

THE INTRATUMORAL MICROBIOME AS A DETERMINANT OF CANCER IMMUNOTHERAPY RESPONSE: MECHANISMS, EVIDENCE, AND TRANSLATIONAL FRONTIERS

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Abstract

Immune checkpoint inhibitors have reshaped the treatment of many advanced cancers, yet most patients derive no durable benefit, and the determinants of this variability remain incompletely understood. The long-held assumption that solid tumours are sterile has been overturned by evidence that human cancers harbour low-biomass, tumour-type-specific communities of bacteria, fungi, and viruses, frequently residing within malignant and immune cells. This narrative review synthesises the evidence linking these intratumoral communities to antitumor immunity and to checkpoint-inhibitor outcomes. First outline the composition and probable origins of the tumour microbiome, then examine the methodological safeguards required to study low-biomass specimens credibly. Describe the mechanisms through which resident microbes shape immune function, including direct metabolism of drugs, presentation of bacteria-derived peptides on tumour human leukocyte antigen molecules, metabolite and pattern-recognition signalling, remodelling of myeloid and dendritic-cell compartments, and modulation of T-cell priming and exhaustion. Then appraise clinical and translational data, including gut-microbiome correlates of anti-programmed cell death protein 1 response, cross-cohort meta-analyses, the prognostic relevance of the intratumoral mycobium, and early-phase faecal microbiota transplantation trials that convert a subset of refractory patients into responders. Therapeutic strategies under investigation, including faecal transplantation, defined bacterial consortia, dietary fibre, and engineered bacteria, are considered alongside their limitations. Finally, address the field's central controversies, particularly contamination and data-reanalysis disputes that have challenged foundational datasets, and argue that causal,

standardised, multi-omic study designs are required. The intratumoral microbiome is a biologically plausible and potentially modifiable determinant of immunotherapy response that merits rigorous, reproducible investigation.

1. INTRODUCTION

Cancer remains one of the leading causes of death worldwide, with an estimated 19.3 million new cases and almost 10 million deaths recorded in 2020, and a projected rise to 28.4 million cases by 2040.¹ Against this burden, immune checkpoint inhibitors have produced a genuine shift in prognosis for several malignancies, most notably melanoma, non-small-cell lung cancer, and renal cell carcinoma. By releasing inhibitory signals that restrain cytotoxic T lymphocytes, antibodies targeting cytotoxic T-lymphocyte-associated protein 4 and the programmed cell death protein 1 axis can induce durable, occasionally curative, responses.² The central clinical problem is that these responses are the exception rather than the rule: a majority of treated patients show primary resistance, and a substantial fraction of initial responders later relapse.² Reliable determinants of who will benefit, beyond tumour mutational burden and programmed death-ligand 1 expression, remain elusive, and this gap has motivated a broad search for host and environmental modifiers of response.

The clinical reach of checkpoint blockade has expanded rapidly, from late-line melanoma to first-line use across numerous tumour types and, increasingly, to earlier disease and the perioperative setting.² This expansion has sharpened rather than resolved the biomarker problem, because each new indication brings patients who will be exposed to immune-related toxicity without benefit. Programmed death-ligand 1 expression and tumour mutational burden capture only part of the variance in response, and neither accounts for the influence of the host environment in which a tumour and its immune infiltrate develop.³ It is against this background that the microbiome has attracted attention: unlike fixed genomic features, it is modifiable, raising the possibility that response might be engineered rather than merely predicted.⁴ That therapeutic promise is precisely

why the strength of the underlying evidence matters so much, and why this review weighs mechanism and causation alongside association. Attention has increasingly turned to the tumour microenvironment as a modifier of immunotherapy outcome, and within it to an unexpected participant. For most of the twentieth century, solid tumours were assumed to be sterile, and microbial sequences recovered from them were dismissed as contamination. That assumption no longer holds. Comprehensive surveys have shown that human tumours contain distinct, tumour-type-specific microbial communities, that these microbes are frequently intracellular, and that microbial DNA signatures can, in principle, distinguish cancer types and even discriminate cancer from healthy tissue.^{5,6} These findings have reframed the microbiome not as an external bystander but as a resident component of the tumour with the potential to influence tumour biology and treatment response.^{7,8}

The idea that microbes inhabit tumours is not itself new. Bacteria were observed within tumours more than a century ago, and deliberate bacterial infection was used therapeutically by William Coley in the 1890s, long before any mechanism could be articulated.⁷ What has changed is the analytical capacity to characterise these communities at scale and with molecular precision, together with the parallel discovery that the gut microbiome shapes systemic antitumor immunity.^{9,4} The convergence of these two lines of enquiry, one centred on microbes within the tumour and the other on microbes in the gut, underlies the current interest in the microbiome as a determinant of immunotherapy response.¹⁰ Whether the relevant microbes act locally, systemically, or through both routes is a question that recurs throughout the evidence discussed below.

