvert therapy. Integrating single-cell immune profiling with spatial microbial mapping will be essential to decode these complex interactions and to harness microbial adjuvants that enhance cancer immunotherapy.

Impact on DNA damage and metastatic fitness

Several intratumoral bacteria produce direct DNA-damaging toxins that can accelerate oncogenic mutagenesis. For example, *Escherichia coli* strains harboring the pks island synthesize colibactin, a cyclomodulin that induces DNA double-strand breaks, chromosomal aberrations, and a unique mutational signature in colorectal epithelial cells, thereby promoting tumor initiation.[75,83,91] Similarly, cytolethal distending toxin (CDT), produced by certain species of *Campylobacter*, induces DNA strand breaks and causes cell cycle arrest. Such damage has been implicated in colorectal cancer, which may also enhance metastatic potential by increasing tumor cell plasticity and genomic diversity.[92,93] These microbial genotoxins establish a mechanistic

Table 4. Clinical associations of intratumoral and circulating microbiomes in major solid tumor types.

Cancer type (stage predominating)	Diversity index and Prognosis	Predictive / enrichment taxa for ICB or targeted Treatment	Chemo-/drug-modifying taxa and metabolites	Circulating microbial DNA / EV signature	Key references
NSCLC	Low alpha diversity ↔ poor RFS	*Roseburia* (Enriched in early recurrence)	Butyrate (Microbial metabolite promoting metastasis)	16S tumor-tissue AUC = 0.887; cmDNA panel AUC = 0.857 (discovery) / 0.717 (validation)	Ma 2024 (Cell Rep Med)[63]
Hepatocellular carcinoma	-	Gut microbiome-derived D-lactate as an immunomodulatory agent	D-lactate (DL) transforms M2 TAMs to M1 phenotype	DL-loaded nanoparticles (DL@NP-M-M2pep) for targeted delivery	Han 2023 (Sci Adv)[85]
CRC	High *Fusobacterium nucleatum* ↔ poor prognosis	*Fusobacterium nucleatum* diminish the response to immunotherapy	Succinic acid induces resistance to immunotherapy	High Serum Succinic acid levels	Mima 2016 (Gut);[72] Jiang 2023 (Cell Host & Microbe)[103]
HNSCC (Advanced/ Recurrent)	HPV-positive status ↔ improved OS	HPV-positive status (p16+) strongly predicts improved response to anti-PD-1	HPV modulates TME inflammation and PD-L1 expression	HPV status determined by p16 staining or DNA PCR	Wang 2019 (Scientific Reports)[104]
PDAC	High alpha diversity →longer OS	Higher microbial diversity correlates with increased $CD8^+$ T cell infiltration	*Gammaproteobacteria* (CDD_L cytidine deaminase) → gemcitabine chemoresistance	-	Riquelme 2019 (Cell);[16] Geller 2017 (Science)[37]

Abbreviations: NSCLC, non-small cell lung cancer; CRC, colorectal cancer; HNSCC, head and neck squamous cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; OS, overall survival; RFS, long recurrence-free survival; TAMs, tumor associated macrophages; PD-1, programmed cell death protein 1; HPV, Human papillomavirus; PCR, polymerase chain reaction; AUC, area under the curve; TME, tumor microenvironment; CDD_L, bacterial enzyme cytidine deaminase.

link between intratumoral bacteria and cancer-associated genomic instability.

Intratumoral microbes can further boost metastatic potential by both cell-intrinsic and niche-modifying mechanisms. In spontaneous breast cancer models, intracellular bacteria derived from primary tumors co-migrate with circulating tumor cells (CTCs), reorganize the actin cytoskeleton via RhoA-dependent pathways, and increase CTC survival under shear stress, leading to enhanced metastatic colonization.[94,95] Similarly, in lung cancer, butyrate from intratumoral Roseburia upregulates the lncRNA H19, which promotes E-cadherin downregulation and M2-like macrophage polarization, creating a pro-metastatic microenvironment.[63]

These findings highlight a dual role for intratumoral microbes: they act not only as initiators of genomic instability but also as modulators of metastatic progression. Future studies employing isotope tracing and single-cell metabolomics will be crucial to map these microbe-tumor metabolic crosstalks with spatial resolution.

Polymicrobial synergies and antagonisms (bacteria–fungi, bacteria–virus)

Solid tumors harbor not only bacteria but also fungi and viruses, which together form polymicrobial consortia. These interkingdom communities engage in cooperative or antagonistic interactions that can modulate tumor progression, immune responses, and treatment outcomes. Recent pan-cancer mycobiome analyses have demonstrated that tumor-resident fungi are not randomly distributed; instead, they exhibit reproducible co-occurrence patterns with bacteria and specific immune microenvironments. Unsupervised clustering has revealed three distinct mycotypes across multiple tumor types: F1 (*Malassezia–Ramularia–Trichosporon*), F2 (*Aspergillus–Candida*), and F3 (multi-genera including *Yarrowia*)—each associated with characteristic shifts in microbial abundance and immune signatures. These fungal-bacterial-immune clusters suggest a coordinated ecological restructuring across microbial kingdoms that may influence tumor biology in a subtype-specific manner.[5] For instance, *Malassezia* species (notably *M. globosa*) often co-occur with *Proteobacteria* in pancreatic tumors and drive oncogenesis via MBL-dependent activation of the complement cascade.[5,6] *Candida* species can support growth of oral bacteria including *Fusobacterium nucleatum* in biofilm models, suggesting that targeted antifungal therapy may destabilize complex communities.[96-98]

