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Gut dysbiosis in oncology: a risk factor for immunoresistance

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The gut microbiome is recognized as a determinant of response to immune checkpoint inhibitor (ICI) therapies in cancer. However, the clinical translation of microbiome science has been hampered by inconsistent definitions of dysbiosis, inadequate biomarker frameworks, and limited mechanistic understanding. In this review, we synthesize the current state of knowledge on how gut microbial composition and function influence ICI efficacy, highlighting both correlative and causal evidence. We discuss computational approaches based on α -diversity or taxonomic abundance and argue for more functionally and clinically informative models, such as the topological score (TOPOSCORE) and other dysbiosis indices derived from machine learning. Using retrospective analyses of metagenomic datasets from thousands of patients and healthy controls, we examine microbial patterns that distinguish responders from non-responders. We also explore how dysbiosis perturbs immunoregulatory pathways, including bile acid metabolism, gut permeability, and mucosal immunomodulation. Finally, we assess emerging therapeutic strategies aimed at correcting microbiome dysfunction — including dietary modification, bacterial consortia, and fecal microbiota transplantation — and describe how they are being deployed in multiple clinical trials. We conclude with a brief discussion of the ONCOBIOME initiative, which works with international partners to incorporate microbiome science into oncology workflows. By refining our understanding of gut–immune interactions and translating it into action, microbiome-informed oncology may unlock new therapeutic potential for patients previously resistant to immunotherapy.

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INTRODUCTION

Intestinal dysbiosis, broadly defined as an imbalance in the composition or function of the gut microbiota, is a significant contributor to resistance against immune checkpoint inhibitor (ICI) therapies.^{1–16} However, despite the significant resources used to map the human gut microbiome through the Human Microbiome Project and bring clarity to these enteric states,^{17–19} there is still no consensus definition of what constitutes a “healthy” (eubiotic) vs a dysbiotic microbiome, complicating translation into biomarkers and therapeutics. Clarifying and operationalizing these concepts is critical for improving patient stratification and the rational use of microbiota-centered interventions (MCIs), such as dietary regimens and fecal microbiota transplantation (FMT), to overcome ICI resistance.

Skepticism about the terms “dysbiosis” and “eubiosis” persists, with critics arguing that they are imprecise or pre-scientific.^{20–22} Nonetheless, in oncology settings, dysbiosis can be measured and correlated with ICI outcomes, and is amenable to intervention.²³ Key metabolic and genomic markers have been proposed and validated in different disease contexts, and more are being investigated. This has led to the realization that dysbiosis may not be a uniform condition; our group has identified at least two distinct forms of dysbiosis, defined by either the close-to-complete absence or overabundance of *Akkermansia muciniphila*.³

This is notable because *A. muciniphila* has been previously linked to improved ICI responses.¹³ Indeed, this trichotomic observation indicates a more complicated, non-linear relationship with anti-tumor immunity.²⁴

In this review, we examine the evolving conceptual frameworks for defining and measuring dysbiosis in the context of ICI therapy. We outline key factors that influence gut microbial health and that need to be considered when attempting to define a “healthy” vs “imbalanced” gut microbial community. We then assess the current toolbox available for detecting and quantifying dysbiosis, ranging from serum and fecal biomarkers to classical diversity metrics and taxonomic signatures, to newer approaches that integrate ecological topology and functional inference. Finally, we discuss how dysbiosis metrics might be translated into clinical practice as biomarkers of response, guides for patient stratification, and rationale for implementing MCIs.

DEFINING A HEALTHY MICROBIOME

The World Health Organization constitution defines health as “a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity”.²⁵ This definition has been elaborated upon by identifying and describing eight key interconnected hallmarks of health within three broader