This review examines whether, and how, the intratumoral microbiome acts as a determinant of cancer immunotherapy response. Consider the

composition and origins of these communities, the methodological caution their study demands, the mechanisms connecting them to antitumor immunity, and the clinical evidence relating microbial features to checkpoint-inhibitor outcomes. Then evaluate strategies to modulate the microbiome therapeutically and confront the controversies that continue to shape the field. Throughout, distinguish causal experimental evidence from observational association, and treat the reliability of the underlying data as a question of first importance rather than an afterthought. Aim is to provide an integrated and appropriately sceptical account for clinical and translational readers, identifying where the evidence is strong, where it is preliminary, and where it is genuinely contested.

2. The Intratumoral Microbiome: Composition and Origins

The most systematic early characterisation of the tumour microbiome analysed more than 1,500 tumours and adjacent normal tissues across seven cancer types, and reported that each tumour type carries a distinct microbial composition, with breast tumour harbouring a particularly rich and diverse community.⁵ Critically, the bacteria detected were predominantly intracellular and were found in both malignant cells and infiltrating immune cells, a localisation that distinguishes the tumour microbiome from transient surface colonisation.⁵ The picture is not limited to bacteria. A pan-cancer survey of more than 17,000 samples across 35 cancer types described tumour-associated fungal communities, present at low abundance but detectable across most major cancers and displaying cancer-type-specific compositions.¹¹ A parallel analysis linked specific fungi, including *Candida* species, to gastrointestinal and lung tumours and to markers of pro-inflammatory signalling and poorer outcome.¹² Bacteria, fungi, and viruses are therefore best understood as co-resident kingdoms within the tumour rather than as isolated phenomena.¹³

The fungal component, or mycobiome, has been the most recently characterised and illustrates how kingdoms interact. Pan-cancer surveys found

that fungal communities co-occur with particular bacterial communities and with specific immune-cell states, implying ecological relationships rather than independent colonisation.¹¹ In gastrointestinal and lung tumours, fungal enrichment was associated with pro-inflammatory signalling and worse survival, and in pancreatic cancer the mycobiome actively promoted tumour growth through activation of the complement cascade, establishing that fungi are not passive passengers.^{12,14} Viruses add a further layer whose contribution to the low-biomass community is least well defined and distinct from the established role of classical oncoviruses. The emerging picture is of a multi-kingdom ecology in which bacteria, fungi, and viruses interact with one another and with host cells, so that the effect of any one organism may depend on its microbial neighbours as much as on the tumour itself.^{13,7} This framing has practical consequences: interventions targeting a single taxon may be buffered or amplified by the surrounding community, and profiling that captures only one kingdom will give an incomplete account of the biology.

Two features of these communities' shape both their biology and their study. The first is scale. Microbial biomass within a tumour is minute; fungal cells have been estimated at roughly one per ten thousand tumour cells in some analyses, and bacterial loads are comparably low.¹¹ Such rarity means that microbial effects are unlikely to arise from sheer mass, and more plausibly reflect potent signalling or localised interactions with neighbouring cells. The second is kingdom breadth. Beyond bacteria and fungi, viruses form part of the intratumoral community, and several classically oncogenic viruses are established causes of specific cancers; the commensal-type, low-biomass populations now under study are conceptually distinct from these oncoviruses but add further complexity to the microbial landscape of the tumour.⁷ A coherent account of the tumour microbiome must therefore accommodate several kingdoms interacting with one another and with host cells.

Spatial studies have refined this understanding by showing that intratumoral bacteria are not

distributed uniformly. Single-cell and spatial profiling of colorectal and oral tumours revealed that microbial communities occupy discrete, poorly vascularised, immunosuppressive niches, and that microbe-associated malignant and immune cells display distinct transcriptional

programmes.¹⁵ Such heterogeneity implies that bulk measurements average over microenvironments that may differ sharply in their local biology, a consideration with direct relevance to how microbial effects on immunity should be interpreted.

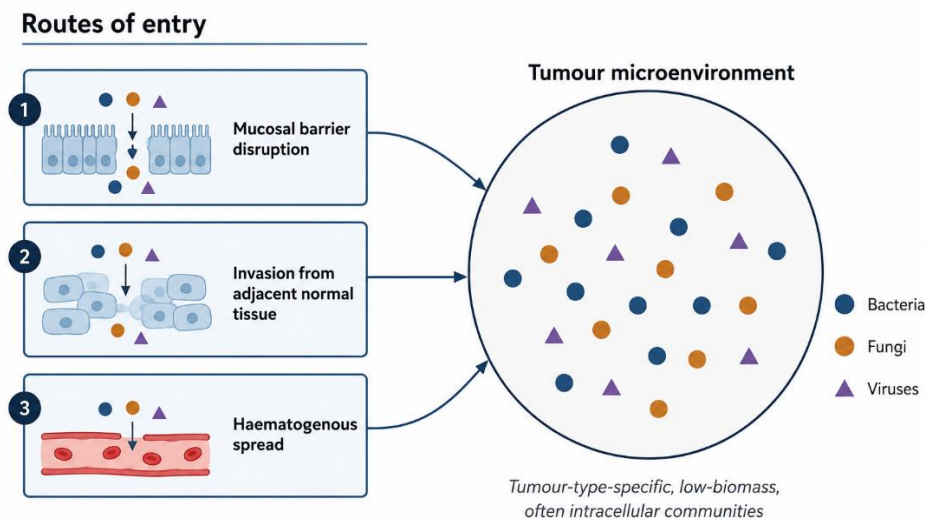


Figure 1. Composition and probable routes of entry of the intratumoral microbiome. Tumourharbour low-biomass, tumour-type-specific communities of bacteria, fungi, and viruses that are frequently intracellular. Proposed routes of entry include disruption of adjacent mucosal barriers, direct invasion from neighbouring normal tissue, and haematogenous spread. Schematic prepared by the authors.