Viral components within the tumor microenvironment further diversify these interkingdom networks. Human papillomavirus (HPV) infection, for example, modulates both vaginal and oral microbiomes, with implications for systemic immunity and local bacterial interactions. Vaginal microbiome profiling has identified distinct cancer-associated microbial signatures, including *Bacteroides, Mycoplasma, Bacillus, Dialister, Porphyromonas, Anaerococcus, and Prevotella*.[99] Notably, increased *Bifidobacterium* abundance has been linked to high-risk HPV clearance, and may be enhanced by focused ultrasound treatment, highlighting potential avenues for therapeutic modulation.[99,100] Similarly, bidirectional interactions between herpesviruses and bacteria have been implicated in periodontal diseases and oncogenesis. Periodontal herpesviruses can promote the proliferation of periodontopathic bacteria, while bacterial-induced inflammation may reactivate latent viral infections. These mutualistic interactions compromise mucosal immunity, enhance bacterial colonization, and contribute to the pathogenesis of aggressive periodontitis.[101] In the context of nasopharyngeal carcinoma (NPC), co-infection with Epstein–Barr virus (EBV) and oral bacteria correlate with disease severity. The abundance of oral translocated microbes in the nasopharynx is positively associated with local EBV load, suggesting that dysbiotic bacterial communities may facilitate EBV reactivation or persistence, collectively promoting a tumor-permissive niche.[102]

These examples underscore the intricate and dynamic nature of polymicrobial interactions within tumors, which may act either synergistically or antagonistically to influence tumor behavior. Future network-based analyses that integrate taxonomic abundance with metatranscriptomic and functional gene expression data will be instrumental in identifying microbial keystone species.[5]

CLINICAL CORRELATES

Prognostic associations: Diversity indices and specific taxa linked to survival or recurrence

Multiple cohorts have demonstrated that intratumoral microbial diversity, quantified by indices such as Shannon or Simpson metrics, correlates with patient outcomes across cancer types.[16] Table 4 highlights that intratumoral and circulating microbiomes are clinically relevant across solid

tumors.[16,37,63,72,85,103-104]

Beyond global diversity, specific microbial taxa have been identified as individual prognostic biomarkers. In colorectal cancer, enrichment of Fusobacterium nucleatum within primary tumors correlates with shorter disease-specific survival and increased recurrence, likely via its ability to modulate Wnt/β-catenin signaling and recruit myeloid-derived suppressor cells.[72,105,106] In NSCLC, Ma et al. identified that intratumoral abundance of butyrate-producing *Roseburia* was significantly associated with early postoperative recurrence, and constructed a six-genus microbial signature that independently predicted recurrence-free survival with high accuracy.[63] In cervical cancer, tumor-resident *Lactobacillus iners* was independently associated with poorer recurrence-free and overall survival and promoted chemoradiotherapy resistance through L-lactate-induced metabolic rewiring.[107] Additional studies have linked intratumoral Fusobacterium nucleatum and Pseudomonas to poorer survival outcomes in cervical carcinoma.[107,108] A broader prognostic landscape has also been observed in gastric cancer, where multivariable analyses identified multiple intratumoral microbial taxa across the phylum, genus, and species levels that were independently associated with overall survival.[109]

Fungal taxa also hold prognostic significance. Pan-cancer mycobiome analyses demonstrate that intratumoral *Malassezia globosa* correlates with reduced overall survival (OS) in breast cancer, while intratumoral *Phaeosphaeriaceae* is associated with shorter progression free survival (PFS) in ovarian cancer.[5] In metastatic melanoma, intratumoral fungal profiles have been associated with response to immune checkpoint inhibitors, with *Capnodiales* and its genus *Cladosporium* significantly enriched in non-responders.[5,110,111]

Leveraging both bacterial and fungal abundance data often outperforms single-kingdom classifiers in prognostic prediction. For example, a composite tumor microbiome risk score incorporating *Pseudoxanthomonas, Streptomyces, Saccharopolyspora* and *Bacillus clausii* abundances effectively stratified pancreatic cancer patients into high- and low-risk groups, demonstrating superior performance compared to clinical staging alone.[16,112] Further enhancing these models by incorporating intratumoral immune cell densities (e.g., CD8+ T cells) improves prognostic accuracy.[4,16,113]

Collectively, these findings strongly suggest that both global diversity metrics and taxon-specific signatures possess robust prognostic value across cancer types. The development of standardized prognostic microbiome panels, validated in large, prospective cohorts, will be critical for translating these insights into clinically applicable risk calculators that can complement established genomic and pathological staging systems.

Predictive markers for therapy response

The composition of the intratumoral microbiome has emerged as a predictor of response to immune checkpoint blockade. In metastatic melanoma, intratumoral fungal profiles have been associated with response to immune checkpoint inhibitors, with Capnodiales and its genus Cladosporium enriched in non-responders.[5] Mechanistically, bacteria-derived peptides can be presented by HLA-I and HLA-II molecules on melanoma cells and antigen-presenting cells, and some of these peptides elicit tumor-infiltrating T-cell reactivity.[45] Preclinical evidence further indicates that intratumoral Lactobacillus reuteri can enhance immune checkpoint inhibitor efficacy through an indole-3-aldehyde–CD8 T-cell axis.[84] Beyond immunotherapy, chemotherapeutic drugs can be inactivated or activated by tumor-resident microbes. Geller et al. first revealed that intratumoral *Gammaproteobacteria* express CDD_L, which metabolizes gemcitabine to its inactive form, thereby leading to chemotherapy resistance in pancreatic cancer.[37] On the other hand, cyclophosphamide can be significantly influenced by the gut microbiome through immune system modulation. Cyclophosphamide treatment facilitates the translocation of specific commensal bacteria, notably Lactobacilli and Enterococci, from the gut lumen to secondary lymphoid organs like the spleen. Once translocated, these bacteria, particularly *Enterococcus hirae* and *Barnesiella intestinihominis*, stimulate crucial anti-tumor immune responses, including the generation of pathogenic T helper 17 (pTH17) cells and the induction of memory Th1 responses. Ultimately, this robust immune stimulation enhances cyclophosphamide's anti-tumor efficacy by increasing the accumulation of cytotoxic T-cells within the tumor microenvironment.[114,115]