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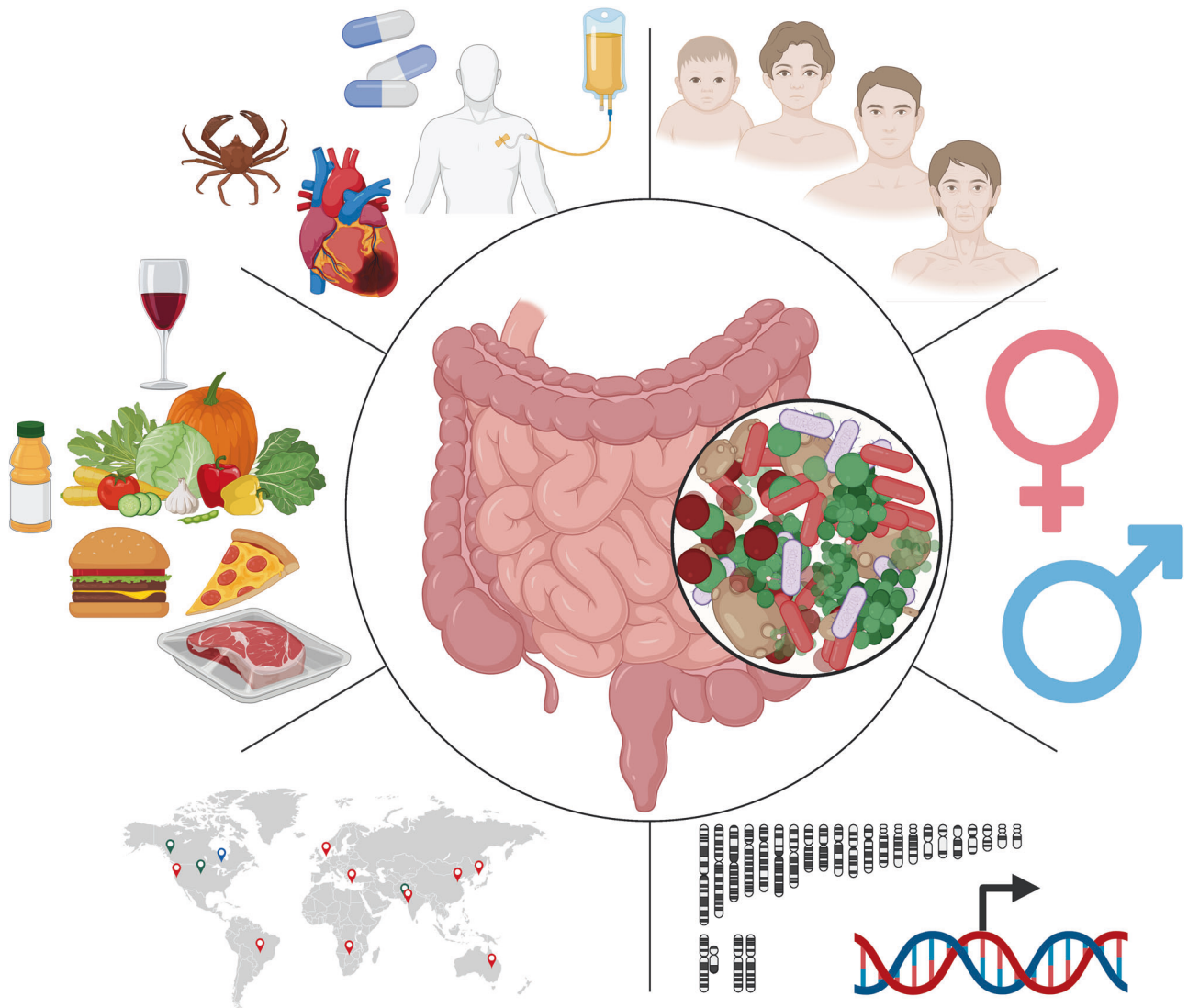


Fig. 1 Multilayered determinants of gut-microbiota ecology. A schematic overview of the principal host-intrinsic and environmental factors known to shape intestinal microbial composition, diversity, and function. Clockwise from top: biological age; sex and sex hormones; host genetics; geography and ethnicity; dietary patterns; chronic diseases; and medications and co-medications, including antibiotics, proton-pump inhibitors, and benzodiazepines. These factors interact to modulate microbial community structure, SCFA production, bile acid metabolism, and immune tone, thereby influencing health trajectories and the efficacy of ICIs. Illustration created with BioRender.com.

categories: spatial organization (maintaining barrier integrity and containing insults); homeostasis (healthy turnover, circuit integration, and rhythmic oscillations); and responses to stress (resilience, repair, and hormones).²⁶

Applying and integrating this holistic view to the gut ecosystem is challenging because a “healthy” or eubiotic microbiome may entail more than simply the absence of dysbiosis or the presence of a few keystone taxa, but likely results from the dynamic interplay of these factors. Indeed, inherent host factors such as age, gender, or genetics can influence the gut microbiota composition, but external factors such as diet, medication, and lifestyle also contribute to continually shaping microbiota composition. Any practical definition must therefore consider both the intrinsic and extrinsic factors that shape microbial communities across an individual’s lifespan (Fig. 1).