How microbes reach the tumour is not fully resolved, but three non-exclusive routes are generally proposed: translocation across disrupted mucosal barriers, particularly for tumours arising at or near mucosal surfaces; migration from adjacent normal tissue; and haematogenous dissemination.^{7,13} Support for a mucosal origin is strongest in colorectal cancer, where *Fusobacterium nucleatum* is enriched in tumours relative to surrounding tissue and is prevalent across independent patient cohorts.^{16,17} The same organism can be traced from primary tumours to distant metastases, indicating that tumour-associated microbes can travel with malignant cells rather than being acquired anew at each site.¹⁸ The functional consequences of this residency may extend beyond immunity: in a breast-cancer model, tumour-resident

intracellular bacteria carried by circulating tumour cells enhanced host-cell survival under mechanical stress and promoted metastatic colonisation, without affecting primary tumour growth.¹⁹ The microbiome may therefore participate in several steps of cancer progression, a breadth of influence that motivates careful mechanistic dissection.

The tissue specificity of these communities is among their most reproducible features and hints at underlying biology. Tumours of the colon and other mucosal sites, which sit adjacent to dense microbial reservoirs, tend to carry heavier and more diverse bacterial loads than tumours of typically sterile organs, consistent with local translocation as one route of seeding.^{5,13} Within a given tumour type, particular organisms recur: *Fusobacterium nucleatum* in colorectal cancer,

Candida and other fungi in gastrointestinal and lung tumours, and a rich, diverse community in breast cancer.^{16,12,5} These recurring associations matter clinically because they suggest that microbial features could, in principle, be measured non-invasively; the detection of microbial DNA in blood and its capacity to discriminate cancer types raised the prospect of microbiome-informed liquid biopsy, although the reliability of that specific approach has since been

contested.^{6,20} At the same time, the presence of the same organism in tumour and in adjacent normal tissue, and its persistence into metastases, cautions against treating any single taxon as a tumour-specific marker without careful controls.¹⁸ The composition of the tumour microbiome is therefore best read as an ecological signature of tissue and microenvironment rather than as a simple binary label.

Table 1. Representative studies characterising the human intratumoral microbiome.

Study	Cancer type(s)	Principal finding	Ref
Nejman et al., 2020	Breast, lung, ovary, pancreas, melanoma, bone, brain	Tumour-type-specific, largely intracellular bacterial communities across 1,526 tumours	5
Poore et al., 2020	Pan-cancer (TCGA)	Blood and tissue microbial DNA signatures can discriminate cancer types and stages	6
Narunsky-Haziza et al., 2022	Pan-cancer (35 types)	Tumour-associated fungal communities with cancer-type-specific composition; bacteriome co-occurrence	11
Dohlman et al., 2022	Gastrointestinal, lung	Candida enrichment linked to pro-inflammatory signalling and reduced survival	12
Galeano Nino et al., 2022	Colorectal, oral	Bacteria occupy discrete immunosuppressive niches; microbe-associated single-cell programmes	15
Kalaora et al., 2021	Melanoma	Bacteria-derived peptides presented on tumour HLA-I and HLA-II molecules	21
Fu et al., 2022	Breast (model)	Tumour-resident intracellular bacteria promote metastatic colonisation	19

HLA, human leukocyte antigen; TCGA, The Cancer Genome Atlas.

3. Detecting a Low-Biomass Community:

Methods and Contamination

Because the tumour microbiome is a low-biomass system, its study is unusually vulnerable to error, and any credible interpretation must begin with the limitations of measurement. When the quantity of genuine microbial nucleic acid is small, DNA introduced by reagents, laboratory surfaces, and sample handling can equal or exceed the biological signal. This problem was demonstrated in dilution experiments showing that reagent and laboratory contamination can dominate sequence-based analyses of low-biomass samples and generate entirely spurious

community profiles.²² For tumour specimens, the implication is that rigorous negative controls, contamination-aware computational filtering, and orthogonal confirmation are prerequisites rather than refinements, and dedicated statistical tools now exist to identify and remove likely contaminant sequences.²³

Landmark studies in the field have taken these concerns seriously. The characterisation by Nejman and colleagues incorporated hundreds of negative controls and used multiple orthogonal methods, including visualisation of bacteria within cells, to distinguish signal from contamination.⁵ Fungal surveys similarly

combined sequencing with histological staining and, where feasible, culture.¹¹ Nonetheless, the reliability of large pan-cancer microbial datasets has been directly challenged. A reanalysis of a widely cited machine-learning study reported that major data-processing errors, including flawed normalisation and misclassified sequences, invalidated the original conclusions, and argued that many microbes reported as cancer-associated were not present in the samples at all.²⁰ This dispute, discussed further below, illustrates that measurement and analysis choices are not peripheral technicalities but can determine whether a reported association is real.