Beyond immunotherapy and chemotherapy, the tumor microbiome also serves as a predictive biomarker for response to targeted therapies. In HER2-positive breast cancer, for example, *Pseudomonas aeruginosa* has been shown to secrete the quorum-sensing molecule 3-oxo-dodecanoyl homoserine lactone (3oc), which induces trastuzumab resistance by activating TGF-β/ErbB2 signaling in cancer cells.[116]

Taken together, these findings underscore that both the compositional and functional attributes of tumor-resident microbes represent robust predictive biomarkers of therapeutic response. As summarized in Table 4,[16,63,85,103] specific microbial taxa are linked to treatment efficacy, while circulating microbial DNA and extracellular vesicle (EV) profiles offer minimally invasive modalities for early detection and dynamic treatment monitoring, often complementing or even outperforming conventional clinical markers. Accordingly, prospective patient stratification that integrates intratumoral microbiome profiling with circulating microbial signatures holds significant potential to refine therapeutic decision-making and improve clinical outcomes.

Minimally invasive diagnostics

Liquid biopsies that capture cell-free microbial nucleic acids offer a noninvasive window into tumor-associated microbiomes. Plasma-derived fungal DNA has emerged as a promising diagnostic biomarker, with pan-cancer analyses demonstrating its ability to discriminate between cancer patients and healthy controls with high accuracy.[5] In addition to conventional cell-free microbial DNA profiling, extracellular-vesicle-associated bacterial DNA profiles are emerging as candidate liquid-biopsy biomarkers.[117-119] For instance, studies in gastric cancer and renal cell carcinoma have shown that 16S rRNA gene profiling of bacterial DNA in serum or urine extracellular-vesicle (EV) fractions can distinguish patients with cancer from controls.[117-119] Bacterial extracellular vesicles can also carry functional small RNAs, although the diagnostic utility of microbial EV RNA profiles in cancer remains to be established.[120]

Developing robust, standardized protocols for microbial cell-free nucleic acid isolation, sequencing, and analyses—alongside appropriate contamination controls—is critical to effectively translate these minimally invasive diagnostic approaches into widespread clinical practice.

Microbiome-informed patient stratification and risk calculators

Building on prognostic and predictive microbiome biomarkers, risk calculators that integrate microbial features with clinical variables (age, tumor stage, genomic alterations) are currently being developed to refine patient stratification. In pancreatic cancer, Riquelme et al. identified a bacterial signature consisting of *Pseudoxanthomonas*, *Streptomyces*, *Saccharopolyspora*, and *Bacillus clausii* that was highly predictive of long-term survival in two independent patient cohorts, and demonstrated that integrating this signature with standard TNM staging significantly improved prognostic accuracy compared to clinical factors alone.[16] Similarly, in NSCLC, Ma et al. constructed a six-genus intratumoral microbial signature whose tumor-tissue 16S panel independently predicted recurrence-free survival with high accuracy (AUC=0.887); a companion plasma cell-free microbial DNA (cmDNA) panel achieved AUC=0.857 (discovery) / 0.717 (validation), providing a minimally invasive complement to tissue-based stratification.[63] Risk scores utilizing both gut and tumor microbiome signatures may help identify patients who could benefit from intensified surveillance or adjuvant therapy, as well as low-risk patients who may be suitable for treatment de-escalation.[121] Rigorous external validation in large-scale, multi-center prospective trials is essential to establish generalizability across diverse populations, sequencing platforms and clinical settings. In future, microbiome-informed risk stratification holds promise to advance truly personalized oncology, guiding cancer screening frequency, therapeutic selection, and post-treatment follow-up intensity.

THERAPEUTIC OPPORTUNITIES

Recent clinical trials and ongoing interventional studies are exploring strategies to modulate the tumor-associated microbiome, with the aim of improving cancer treatment outcomes (Table 5).[122-126] These efforts underscore the translational potential of the microbiome as a novel therapeutic target or synergist. The array of strategies under investigation includes Fecal

Table 5. Ongoing or planned interventional trials that manipulate the tumor-associated microbiome.

Registry ID / Sponsor	Intervention class and specific agents	Cancer indications	Phase / Design	Primary endpoint(s)	Status / est. completion	Key references
NCT03341143 (Zarour, Hassane, MD)	Drug: Fecal Microbiota Transplant with Pembrolizumab	Stage III or IV melanoma	Ph II, Single Group Assignment	ORR	Completed	Davar 2021 (Science)[122]
NCT03353402 (Sheba Medical Center)	FMT	Metastatic melanoma	Ph I, Single Group Assignment	Incidence of FMT-related Adverse Events	Unknown (2019)	ClinicalTrials.gov, Baruch 2021(Science)[123]
NCT04116775 (Julie Graff, MD)	FMT+ Pembrolizumab+ Enzalutamide	Prostate cancer	Ph II	Anticancer effect of FMT from pembrolizumab responders to non-responders	Recruiting	Yang 2024 (EBioMedicine)[124]
NCT03817125 (Parker Institute for Cancer Immunotherapy)	Nivolumab+ SER-401	Metastatic melanoma	Ph Ib	Percentage of Patients with Adverse Events	Completed	Glitza 2024 (Cancer Discov)[125]
NCT06349590 (University of Chicago)	Dietary Intervention (High-fiber / Low-fat meals)	CRC	Ph I/II	Recurrence and Metastasis prevention; Organoid validation	Recruiting	ClinicalTrials.gov
NCT05748145 (Oncology Institute of Southern Switzerland)	Antibiotic Intervention (Metronidazole Oral)	CRC	Ph II, Interventional , Proof-of-concept	Reduction of *Fusobacterium nucleatum* load in tumor tissues	Recruiting	De Dosso 2025 (ESMO Gastrointestinal Oncology)[126]

Abbreviations: ORR, objective response rate; FMT, fecal microbiota transplantation; CRC, colorectal cancer; Ph, phase.