Age and microbiome trajectories

Biological age is one of the strongest determinants of gut microbiota composition.²⁷ Across a person’s lifespan, three broad successional phases can be distinguished. Primary succession

involves rapid assembly of a community — set in utero and at delivery — that settles into a relatively stable consortium dominated by Firmicutes and Bacteroidetes.^{28,29} As pregnancy advances, the vaginal microbiota diversifies,³⁰ and delivery provides a critical first inoculum for the infant gut. Vaginally delivered infants acquire maternal Bacteroides, whereas Cesarean birth favors early colonization by *Enterococcus*, *Enterobacter*, and *Klebsiella* pathobionts.²⁷ Cesarean delivery also predicts a more unstable neonatal microbiome and a heightened risk of infections, immune disorders, obesity, and neuroendocrine disturbances.^{31–33} Breastfeeding tempers these effects by delivering secretory immunoglobulin A (IgA), antimicrobial peptides, and prebiotic human milk oligosaccharides, which foster *Bifidobacterium* dominance and direct the orderly succession from pioneer taxa to a toddler-age consortium rich in *Bifidobacterium*, *Clostridium*, and *Bacteroides*.³⁴ By 3–6 years, the microbiota approaches an adult-like “climax” community that remains comparatively stable thereafter.

Secondary succession in early to mid-adulthood reflects dietary diversification, lifestyle factors, and environmental exposures.

However, day-to-day oscillations persist. Circadian cues shift the abundance of *Clostridiales*, *Lactobacillaceae*, and *Bacteroidales* in mice, and ~10% of human operational taxonomic units display similar time-of-day variation.^{35,36} Psychosocial stress likewise skews the community, increasing *Clostridium* and depleting *Bacteroides*.³⁷ Despite such perturbations, adult microbiomes co-evolve with their hosts, as *in situ* bacterial genome adaptation fosters long-term colonization.³⁸

Finally, late life ushers in tertiary succession, which is marked by declining α - and β -diversity and loss of keystone commensals like *Faecalibacterium* and *Bifidobacterium*.^{39,40} Healthy ageing is associated with butyrate-producing taxa, an expanded *Bacteroides* enterotype (discussed below), and enrichment of *Alistipes* spp. or *A. muciniphila*, which generates anti-inflammatory polyamines and bile acid (BA) derivatives.^{41,42} By contrast, frailty and multimorbidity are correlated with blooms of *Enterocloster*, *Eggerthella*, and other opportunists,⁴³ which can be associated with a dysbiotic gut.

Sex and the gut microbiome

Women generally harbor richer and more diverse intestinal communities than men.^{44–46} They are relatively enriched in *Alistipes* spp., *A. muciniphila*, *Clostridium symbiosum*, and butyrate-biosynthesis genes, whereas men carry more *Prevotella* spp. and BA transporter genes.^{44,45} The endocrine milieu helps explain these patterns. For example, hormonal contraception and menstrual status shift microbial profiles in women.⁴⁴ Moreover, a meta-analysis of 13 studies and 10,468 samples found that higher circulating estrogen was correlated with increased microbial diversity, greater *Bacteroides* abundance, and lower *Ruminococcaceae* abundance, whereas testosterone levels were correlated with *Ruminococcus*, *Acinetobacter*, and overall diversity in men.⁴⁷ Menopause further reshapes the community: premenopausal women display higher Firmicutes-to-Bacteroidetes ratios and higher levels of *Roseburia*, whereas the post-menopausal decline in estrogen is accompanied by lower diversity and expansions of taxa linked to cardiometabolic risk.^{48–50}

Whether this variation is enough to cause differential responses to ICIs between men and women is not understood. If present, the influence of sex-specific microbial variation would be very small. The total variance in gut microbial composition attributable to sex is modest (~0.5%) but biologically meaningful alongside stronger drivers such as diet or medication.⁴⁴ Some studies have investigated whether sex-specific variations influence a patient's outcome following ICI therapy. Two meta-analyses of 20 and 14 randomized clinical trials, involving more than 15,000 patients diagnosed with different indications, found that although ICIs enhance anti-cancer responses in both men and women, men appear to experience a modestly greater benefit.^{51,52} However, the generalizability of these findings has been debated^{53,54} owing to some inconsistencies between studies.^{55–57}

Host genetics and the microbiome

Host genetics leaves a discernible, though limited, imprint on gut microbial composition. Genome-wide analyses estimate that host variants account for roughly 2%–8% of interindividual compositional variation,^{58,59} and a cohort of over 1500 healthy adults revealed that nearly one-third of fecal taxa are heritable.⁶⁰ Notably, the most robust gene–microbe associations cluster around loci that govern diet, metabolism, immunity, and mucosal glycosylation. Lactase-persistence alleles at the *LCT* gene consistently track with higher abundances of *Bifidobacterium* spp.,^{61,62} whereas polymorphisms in the *FUT2/FUT1* secretor locus and variants near the *ABO* gene modulate fucosylated mucin production and favor mucin specialists like *Ruminococcus torques*.^{63,64} Twin studies extend this picture, showing that highly heritable taxa often co-segregate with alleles involved in barrier defense, olfaction, and short-chain fatty acid (SCFA) metabolism. For example, *Christensenella* spp. is associated with lean body mass

and variants in genes such as *ALDH1L1* and *GNA12*.^{65,66} Although genotype shapes the niche space for specific microbes, environmental exposures, lifestyle, and stochastic colonization dominate the wider landscape of the adult gut ecosystem.