Several practical principles follow for anyone generating or evaluating this evidence. Amplicon sequencing of the 16S ribosomal RNA gene or the fungal internal transcribed spacer is inexpensive but offers limited taxonomic resolution and is highly susceptible to contamination, whereas shotgun metagenomics provides finer resolution at the cost of an overwhelming host-DNA background in tumour tissue. Detection of microbial DNA does not by itself establish that viable organisms are present, so methods that capture transcription or viability, together with direct visualisation by microscopy or fluorescence in situ hybridisation, materially strengthen claims of genuine colonisation. The studies that have proved most durable are precisely those that combined orthogonal methods rather than relying on sequencing alone, and this triangulation remains the most reliable safeguard against artefact.^{5,11} A recurring theme of this review is that mechanistic evidence from controlled models is often more persuasive than associations drawn from bulk sequencing alone.

Positive controls and independent validation are as important as the exclusion of contaminants. Approaches that localise microbes in situ, such as fluorescence in situ hybridisation, immunohistochemistry for bacterial components, correlative microscopy, and RNA-based detection of metabolically active organisms, provide evidence that sequenced taxa correspond to genuine tumour residents rather than free-floating nucleic acid.^{5,15} Cultivation, where achievable, offers the strongest confirmation

because it demonstrates viability and enables functional study. The most influential datasets have combined several of these strategies, and future work will be judged partly on whether it does the same.¹¹ Standardised reporting of controls, sequencing depth, and analytical parameters would allow readers to assess reliability directly and would facilitate the independent reanalysis that the field now recognises as indispensable.^{20,23}

4. Mechanisms Linking Intratumoral Microbes to Antitumor Immunity

Intratumoral microbes can influence tumour biology through several partly overlapping mechanisms, summarised in Figure 2. The first is direct metabolic interference with therapy. Bacteria within pancreatic tumours were shown to express a bacterial enzyme that metabolises and inactivates the chemotherapeutic gemcitabine, and antibiotic treatment restored drug sensitivity in a preclinical model.²⁴ Although this example concerns chemotherapy, it establishes the broader principle that resident microbes can chemically modify the local pharmacological environment, and complementary work has shown that commensal bacteria more generally control the response to chemotherapy and immunotherapy by shaping the tumour microenvironment.^{25,26}

A second mechanism operates through modulation of innate and adaptive immunity within the tumour. In pancreatic cancer, the tumour microbiome was found to promote oncogenesis by inducing innate and adaptive immune suppression, and its ablation reprogrammed the immune microenvironment and enhanced sensitivity to checkpoint blockade.²⁷ The prognostic weight of these communities is illustrated by long-term survivors of pancreatic cancer, whose tumours carried a distinct, more diverse microbiome associated with stronger CD8 T-cell infiltration.²⁸ Fungi contribute in parallel: in pancreatic cancer, mycobiome expansion promoted oncogenesis through activation of the mannose-binding lectin and complement cascade.¹⁴ In colorectal cancer, *F. nucleatum* recruits tumour-infiltrating myeloid

cells and generates a pro-inflammatory, pro-tumorigenic microenvironment, in part through the adhesin FadA, which engages epithelial signalling, and through the Fap2 protein, which binds the inhibitory receptor TIGIT to shield tumours from natural-killer- and T-cell attack.^{17,29,30} The same principle extends beyond the gut: in lung cancer models, commensal bacteria promoted tumour development by stimulating gamma-delta T cells and local inflammation.³¹

A third and conceptually striking mechanism is antigenic. Peptides derived from intracellular bacteria have been identified bound to both class I and class II human leukocyte antigen molecules on the surface of melanoma cells, demonstrating that microbial proteins can be processed and presented by tumour cells and can elicit reactivity from tumour-infiltrating lymphocytes.²¹ This raises the possibility that bacteria-derived antigens constitute a previously unrecognised class of tumour antigens with therapeutic relevance.³² A related route is the shaping of organised anti-tumour immune structures: in colorectal cancer models, introduction of an immunogenic commensal drove microbiota-specific T follicular

helper cells and the maturation of tertiary lymphoid structures, enhancing tumour control.³³

Fusobacterium nucleatum in colorectal cancer serves as a useful case study because several of these mechanisms have been dissected for a single organism. It is enriched in colorectal tumours across independent cohorts and is present in both early lesions and established carcinomas.^{16,17} Mechanistically, its FadA adhesin engages epithelial E-cadherin and activates oncogenic signalling, its Fap2 protein binds the inhibitory receptor TIGIT to protect tumour cells from immune attack, and it recruits immunosuppressive myeloid cells into the tumour.^{29,30,17} It also persists from primary tumours into liver metastases, and antibiotic treatment can reduce tumour burden in models, linking the organism causally to disease progression rather than merely marking it.¹⁸ No comparable depth of mechanistic understanding yet exists for most tumour-associated microbes, and the *Fusobacterium* example both demonstrates what rigorous dissection can achieve and highlights how much remains unknown for other organisms and cancer types.