Microbiota Transplantation (FMT), rationally designed live biotherapeutics, phage-mediated targeted therapies, and the modulation of metabolites or their precursors to influence the TME.

Microbiota modulation strategies

Modulating tumor-associated microbiota represents a promising avenue for altering tumor progression and enhancing therapeutic responses. Several strategies, including antimicrobial agents, engineered microbes, and bacteriophage therapy, are being explored to reshape the tumor microbial landscape and its interaction with the host immune system.

Antibiotics, antifungals and ecological rebounding. Broad-spectrum antibiotics have been used to deplete tumor-resident bacteria, reversing microbe-mediated drug resistance and modulating the tumor microenvironment. Geller et al. showed that intratumoral *Gammaproteobacteria* confer resistance to gemcitabine by expressing CDD_L, and that concurrent antibiotic treatment restores chemosensitivity in PDAC models.[15,37] Similarly, antifungal targeting of Malassezia species in murine PDAC reduced tumor growth through a mechanism involving MBL, mediated complement activation and oncogenic inflammation.[5,6] However, indiscriminate microbial eradication risks ecological rebounding—overgrowth of resistant or opportunistic species—and collateral damage to beneficial commensals.[127] Therefore, precision antimicrobials that selectively target pathogenic tumor-resident taxa while sparing symbionts are under active development.[128,129]

Engineered bacteria and bacteriophage therapy. In contrast to microbial depletion, engineered probiotic strains have further expanded the therapeutic potential of microbiota manipulation. For instance, a genetically modified Escherichia coli Nissle 1917 strain engineered to convert ammonia into L-arginine within tumors increased tumor-infiltrating T cells and synergized with PD-L1 blockade to promote tumor clearance.[130] Another approach utilized non-pathogenic E. coli engineered to selectively lyse within the tumor microenvironment and release a nanobody antagonist against CD47, leading to tumor regression, metastasis prevention, and durable survival in syngeneic models.[131] Moreover, bacteriophage therapy—the use of viruses that specifically lyse bacterial pathogens—represents a precision tool to deplete resistance-conferring tumor residents without disrupting the broader microbiome. Phage-guided targeting of *Fusobacterium nucleatum* has shown preclinical potential in colorectal cancer. In mouse models, *Fusobacterium nucleatum*-targeting phages linked to irinotecan-loaded nanoparticles enhanced chemotherapy efficacy.[132,133]

Collectively, these microbiota modulation strategies, ranging from microbe depletion to microbial augmentation, offer a suite of interventions to remodel the tumor ecosystem. Their clinical translation will depend on rigorous assessment of safety, delivery methods, and microbial rebound dynamics, ideally guided by companion microbiome diagnostics to tailor interventions to individual tumor microbiomes.

Microbial metabolite mimetics or inhibitors

Microbial metabolites—including SCFAs, tryptophan catabolites and secondary bile acids—exert profound effects on tumor biology and host immunity. Targeting these metabolites, either by mimicking beneficial compounds or inhibiting deleterious ones, offers a precision strategy to reprogram the TME without eradicating the microbiota.

Among SCFAs, butyrate—produced by commensal bacteria such as *Roseburia*—exerts epigenetic regulation through HDAC inhibition. In cytotoxic T lymphocytes (CTLs) and chimeric antigen receptor (CAR) T cells, in vitro exposure to butyrate or pentanoate enhances mTOR signaling and suppresses class I HDAC activity. This metabolic reprogramming promotes the expression of activation markers and effector cytokines, such as CD25, IFN-γ, and TNF-α, thereby significantly boosting anti-tumor efficacy in murine melanoma and pancreatic cancer models.[134] However, the role of butyrate is not universally protective. In lung cancer, bacteria-derived butyrate has been shown to promote metastasis by upregulating the long non-coding RNA H19 via HDAC2 inhibition and H3K27 acetylation at the H19 promoter, while simultaneously inducing M2 macrophage polarization.[63]

Tryptophan-derived metabolites also play important roles in modulating tumor immunity. Dietary interventions rich in fiber and anthocyanins have been shown to enhance probiotic abundance and elevate ILA levels, thereby suppressing colorectal cancer progression.[135,136] Another key metabolite, indole-3-propionic acid (IPA), generated via cooperative metabolism between *Lactobacillus johnsonii* and *Clostridium sporogenes*, improves responsiveness to anti-PD-1 therapy by promoting progenitor-exhausted $CD8^+$ T cells. This occurs through enhanced H3K27 acetylation at Tcf7 super-enhancers, thereby potentiating immune checkpoint blockade efficacy across melanoma, breast, and colorectal cancers.[137]

Secondary bile acids, such as deoxycholic acid (DCA), are microbial transformations of host-derived primary bile acids, which have pro-carcinogenic and immune-suppressive effects.[138,139] Elevated DCA levels are associated with immunosuppression and carcinogenesis, particularly in hepatocellular carcinoma (HCC), and may result from reduced abundance of bile salt hydrolase (BSH)-rich commensals.[140] Modulation of bile acid metabolism can have

therapeutic benefits. *Weissella cibaria* supplementation suppresses colitis-associated colorectal cancer by reshaping the gut microbiota–bile acid–farnesoid X receptor (FXR) axis. This intervention restores intestinal barrier integrity, remodels the gut microbiota–bile acid axis, is associated with activation of intestinal FXR signalling, and suppresses NF-κB-driven inflammation.[141]

Collectively, these findings highlight the therapeutic potential of microbial metabolite-based interventions. By selectively enhancing beneficial metabolites (e.g., ILA, IPA) or inhibiting harmful ones (e.g., DCA, context-dependent butyrate), clinicians may precisely reprogram the TME and synergize with existing cancer therapies. Future efforts should focus on identifying robust pharmacodynamic biomarkers—such as histone acetylation profiles or AhR target gene expression—to optimize dosing regimens and develop rational combinatorial treatments for clinical application.