Geography and lifestyle

Geography and ethnicity shape gut microbial composition, but their influence is channeled largely through diet and lifestyle. Comparative surveys show that the fiber-rich, agrarian diets typical of rural Africa promote Bacteroidetes-enriched, *Prevotella*-dominated communities that maximize fermentation and suppress inflammatory pathobionts, whereas western cohorts — characterized by higher fat and lower fiber intake — display reduced α -diversity, depletion of *Prevotella*, and expansion of Enterobacteriaceae like *Escherichia* and *Shigella*.^{67,68} Even within a single continent, country-specific differences persist; ethnicity alone accounts for about 6% of interindividual variance, and migration studies show that acculturation rapidly shifts the microbiome toward the host country's profile.^{69–72} Lifestyle compounds these effects: regular physical activity enhances diversity, enriches SCFA producers, and correlates with favorable cardiometabolic markers.^{70,73–75} This is particularly salient for cancer patients undergoing ICI therapy, as exercise has been demonstrated to modulate gut microbial composition and correlate with increased levels of formate, which enhances the cytotoxic activity of CD8⁺ T-cells.¹⁰ Geography, ethnicity, and lifestyle intersect to generate distinct, yet flexible, microbial landscapes.

Dietary influences

Diet is the most malleable — and most powerful — determinant of gut microbiota composition. Poor diets now surpass other behavioral risk factors for global mortality,⁷⁶ and controlled feeding studies show that the gut microbiota can shift within days of a dietary change.⁷⁷ Western-style, high-fat, animal-based diets promote bile-tolerant, pro-inflammatory taxa (e.g., *Alistipes*, *Bifidobacterium wadsworthia*, *R. torques*), reduce diversity, deplete *Prevotella*, and expand *Proteobacteria*, driving barrier dysfunction, endotoxemia, and chronic inflammation.^{77–80} Fiber-rich, plant-based diets have the opposite effect, nurturing *Bifidobacterium* and *Lactobacillus*, increasing butyrate producers like *Roseburia* and *Eubacterium rectale*, and shielding against obesity and insulin resistance via SCFA signaling.^{15,70,81–83} Very-low-carbohydrate ketogenic regimens selectively expand mucin specialists like *A. muciniphila* and *Roseburia intestinalis*,⁸⁴ whereas Mediterranean diets — abundant in vegetables, legumes, fruits, fish, and olive oil — increase *Faecalibacterium prausnitzii*, *Bacteroides cellulosilyticus*, and *Roseburia* spp., lower secondary BAs, attenuate systemic inflammation, and correlate with reduced risks of disease.^{70,85,86}

Diet continually shapes the gut ecosystem, with downstream consequences for immune tone, metabolic health, and disease trajectory. Dietary modulation has therefore been proposed as a strategy to enhance ICI efficacy and is arguably the least intrusive MCI.^{15,87} Several clinical trials are currently underway to determine its potential to improve cancer immunotherapies. We have recently published a tabulated list of current MCI trials and encourage readers to consult it for further insights.⁸⁸

Medications and co-medications

Medications rival diet as modulators of gut ecology. Together, they explain nearly 20% of interindividual variation in microbial composition, with drugs often exerting the stronger influence.^{59,70} Broad surveys identify antibiotics,¹⁶ proton-pump inhibitors (PPIs),⁸⁹ benzodiazepine derivatives,⁹⁰ antidiabetics, non-steroidal anti-inflammatories, and immunosuppressants as major drivers of microbial richness, diversity, and composition.^{43,58,70,91} PPIs, for instance, enrich Firmicutes and oral taxa like *Streptococcus salivarius* and *Micrococcaceae*, expand *Veillonella* and *Enterocloster*, and reduce overall diversity, a profile linked to *Clostridioides*

difficile infection and adverse metabolic shifts.^{43,92–97} Antibiotics predictably inflict the most acute damage, reducing diversity, depleting beneficial commensals, and permitting blooms of pathobionts like *Enterocloster* and *Streptococcus*.^{4,27,98} Indeed, long-term use of these medications can leave persistent alterations in the gut microbiome that can endure years beyond treatment discontinuation.⁹⁹

High-throughput transcriptomics and machine learning (ML) approaches can now map these drug–microbe interactions in finer detail. *Bacteroides* appear particularly susceptible to non-antibiotic toxicities, and agents like simvastatin can downregulate bacterial riboflavin biosynthesis.¹⁰⁰ In silico models further reveal that lipophilicity and charge distribution best predict a molecule's anti-commensal potency, with PPIs, immunosuppressants, and antineoplastic drugs ranking among the most dysbiotic.¹⁰¹ Polypharmacy amplifies these effects in older adults, eroding biodiversity, curtailing SCFA production, and fostering expansions of *Streptococcus* and *Lactobacillus* spp.^{102,103}