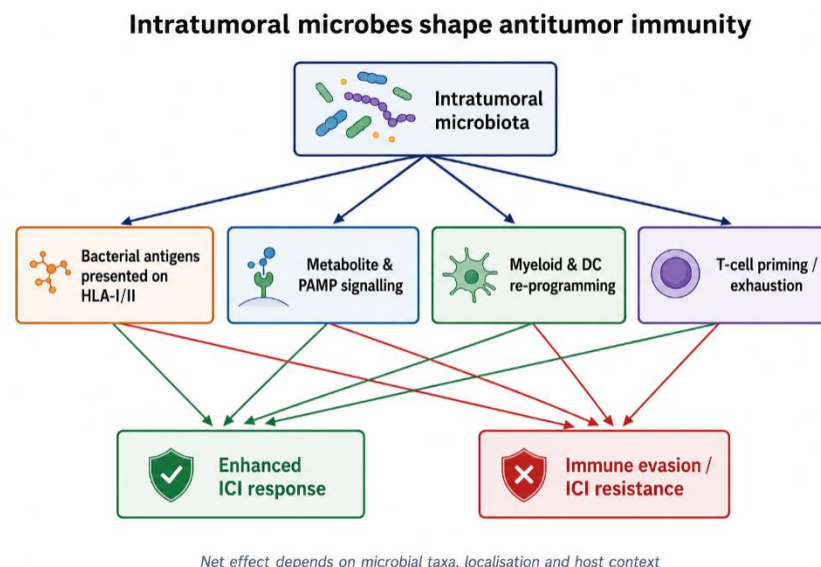


Figure 2. Mechanisms by which intratumoral microbes shape antitumor immunity. Resident microbes can act through presentation of bacteria-derived peptides on tumour HLA molecules, metabolite and pattern-recognition (PAMP) signalling, remodelling of myeloid and dendritic-cell (DC) compartments, and modulation of T-cell priming versus exhaustion. The net effect on checkpoint-inhibitor response

depends on microbial taxa, localisation, and host context. HLA, human leukocyte antigen; ICI, immune checkpoint inhibitor; PAMP, pathogen-associated molecular pattern. Schematic prepared by the authors.

Microbial metabolites provide a further, mechanistically distinct route of influence. Products of microbial metabolism, including short-chain fatty acids such as butyrate, modulate the differentiation and function of immune cells, for example by promoting regulatory T-cell differentiation in the intestine.³⁴ More directly relevant to immunotherapy, the microbial metabolite inosine was shown to enhance checkpoint-blockade efficacy by acting on T cells once increased gut permeability allowed its systemic translocation.³⁵ Within the tumour, microbial pathogen-associated molecular patterns engage innate sensors on myeloid and dendritic cells and, depending on context, can either reinforce an immunosuppressive, tumour-permissive state or help prime effective antigen presentation.^{27,36} The same signal can thus pull in opposite directions according to setting, which is one reason microbial effects on immunity resist simple generalisation.

The myeloid compartment is a recurring point of convergence for these mechanisms. Across several tumour types, microbial signals skew infiltrating myeloid cells toward immunosuppressive phenotypes, expand myeloid-derived suppressor cells, and dampen the antigen-presenting capacity of dendritic cells, thereby restraining the cytotoxic T-cell responses that checkpoint inhibitors aim to unleash.^{27,17} Conversely, when the balance of signals favours innate activation, the same cells can enhance antigen presentation and support productive T-cell priming, which helps explain why microbial depletion improves outcomes in some models yet worsens them in others.^{27,37} This context dependence is not merely a nuisance; it implies that the therapeutic goal is not simply to add or remove microbes but to shift the qualitative character of local immune signalling. Reviews of the field have emphasised that intratumoral and gastrointestinal microbiota act on overlapping myeloid and lymphoid targets, and that a unified account will need to integrate

both compartments rather than privileging one.^{36,4}

Much of the strongest causal evidence comes from the gut rather than the tumour itself, and bears on systemic immune tone. In mouse models, commensal *Bifidobacterium* enhanced antitumor immunity and augmented the efficacy of anti-programmed death-ligand 1 therapy, an effect transferable between animals.³⁷ Independently, the antitumor activity of cytotoxic T-lymphocyte-associated protein 4 blockade was shown to depend on distinct gut bacteria, with germ-free or antibiotic-treated mice responding poorly until the relevant taxa were restored.³⁸ These experiments moved the field from correlation toward causation by showing that manipulating microbial composition can change immunotherapy outcomes in controlled systems.³ Whether the operative microbes act primarily from the gut, from within the tumour, or through circulating signals that connect the two remains an important open question, and the metastasis-promoting role of tumour-resident bacteria suggests the compartments are not independent.¹⁹

Reconciling the gut and the tumour is an active area of investigation. One influential possibility is that intestinal microbes set a systemic immunological baseline, priming or restraining T cells that then act at the tumour, while intratumoral microbes exert additional local effects on the microenvironment.^{3,4} Bacterial translocation from mucosal surfaces to tumours, and the transport of tumour-resident bacteria within circulating cells, suggest physical as well as signalling connections between the compartments.^{19,18} Whichever mechanism predominates in a given cancer, the practical message is that sampling the gut and the tumour together, rather than either alone, will give the most complete picture of how the microbiome shapes response.