Fecal or intratumoral microbiota transplantation

FMT has emerged as a powerful tool to restore healthy gut communities and bolster antitumor immunity. In murine models of melanoma, transfer of stool from ICB-responding patients into germ-free mice improved anti–PD-1 efficacy, increasing $CD8^+$ T cell infiltration and tumor control.[142,143] Early clinical series in metastatic melanoma have similarly reported enhanced ICB responses post-FMT in refractory patients.[122,123] While these studies target the gut microbiome, they establish proof-of-principle that microbiota transplantation can be harnessed in oncology.

Building on the concept of direct tumor colonization by commensals, intratumoral injection of defined bacterial consortia (e.g., attenuated Salmonella or Lactobacillus strains) has shown potent antitumor effects in preclinical models. A two-component delivery system combining Salmonella SL7207 with an albumin–IL-2 fusion protein (Alb-IL2) induced durable tumor regression in the murine CT26 colon cancer model through localized T cell activation, with manageable toxicity.[144] Systemically administered defined Bifidobacterium strains preferentially accumulated in the tumor microenvironment and, via the STING–IFN pathway, enhanced dendritic cell cross-presentation, potentiating anti-CD47 therapy responses and improving antitumor efficacy and survival in the MC38 colon adenocarcinoma model.[145]

Despite their promise, both gut- and tumor-targeted microbiota transplantation face several translational challenges. For FMT, key concerns include ensuring engraftment of beneficial taxa, avoiding pathogen transfer, and optimizing donor selection. Intratumoral transplantation demands safe vectors, precise dosing, and compatibility with standard therapies.[124,146,147] Nonetheless, these approaches offer a direct route to reshape tumor-resident microbiota and immune contexture. Integration with microbiome diagnostics will enable patient-tailored transplants, potentially converting "cold" tumors into immunologically active lesions amenable to checkpoint blockade.

Combination regimens with existing oncologic treatments

Chemotherapeutic agents often induce immunogenic cell death, releasing danger-associated molecular patterns (DAMPs) that can be amplified by beneficial microbes. For instance, cyclophosphamide disrupts intestinal barrier integrity, enabling translocation of Gram-positive bacteria such as *Lactobacillus johnsonii* into secondary lymphoid organs, which prime Th17 responses and augment antitumor efficacy.[114,115,148] Co-administration of specific probiotic strains alongside chemotherapy restored commensal diversity and reduced mucositis severity without diminishing cytotoxicity.[149,150] Likewise, co-administration of probiotics such as Lactobacillus rhamnosus GG enhanced chemotherapeutic responses and reduced mucositis in preclinical colorectal cancer models.[149,151,152]

In radiotherapy, microbial modulation can influence both radiosensitivity and tissue recovery.[153] Pre-treatment with *Bifidobacterium infantis* or its specific antibodies enhanced radiosensitization in murine models of lung cancer by augmenting DNA damage responses.[154] Additionally, selective radiosensitization has been achieved through gene-directed enzyme prodrug therapy using E. coli cytosine deaminase, which converts 5-fluorocytosine into cytotoxic 5-fluorouracil within tumors expressing the CD gene.[155] A recent strategy involved the use of hyaluronic acid–decorated nanoparticles encapsulating cytolethal distending toxin B (CdtB), a genotoxic bacterial effector from Campylobacter jejuni. These HA-CdtB nanoparticles selectively sensitized radioresistant prostate cancer cells by inducing DNA double-strand breaks and G2/M arrest, with minimal toxicity to normal prostate epithelial cells.[156] Moreover, administration of *Akkermansia muciniphila* post-irradiation accelerated epithelial repair and reduced gastrointestinal toxicity.[157-159]

To fully harness microbiota-targeted strategies alongside standard oncologic treatments, it is essential to consider the timing, dosing, and sequencing of interventions, as well as dynamic monitoring of the microbiome. This is particularly critical to avoid detrimental interactions—for instance, antibiotic-induced depletion of taxa crucial for ICB efficacy—and to identify therapeutic windows where microbial modulation can most effectively synergize with chemotherapy, radiotherapy, or immunotherapy. Future prospective trials incorporating integrated multi-omics profiling (microbiome, immunome, metabolome) will be pivotal in defining optimal combination regimens and advancing precision microbiome-oncology.