Several large-scale meta-analyses have demonstrated that these iatrogenic effects have practical clinical implications. A meta-analysis of 105 studies and 46,000 cancer patients treated with ICIs convincingly showed that exposure to antibiotics was associated with shorter progression-free survival and overall survival in NSCLC patients.¹⁰⁴ Another meta-analysis that examined 41 studies and 20,000 patients similarly demonstrated the negative impact of PPI treatment on patients treated with ICIs.¹⁰⁵ Benzodiazepine has also been shown to compromise immunotherapy, with preclinical and clinical data demonstrating that these agents suppress anti-tumor immune responses and correlate with reduced survival in NSCLC patients receiving programmed death protein-1 (PD-1) blockade.^{90,106} These large-scale studies demonstrate that medication-induced dysbiosis has a negative impact on the outcome of patients treated by immunotherapy. Drug exposure, therefore, represents a risk factor for a dysbiotic microbiota.

Diseases

Disease states almost invariably influence the gut ecosystem, and, in many conditions, microbial shifts appear to participate in pathogenesis. Metabolic disorders illustrate this reciprocity: obesity is correlated with an elevated Firmicutes-to-Bacteroidetes ratio and blooms of *Roseburia faecis* and *Ruminococcus gnavus*;^{43,107} non-alcoholic liver dysfunction is associated with diminished *E. rectale* and higher circulating secondary BAs,¹⁰⁸ and type 2 diabetes features expansions of Enterobacteriaceae, *Escherichia coli*, *Bacteroides caccae*, and *Prevotella copri*.¹⁰⁹ Inflammatory bowel disease (IBD) is marked by surges of pro-inflammatory Veillonellaceae and Proteobacteria and a concomitant loss of SCFA producers like *Ruminococcus bromii*, *Bifidobacterium* spp., and *F. prausnitzii*.¹¹⁰ Cardiovascular pathology is likewise linked to microbial signatures, as atherosclerosis is accompanied by increased *Streptococcus*, *Escherichia*, and *Shigella* spp.¹¹¹

Cancer progression exerts systemic effects that profoundly alter host–microbe interactions in the gut. Murine models of cancer cachexia show a dysbiosis characterized by expansion of *Enterobacteriaceae*¹¹² such as *Klebsiella oxytoca*, a gut pathobiont whose growth follows the depletion of butyrate-producing taxa.¹¹³ These microbial perturbations are associated with elevated systemic interleukin-6 (IL-6) and lipopolysaccharide-binding protein (LBP), biomarkers predictive of cachexia and poor survival in colorectal and lung cancer.¹¹² Murine studies have also demonstrated that tumor growth can induce a β -adrenergic receptor (β AR)-dependent “stress ileopathy” marked by villous atrophy, epithelial permeability, and a persistent *Clostridium* bloom at the expense of *Lactobacillus*.¹¹⁴ Tumor progression has also been associated with a reduction in fecal and systemic tryptophan — an essential amino acid and microbial substrate. Restoring tryptophan, either through dietary supplementation or

colonization with *Duncanella dubosii*, appears to re-establish immune balance and support antitumor T-cell activity.¹¹⁵ Similar patterns emerge in pediatric neuroblastoma patients: reduced richness, depletion of butyrate producers, and enrichment of proteolytic fermenters define a conserved “oncomicrobiome” signature.¹¹⁶

This dysbiotic state has direct consequences for ICI efficacy. Chronic activation of the sympathetic axis through β AR signaling suppresses cytotoxic T-cell function, whereas β -blockade restores effector activity and improves survival in patients receiving immunotherapy.¹¹⁷ Sustained adrenergic signaling also shapes microbial ecology, promoting *Clostridium*-dominated dysbiosis that blunts anti-PD-1 responses.¹¹⁴ Conversely, tryptophan supplementation or *D. dubosii* colonization in glioblastoma models reinstates CD8⁺ T-cell circulation from the bone marrow to the tumor bed, enhancing ICI efficacy.¹¹⁵ This neuro-immune-microbial axis, linking stress, dysbiosis, and immune suppression, provides a mechanistic continuum from barrier disruption to therapeutic resistance. Historical pathology foreshadowed this interplay: in 1965, Wang and Deller described villous atrophy and steatorrhea in a patient with bronchial carcinoma, attributing it to a tumor-derived systemic factor.¹¹⁸ The convergence of such early observations with contemporary mechanistic insights reinforces the notion that any operational definition of a “healthy” microbiome must account for distant (extraintestinal) disease states.