5. The Microbiome and Checkpoint-Inhibitor Efficacy

The translation of these ideas to patients began with three influential cohort studies published together in 2018, each relating the composition of the gut microbiome to response to anti-programmed cell death protein 1 therapy. In metastatic melanoma, responders were distinguished from non-responders by the diversity and composition of their faecal microbiota, with particular taxa enriched in those who benefited, and favourable communities associated with enhanced systemic and antitumor immunity.³⁹ A separate melanoma cohort likewise identified commensal species associated with response.⁴⁰ In epithelial tumours, including lung and renal cancers, the composition of the gut microbiome influenced the efficacy of programmed cell death protein 1 blockade, and faecal transplantation from responding patients into mice improved the antitumor effect of therapy.⁴¹ Together, these studies established a reproducible, if taxonomically inconsistent, association between microbial features and clinical benefit.

The immunological correlates reported in these cohorts are instructive. Favourable gut communities were associated with higher densities of tumour-infiltrating immune cells, enhanced antigen presentation, and systemic signatures of improved T-cell function, connecting microbial composition to the effector arm of the antitumor response rather than to an isolated marker.³⁹ At the same time, the specific taxa nominated as beneficial differed between studies conducted in different centres and populations, a discordance that has complicated efforts to define a single therapeutic microbial signature.^{39,41,40} Two large cross-cohort analyses addressed this problem directly. A meta-analysis integrating multiple melanoma cohorts found consistent but modest microbial signals and cautioned that cohort-specific effects and methodological differences limit the reproducibility of individual taxa.⁴² A companion study, combining metagenomics with immune profiling, linked particular community features not only to response but also to immune-related

adverse events, indicating that the same microbial landscape may influence both efficacy and toxicity.⁴³ A specific candidate to emerge across studies is *Akkermansia muciniphila*, whose intestinal presence has been associated with improved outcomes on programmed cell death protein 1 blockade in lung cancer.⁴⁴

Consistent with a functional role, exposure to antibiotics around the time of treatment has been linked to worse outcomes. In patients with advanced renal cell and non-small-cell lung cancer, antibiotic use shortly before or during checkpoint-inhibitor therapy was associated with shorter progression-free and overall survival.⁴⁵ A larger prospective analysis across tumour types reached the same conclusion, with antibiotic exposure before immunotherapy associated with substantially reduced survival.⁴⁶ Such observational data cannot establish causation, because antibiotic use may mark sicker patients, but they align with the experimental evidence that microbial composition shapes immunotherapy efficacy.^{38,37}

Beyond taxonomy, the mechanistic texture of these clinical observations has begun to emerge. Patients with favourable microbiomes tend to show broader circulating T-cell repertoires, higher frequencies of activated effector cells, and transcriptional signatures of enhanced antigen presentation, suggesting that the microbiome influences systemic immune tone rather than acting only at the tumour.^{39,41} Integrated metagenomic and immune analyses have reinforced this view while also revealing that microbial features track with immune-related toxicity, complicating any simple narrative in which a beneficial community is uniformly desirable.⁴³ For biomarker development, the practical consequence is sobering: a clinically useful microbial predictor must be reproducible across populations, robust to diet and geography, and additive to established markers such as programmed death-ligand 1 expression and tumour mutational burden. Current signatures do not yet meet that standard, and the cross-cohort analyses that have tested them most rigorously have found consistent but modest effects.⁴²

Evidence relating the intratumoral compartment specifically to immunotherapy response is more recent and more limited. The original tumour-microbiome survey noted correlations between intratumoral bacteria and response to immunotherapy in melanoma, though these observations were associative.⁵ Fungal and bacterial signatures have shown prognostic and, in some analyses, predictive value, but their independence from established biomarkers and their reproducibility across cohorts remain to be confirmed.^{11,28} Table 2 summarises representative clinical and translational evidence, and the outcome column deliberately distinguishes controlled interventional findings from observational associations. Separating predictive value, the ability to forecast response to a specific therapy, from prognostic value, a general association with outcome, remains an unmet requirement for clinical use.

It is worth distinguishing two related but separate questions that the literature sometimes conflates: whether the gut microbiome predicts response, and whether the intratumoral microbiome does. Most robust clinical data concern the gut, whereas evidence for the intratumoral compartment is largely correlative and derived from smaller series.^{39,5} The two are likely connected but not interchangeable, and a biomarker built on stool sampling would have very different practical characteristics from one requiring tumour tissue. Clarifying which compartment carries the predictive signal, and whether the two are additive, is a prerequisite for deploying microbial biomarkers in routine practice, and it will require studies that profile both compartments in the same patients alongside standard immune markers.^{43,44}

Table 2. Selected evidence linking the microbiome to checkpoint-inhibitor outcomes.