CHALLENGES AND FUTURE DIRECTIONS

Standardization of sampling, sequencing, and analytical pipelines

One of the most significant barriers to clinical translation is the pervasive lack of standardized protocols across the entire workflow—from specimen collection and nucleic-acid extraction to library preparation and bioinformatic analysis. In the context of low-biomass tumor tissues, minute variations in sample handling—e.g., excision method, time to freezing, or formalin-fixation duration—can drastically alter microbial profile.[5] Similarly, the choice of DNA extraction kits often varies in their lysis efficiency for diverse microbial kingdoms, particularly Gram-positive bacteria and fungi. This necessitates the implementation of optimized mechanical disruption methods, such as bead-beating, tailored for the extraction of mixed microbial communities.[4,36]

On the sequencing front, choice of 16S/ITS primers (V1–V2 vs. V3–V4 regions) influences taxonomic resolution and introduces amplification bias.[160] Shotgun metagenomics confers strain-level detail but requires rigorous negative controls (extraction blanks, paraffin-only controls) and computational decontamination. To benchmark performance and enable robust cross-study comparisons, there is an urgent need for widespread adoption of standardized mock-community spikes and participation in inter-laboratory ring-trials, such as those championed by the Microbiome Quality Control Project.[161]

Analytically, the absence of consensus on contaminant removal—whether by frequency-based statistical filters (e.g., Decontam), batch-aware supervised normalization (SNM), or manual curation against public fungal- and bacterial-free DNA atlases—hampers reproducibility. Moreover, disparities in taxonomic databases (SILVA vs. UNITE vs. RefSeq) and fledgling fungal genome coverage necessitate community-driven curation.[11] Harmonization of pipelines—from raw-read QC to diversity metrics to differential abundance testing—will be essential to translate intratumoral microbiome insights into robust biomarkers and therapeutic targets.[162]

Causality versus correlation: Experimental models and longitudinal cohorts

A central and persistent challenge in tumor microbiome research is the critical need to distinguish causal roles of intratumoral microbes from mere associations. While the majority of human studies to date eloquently document correlations between specific microbial taxa and various clinical features, these observations inherently fall short of proving direct mechanistic involvement.[4,163] Robust demonstration of causality requires complementary experimental models in which defined microbes or consortia can be introduced, ablated, or engineered in vivo, coupled with longitudinal human cohorts that track microbiome dynamics over disease course.[94]

Germ-free or antibiotic-treated mice recolonized with patient-derived isolates (humanized models) enable direct testing of tumor-promoting or –suppressive functions. For instance, transplantation of *Fusobacterium nucleatum* into $Apc^{Min/+}$ mice accelerated colorectal tumorigenesis and altered local immunity.[46,164] However, murine tissues differ markedly from human tumors, and many intratumoral taxa (e.g., *Malassezia* in pancreatic cancer) lack well-characterized murine analogues.[6]

3D in vitro models, such as spheroids and organoids, maintain native architecture and immune-cell interactions, allowing controlled microbial colo-

nization.[165] Microinjection of intact tumor-associated microbiota into organoids enables high-throughput screening of microbial effects on proliferation, gene expression, and drug sensitivity. Integration with single-cell transcriptomics (e.g., INVADE-seq) can reveal cell-type–specific responses to microbial stimuli.[29]

Cross-sectional tumor sampling conflates temporal variability and cannot resolve whether microbial shifts precede or follow oncogenic changes. Prospective cohorts that collect serial tumor biopsies, paired blood/plasma, and stool samples before, during, and after therapy are critical. Early longitudinal work in melanoma patients undergoing immune checkpoint blockade demonstrated that early shifts in gut microbiome composition predict response.[166] Applying similar rigorous designs to the intratumoral microbiome—with sampling at diagnosis, post-neoadjuvant therapy, and at recurrence—will be paramount to clarifying causality and identifying optimal therapeutic windows for microbiome modulation.

Correlations are significantly strengthened when supported by orthogonal evidence. For instance, the concurrent detection of microbial metabolites (e.g., butyrate levels measured by mass spectrometry), histone lactylation marks, or microbial antigens presented on MHC molecules—bolsters causal inference.[45,81] CRISPR-mediated knockout of microbial sensing pathways (e.g., TLR4 for LPS, or GPR109A for butyrate) in tumor or immune cells, combined with microbial colonization models, will delineate mechanistic pathways.[167]

In conclusion, moving beyond mere correlation and establishing definitive causal links between intratumoral microbes and the hallmarks of cancer will necessitate a concerted and rigorous integration of cutting-edge experimental validation and well-powered longitudinal human studies. This holistic approach is crucial for unlocking the full therapeutic potential of the tumor microbiome.

Integration of multi-modal data (host genomics, epigenomics, immunomes, metabolomics)

A holistic understanding of tumor–microbiome crosstalk demands simultaneous interrogation of host genetic/epigenetic states, immune landscape, and microbial functions/metabolites. Integrative multi-omics approaches have begun to reveal how microbial signals intersect with cancer cell signaling, chromatin modifications, and immune cell phenotype.[5]

Host genomics and microbiome interaction is a bidirectional relationship. Somatic alterations in oncogenes or tumor suppressors can shape the intratumoral niche; conversely, microbial metabolites modulate host gene expression. For example, *Fusobacterium nucleatum*–derived FadA adhesin activates Wnt/β-catenin signaling in colorectal cancer cells via E-cadherin binding.[46,168] Integrated analysis of tumor exome data with bacterial abundance profiles identified correlations between mutational signatures (e.g., MSI status) and microbial community structure.[15] This highlights a dynamic, bidirectional relationship where host genetics influence the microbiome, and vice versa.

Microbial metabolites, notably SCFAs such as butyrate and propionate, serve as HDAC inhibitors and histone acylation donors. Zhang *et al.* demonstrated that histone lysine lactylation, induced by lactate, regulates gene expression programs in macrophages.[81] Mapping histone modification landscapes (ChIP-seq) alongside metagenomic profiling can pinpoint microbial metabolites' direct epigenetic targets in tumor cells.[169]

Integrating single-cell RNA sequencing with sophisticated microbial detection methods (e.g., INVADE-seq) reveals cell-type–specific host responses to internalized bacteria.[13] Integrative analysis of T-cell receptor repertoire, myeloid cell transcriptional states, and microbial gene expression uncovers how microbes modulate antigen presentation, checkpoint molecule expression, and immune exhaustion signatures.