SERUM AND FECAL MARKERS OF DYSBIOSIS

In oncology, some of the most clinically tractable indicators of dysbiosis are metabolites and host-derived proteins measurable in stool or blood. These biomarkers capture functional consequences of microbial imbalance — such as mucosal inflammation, barrier disruption, and altered metabolism — and several have been linked to cancer progression and response to therapy. Although a precise definition of dysbiosis remains elusive, combining systemic, fecal, and taxonomic markers (Fig. 2) enables researchers to assess microbial diversity, abundance, and activity in ways that can provide insight into the microbiome's influence on immunotherapy outcomes. In the following section, we discuss some of the systemic and fecal biomarkers that can be used to diagnose dysbiosis by routine serological or stool assays.

Fecal biomarkers

Dysbiosis often manifests locally as intestinal inflammation and disrupted barrier function, which can release host proteins into stool. One classical example is fecal calprotectin, a calcium-binding heterodimer (S100A8/S100A9) from neutrophils that is elevated in the stool during gut inflammation (Fig. 2).^{119,120} It is an established, sensitive marker used to monitor IBD activity and, by extension, reflects microbiota-driven mucosal inflammation.^{119,121,122} Elevated fecal calprotectin has been correlated with dysbiotic alterations in microbiota composition among IBD patients,¹²³ highlighting that shifts in commensal populations can incite neutrophilic inflammation. Fecal lactoferrin and lipocalin-2 are additional neutrophil-derived proteins detectable in stool and are well-validated proxies for gut inflammation and barrier stress in IBD.^{124,125} Although these stool inflammatory markers were originally developed in the context of IBD, they can be translatable to other contexts of dysbiosis (e.g., cancer) as non-invasive proxies for microbiota-driven mucosal immune activation.

Microbiota-derived metabolites in stool are also being studied as functional dysbiosis markers in oncology. SCFAs are key immunomodulatory microbial metabolites produced by fermentative commensals (e.g., *Faecalibacterium* and *Roseburia* spp.). They signal through G protein-coupled receptors to promote anti-inflammatory regulatory T-cells and enhance CD8⁺ T-cell effector functions.¹²⁶ Butyrate is a frequently studied SCFA compound that has been shown to boost anti-tumor immunity in preclinical