Study	Setting	Observation / intervention	Nature	Ref
Gopalakrishnan et al., 2018	Metastatic melanoma	Gut microbiome diversity/composition associated with anti-PD-1 response	Clinical, observational	39
Routy et al., 2018	NSCLC, RCC	Gut microbiome influences PD-1 blockade efficacy; FMT to mice reproduces effect	Clinical + preclinical	41
Matson et al., 2018	Metastatic melanoma	Commensal taxa associated with response	Clinical, observational	40
Derosa et al., 2018; Pinato et al., 2019	NSCLC, RCC; pan-cancer	Antibiotic exposure associated with shorter PFS and OS	Clinical, observational	45,46
Lee et al.; McCulloch et al., 2022	Melanoma (multi-cohort)	Cross-cohort microbial signatures of response and immune-related adverse events	Clinical, meta-analytic	42,43
Baruch et al.; Davar et al., 2021	ICI-refractory melanoma	FMT from responders induced or restored responses in a subset	Clinical trials (phase 1/2)	47,48
Routy et al., 2023	Advanced melanoma, first line	Healthy-donor FMT plus anti-PD-1 feasible with encouraging response	Clinical trial (phase 1)	49

FMT, faecal microbiota transplantation; ICI, immune checkpoint inhibitor; NSCLC, non-small-cell lung cancer; OS, overall survival; PD-1, programmed cell death protein 1; PFS, progression-free survival; RCC, renal cell carcinoma.

6. Therapeutic Modulation of the Microbiome

If the microbiome helps determine immunotherapy response, then modifying it becomes a therapeutic strategy. Several approaches are under investigation, summarised in Figure 3. The most direct is faecal microbiota transplantation. Two early-phase trials provided proof of concept that transplantation from patients who had responded to immunotherapy could sensitise refractory melanoma to anti-programmed cell death protein 1 therapy, with responses accompanied by favourable shifts in

immune-cell infiltration and gene expression in a subset of recipients.^{47,48} A subsequent phase 1 trial extended the concept to the first-line setting using healthy donors, reporting feasibility, acceptable safety, and encouraging response rates when transplantation was combined with checkpoint blockade.⁴⁹ These studies are small and, in the refractory setting, uncontrolled, and not all recipients benefited, but together they represent the first demonstration in patients that engineering the microbiome can alter the trajectory of immunotherapy.

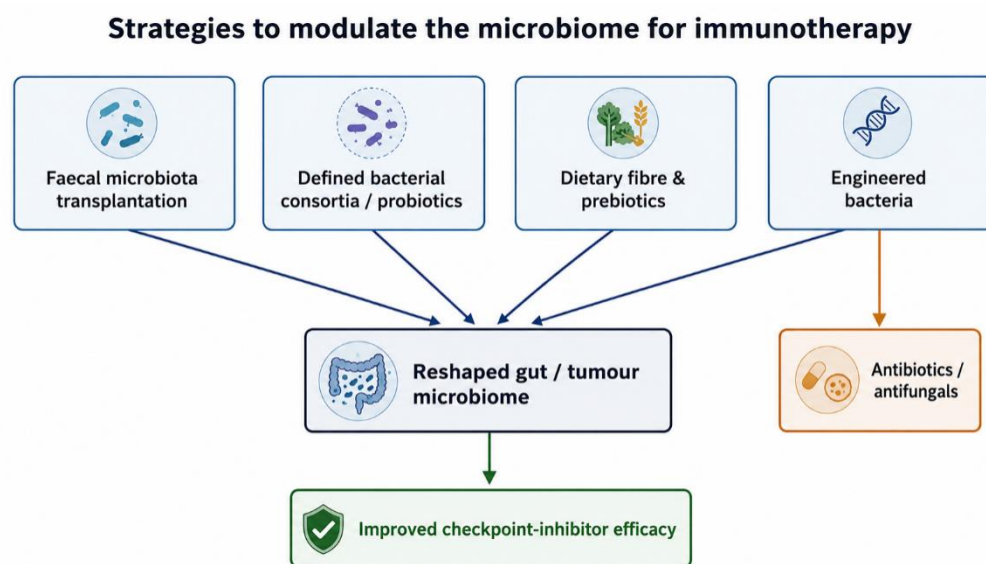


Figure 3. Strategies to modulate the microbiome for immunotherapy. Faecal microbiota transplantation, defined bacterial consortia or probiotics, dietary fibre and prebiotics, and engineered bacteria can reshape the gut and tumour microbiome to improve checkpoint-inhibitor efficacy, whereas antibiotics and antifungals modify the microbial community in ways that may help or hinder therapy. Schematic prepared by the authors.

A more defined alternative is administration of specific bacterial strains rather than whole faecal communities. A rationally selected consortium of eleven human commensal strains was shown to induce interferon-gamma-producing CD8 T cells in the intestine and to enhance the efficacy of checkpoint inhibitors in mouse tumour models, without provoking inflammation.⁵⁰ Such defined consortia offer reproducibility and safety advantages over faecal transplantation and are attractive candidates for clinical translation. Diet provides a further lever: in patients with

melanoma, higher dietary fibre intake was associated with improved progression-free survival on immunotherapy, whereas probiotic supplement use showed a non-significant trend toward worse outcomes, and preclinical models supported a causal role for fibre acting through the gut microbiome.⁵¹