Spatial metabolomics (e.g., mass spectrometry imaging) co-registered with microbial taxa maps (via multiplexed FISH or spatial transcriptomics) can localize metabolite gradients such as butyrate hotspots adjacent to butyrogenic bacteria.[170-172] Correlating metabolite levels with gene expression (metabolite-gene correlation matrices) and immune cell infiltration (immunohistochemistry) elucidates functional micro-niches within tumors.

Analytical platforms such as Multi-Omics Factor Analysis (MOFA)[173] and network-based integration[174] enable unsupervised discovery of cross-modal associations. Building comprehensive atlases that overlay genomic, epigenomic, immunomic, metabolomic, and microbiomics layers—ideally at single-cell and spatial resolution—will uncover mechanistic circuits underpinning microbe-driven oncogenesis and therapeutic response. Harmonized data standards and shared repositories are essential to facilitate integrative analyses and cross-cohort validation.

Ethical and regulatory considerations for microbiome-based interventions

As tumor microbiome–directed therapies rapidly advance towards clinical implementation, their development must be meticulously guided by rigorous ethical and regulatory frameworks. Microbiota modulation strategies—ranging from broad-spectrum antibiotics to precision-engineered probiotics, bacteriophage therapeutics, and live biotherapeutic products (LBPs)—pose unique safety, manufacturing, and consent challenges distinct from conventional pharmacologics.

Clinical protocols involving microbiota transplantation or live microbial administration require comprehensive informed consent detailing potential risks—such as transmission of latent pathogens, perturbation of host immunity, and horizontal gene transfer of antibiotic resistance markers. Retrospective analysis of FMT adverse events underscores the need for stringent donor screening and transparent communication of uncertain long-term sequelae.[175,176] Furthermore, in oncology settings, any form of FMT or intratumoral microbiome transfer must carefully account for the patient's often immunocompromised status and the potential for tumor-microbe interactions that could inadvertently exacerbate disease progression.

The U.S. Food and Drug Administration (FDA) issued guidance on LBPs defining them as biological products that contain live organisms intended for prevention, treatment, or cure of disease. Early-phase trials must adhere to Investigational New Drug (IND) regulations (21 CFR 312) with demonstration of Good Manufacturing Practice (cGMP)–compliant production, rigorous characterization of microbial strains (genomic sequencing, absence of virulence factors), and potency assays (FDA Guidance; European EMA Position Paper, 2018). Engineered bacterial therapeutics, including probiotic strains encoding immunomodulatory factors, require additional oversight under gene therapy frameworks, with attention to genetic stability, horizontal gene transfer, and environmental release.

While antibiotics can transiently reshape tumor microbiomes, off-target effects on commensal ecosystems and emergence of multidrug-resistant organisms must be minimized. Antibiotic use in oncology must follow stewardship principles—narrow spectrum, minimal duration, and susceptibility testing—to reduce collateral ecological damage.[123,177,178] Prophylactic microbiome depletion preceding immunotherapy has shown mixed clinical benefits, highlighting the need for judicious, evidence-based deployment.[123]

Multi-omics profiling captures extensive microbial and host genetic data; patient privacy concerns extend beyond human genomic information to potentially identifiable microbial signatures linked to geography or lifestyle. Data sharing must comply with HIPAA, GDPR, and NIH Genomic Data Sharing Policy, balancing open science with confidentiality.

Institutional Review Boards (IRBs) and Data Safety Monitoring Boards (DSMBs) should include microbial ecology expertise to evaluate trial protocols. Equitable access considerations are paramount; early adopters of microbiome therapies must avoid exacerbating healthcare disparities. Community engagement and culturally sensitive donor recruitment for FMT banks will promote inclusivity. By proactively addressing these ethical and regulatory dimensions—rooted in drawing upon established frameworks for biological, gene therapies, and antimicrobial products—researchers and clinicians can safely translate tumor microbiome discoveries into impactful, patient-centered oncology interventions.

CONCLUSIONS

Over the past decade, groundbreaking advances in high-throughput sequencing, spatial omics, and single-cell technologies have shattered the long-held dogma of the sterile tumor. These innovations have revealed solid malignancies not as isolated masses, but as complex, dynamic polymicrobial ecosystems. Studies conducted across diverse cancer types consistently demonstrate that bacteria, fungi, viruses, and even archaea inhabit