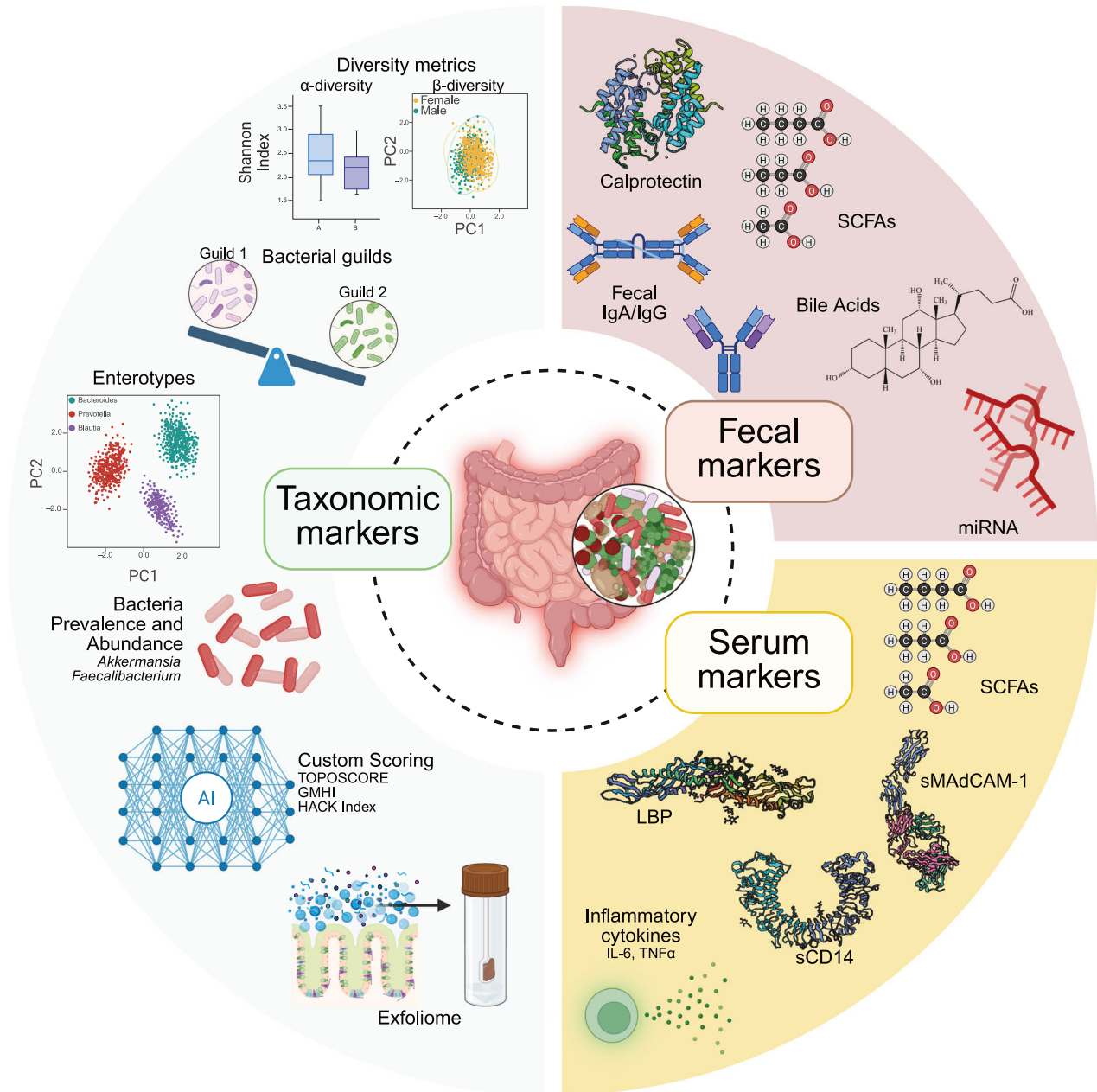
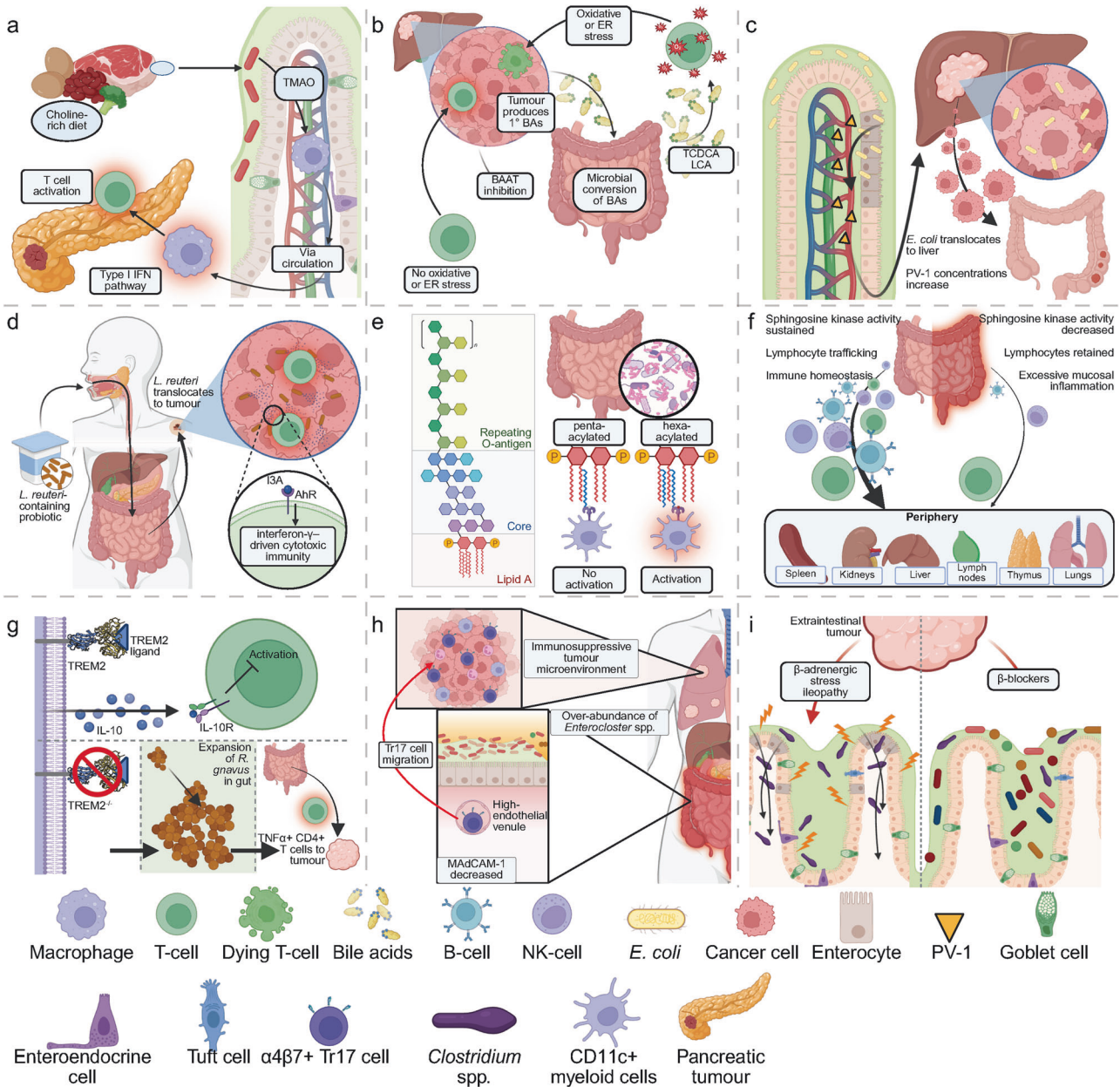