Synthetic biology has enabled a further approach, engineering bacteria as living therapeutics designed to colonise tumours and deliver immunomodulatory payloads. Engineered probiotic strains have been programmed to

release checkpoint-blockade nanobodies locally within tumours, and other designs use synchronised lysis circuits to deliver immune agonists such as CD47 antagonists in pulses, producing durable regression and systemic antitumor immunity in preclinical models.^{52,53} Several such candidates have advanced from preclinical models toward early clinical testing, and the broader strategy of exploiting bacteria for cancer immunotherapy has been reviewed in detail.⁵⁴ Because microbiome-directed and checkpoint-blockade strategies act through complementary mechanisms, rational combinations, whether faecal transplantation, defined consortia, dietary modification, or engineered bacteria paired with checkpoint inhibitors, provide a logical basis for trial design, provided that safety, and in particular infection risk in immunocompromised patients, is addressed carefully.^{50,49} Antibiotics and antifungals sit awkwardly within this landscape, since they can deplete beneficial taxa and blunt immunotherapy, yet might also be exploited to remove specific detrimental microbes.⁴⁵ The clinical translation of these strategies is proceeding cautiously and unevenly. Faecal transplantation is the most clinically advanced, but it raises practical questions of donor selection, standardisation, and long-term safety, and its early trials remain small.^{47,48,49} Defined consortia and single next-generation probiotic strains promise greater reproducibility and are more tractable for regulatory approval as live biotherapeutic products, yet human efficacy data remain limited.⁵⁰ Dietary intervention is attractive because fibre is inexpensive and safe, but dietary studies are difficult to control and their effects are modest.⁵¹ Engineered bacteria offer the most precise control over therapeutic activity but must clear a high bar for biosafety and containment before broad use.^{52,53,54} A realistic near-term expectation is not that any single approach will transform outcomes, but that microbiome modulation will become one adjunct among several used to convert non-responders into responders, tested in rationally designed combination trials.

7. Challenges, Controversies, and Future Directions

Enthusiasm for this field must be tempered by unresolved problems, the most fundamental of which is the reliability of the underlying data. As noted above, a reanalysis of a prominent pancreatic microbial dataset reported that data-processing errors invalidated its conclusions and cast doubt on numerous follow-up studies that had relied on the same resource.²⁰ The original demonstration that contamination can overwhelm low-biomass signals remains the essential cautionary reference, and dedicated decontamination tools should be applied as a matter of routine.^{22,23} The controversy underscores that computational rigour and transparent, reproducible pipelines are not optional, and readers and reviewers should weight studies according to the strength of their controls and the availability of orthogonal validation.

A second challenge is distinguishing correlation from causation. Many clinical findings are observational, and microbial features may reflect rather than cause differences in host physiology, prior treatment, or disease burden. The most convincing causal evidence comes from experiments in which microbial manipulation changes outcome, whether through faecal transplantation, defined consortia, gnotobiotic models, or identification of a specific mediating metabolite.^{38,50,48,35} A third challenge is heterogeneity and standardisation: taxa associated with response differ across cohorts, sampling and sequencing methods vary, and spatial variation within tumours complicates bulk measurement.^{42,15} The tracing of tumour-associated bacteria from primary tumours to metastases further indicates that microbial communities are dynamic across the disease course rather than fixed.¹⁸

The field would also benefit from shared reference standards and open data. Coordinated benchmarking of computational pipelines, agreed contamination thresholds, and public deposition of raw sequences would enable the kind of independent reanalysis that has already reshaped the field's conclusions.²⁰ Integration across omic

layers, pairing taxonomic profiling with function, metabolism, spatial context, and host immune phenotyping, is needed to convert descriptive catalogues into testable mechanisms.^{15,36} Prospective, adequately powered studies should test whether microbial features add value over established biomarkers, and interventional trials should carry clear, preregistered endpoints. These requirements are demanding, but they are the same standards that underpin credible biomarker development elsewhere in oncology, and meeting them is what separates a durable clinical tool from a transient literature.⁴

Several further priorities deserve emphasis. The human microbiome varies with geography, diet, and ancestry, so signatures derived largely from cohorts in high-income countries may not generalise, and inclusive study designs are needed if microbiome-informed care is not to widen existing disparities.⁴² Spatially resolved, multi-kingdom profiling that captures where microbes sit relative to immune cells will be more informative than bulk taxonomy, particularly given the niche structure already documented within tumours.¹⁵ Live biotherapeutic products will require frameworks for manufacturing consistency, potency assays, and containment that differ from those governing conventional drugs.⁵⁴ And because microbial communities change over the course of disease and treatment, longitudinal sampling rather than single snapshots will be essential to link dynamics to outcome.¹⁸ Progress on these fronts will determine whether the intratumoral microbiome becomes an actionable axis of precision oncology or remains a compelling but unfulfilled hypothesis.

8. Conclusion

The recognition that human tumour harbour resident microbial communities has opened a genuinely new dimension in cancer biology, and a plausible explanation for part of the variability in immunotherapy response. Bacteria and fungi within tumours can interfere with drug metabolism, remodel innate and adaptive immunity, and even supply antigens presented by tumour cells, while the gut microbiome exerts a demonstrable, causally supported influence on

checkpoint-inhibitor efficacy.^{24,21,38} Early clinical trials of faecal transplantation, together with defined consortia, dietary intervention, and engineered bacteria, indicate that this biology may be therapeutically actionable.^{48,49,51} At the same time, the field carries a heavy burden of methodological caution, and prominent datasets have been seriously contested.²⁰ The intratumoral microbiome should therefore be regarded as a promising and potentially modifiable determinant of immunotherapy response whose clinical value will be established only through rigorous, reproducible, and causal investigation. Realising that promise, rather than assuming it, is the central task ahead.

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