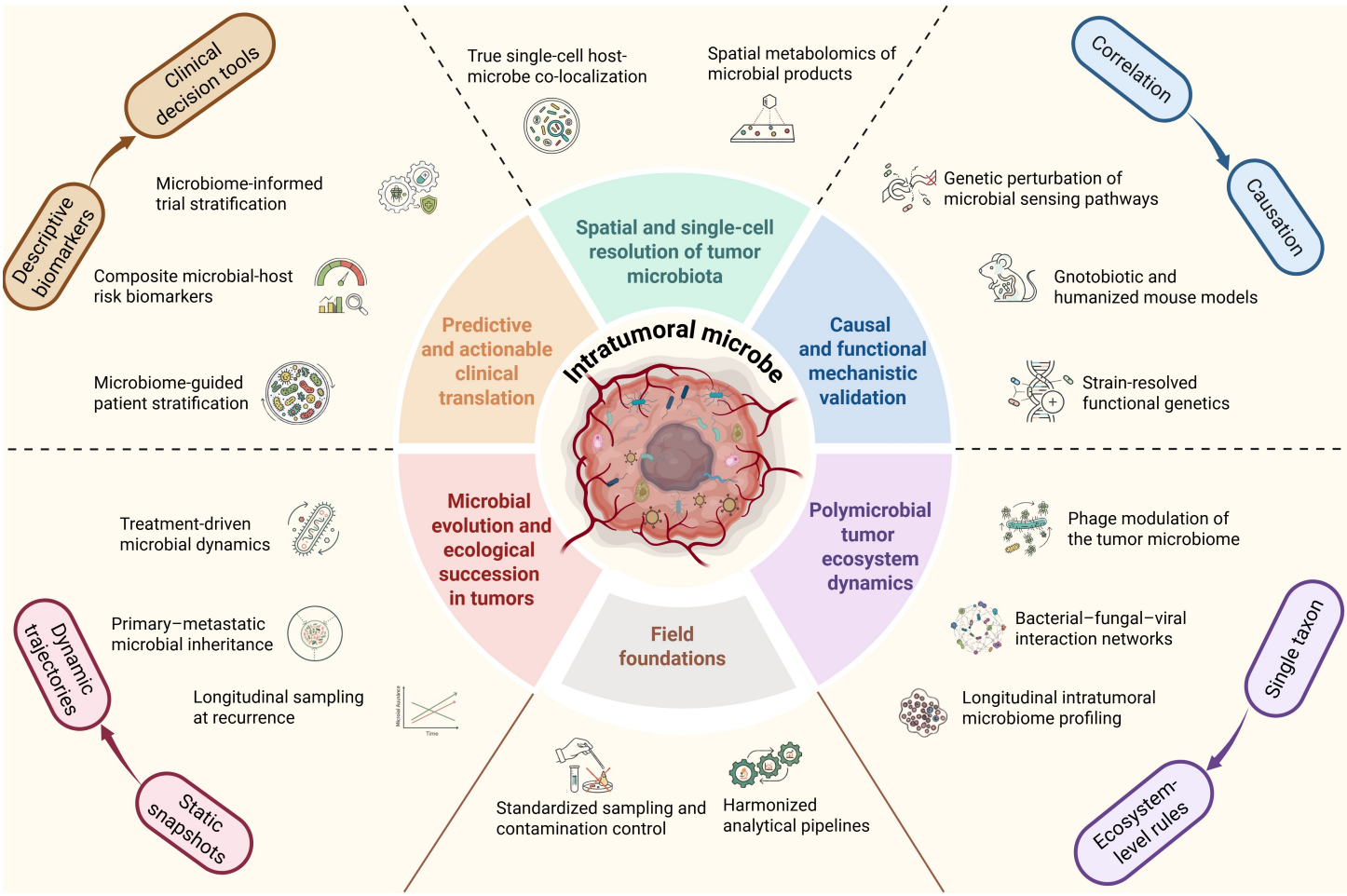

Figure 4. A five-sector roadmap for intratumoral microbiome research The wheel partitions the field's next decade into five interlocking domains radiating from a central tumor cross-section. (1) Spatial and single-cell resolution of tumor microbiota, encompassing true single-cell host–microbe co-localization and spatial metabolomics of microbial products at micrometre scale. (2) Causal and functional mechanistic validation, including genetic perturbation of microbial sensing pathways, gnotobiotic and humanized mouse models with defined colonization, and strain-resolved functional genetics that distinguish pathogenic from commensal clades. (3) Polymicrobial tumor ecosystem dynamics, spanning phage modulation of the tumor microbiome, bacterial–fungal–viral interaction networks, and longitudinal profiling of microbial succession. (4) Microbial evolution and ecological succession in tumors, covering treatment-driven microbial dynamics, primary–metastatic microbial inheritance and longitudinal sampling at recurrence. (5) Predictive and actionable clinical translation, comprising microbiome-informed trial stratification, composite microbial–host risk biomarkers and microbiome-guided patient stratification. Two field-wide foundations — standardized sampling with contamination control, and harmonized analytical pipelines — underpin all five sectors. Four outer-arrow chips frame the overall trajectory: descriptive biomarkers to clinical decision tools, correlation to causation, static snapshots to dynamic trajectories, and single taxon to ecosystem-level rules. Created with BioRender.com.

tumors in cancer-type–specific patterns, localized to micrometers and even within intracellular compartments. These tumor-resident microbes are far from passive bystanders; they exert a profound and multifaceted influence on core cancer hallmarks—modulating epigenetics via microbial metabolites, fine-tuning both innate and adaptive immunity, significantly affecting DNA damage and repair pathways, and actively shaping metastatic capacity.

Clinically, the emerging tumor microbiome signatures, detectable both within tissue and in circulation, are proving to be powerful prognostic and predictive biomarkers. Increasingly supported associations exist between microbial diversity or specific microbial taxa and critical clinical outcomes, including patient survival, recurrence risk, and crucial responses to therapeutic interventions, particularly immune checkpoint blockade and chemotherapy. Integration of microbial features with host genomic and immunologic markers in composite risk calculators holds promise for precision oncology, enabling refined patient stratification and personalized therapeutic guidance.

Looking forward, the transformative potential of tumor microbiome research hinges on overcoming several key challenges: standardization of low-biomass sampling and decontamination protocols; rigorous experimental validation to distinguish causality from correlation; harmonization and integration of multi-modal host–microbial omics data; and establishment of ethical, regulatory, and manufacturing frameworks for microbiome-based interventions. Here, we outline a strategic roadmap for advancing the field, emphasizing spatial and single-cell mapping, causal validation, characterization of polymicrobial ecosystem dynamics, microbial evolutionary tracking, and predictive clinical translation, all supported by standardized methodologies and reference atlases (Figure 4). Through focused, interdisciplinary efforts, these directions can be translated into mechanistic insights and microbiome-informed therapies, ushering in a new era of precision microbiome oncology.

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AUTHOR CONTRIBUTIONS

J.W. and L.H. supervised this review. L.C. and G.B. drafted the manuscript. All authors revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Not applicable.