Fig. 2 Multi-omic framework for detection of intestinal dysbiosis. The dysbiotic gut is at the center of three complementary biomarker domains. Taxonomic markers (green) encompass ecological read-outs — α - and β -diversity, enterotype and bacterial-guild stratifications, prevalence/abundance of signature taxa such as *Akkermansia* and *Faecalibacterium*, and AI-enabled composite scores. Emerging “exfoliome” assays also capture host and microbial material shed from the gut epithelium, providing additional insight into enteric health. Fecal markers (pink) quantify host-derived inflammation (calprotectin), mucosal immune activity (fecal IgG and IgA), metabolic outputs of microbiota such as SCFAs and BAs, and regulatory miRNAs. Serum markers (yellow) extend surveillance systemically, measuring translocated microbial metabolites, classic “leaky gut” markers, endothelial activity (sMAdCAM-1), and pro-inflammatory cytokines. Collectively, these markers enable a holistic, compartmentalized assessment of dysbiosis. Three-dimensional protein structures were obtained from the Protein Data Bank, and the figure was created with BioRender.com.

models.¹²⁷ Patients with dysbiosis often have a reduced abundance of butyrate producers and lower overall SCFA levels due to a loss of beneficial fermenters, and their depletion in dysbiosis could thus signal an impaired immunological tone. Clinically, measurement of fecal SCFAs or their ratios is an emerging approach for functional characterization of dysbiosis in diseases like IBD and colorectal cancer (CRC).^{128,129}

SCFAs are not the only fecal metabolites being investigated in a cancer context. Abnormal profiles of BAs in stool can also reflect dysbiosis. Gut microbes convert host BAs into secondary BAs, and

a dysbiotic microbiome can skew this conversion, resulting in excess production of immunosuppressive BA species that may contribute to disease (Fig. 3b).¹³⁰ For instance, our lab has shown that antibiotic-induced dysbiosis spurs the downregulation of mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1; discussed below) via altered BA metabolism (Fig. 3h).⁴ Fecal BA composition is therefore being investigated as a proxy for dysbiosis. Another less conventional fecal dysbiosis marker is the fecal microRNA (miRNA) profile. The gut can shed host miRNAs into the stool, and recent studies indicate that they might reflect



the state of the gut mucosa and microbiome.¹³¹ Although not yet used clinically, stool miRNA panels are a novel dimension of the “exfoliome” of the gut epithelium that could furnish indirect readouts of microbiome status in disease.^{24,132}

Collectively, these fecal markers represent neutrophil-driven mucosal inflammation, while metabolite profiles provide a window into microbial metabolic capacity. Together, they demonstrate that dysbiosis is not defined by any single marker but by the convergence of immune, barrier, and metabolic perturbations.

Systemic biomarkers

Because gut dysbiosis and cancer¹¹⁴ have been associated with a compromised intestinal barrier, markers of intestinal permeability and microbial translocation are of great interest. Zonulin, a regulator of gut permeability, is released when barrier integrity is compromised; elevated serum zonulin has therefore been investigated as a proxy for “leaky gut” in cancer patients.¹³³ Other systemic indicators of epithelial injury include intestinal fatty acid-

binding protein, which reflects enterocyte damage, and circulating citrulline, a marker of functional mucosal surface area.⁸⁷ Together, these markers provide clinically accessible readouts of barrier dysfunction that may help stratify patients at risk of dysbiosis-associated inflammation and poor therapeutic response. Perhaps the most widely studied blood markers of dysbiosis are those that reflect endotoxin leakage from the gut. An outgrowth of Gram-negative bacteria leads to excess lipopolysaccharide (LPS) in the gut lumen, which can translocate into the bloodstream if the mucosal barrier is compromised.

Notably, bacteria can present diverse variations of the LPS molecule, each with distinct chemical, structural, and functional heterogeneity and varying immunogenicity (Fig. 3e).¹³⁴ These factors can influence immunotherapy outcomes. For example, the number of acyl chains attached to lipid A — the most conserved and immunologically active portion of LPS — can modulate host toll-like receptor 4 (TLR4) signaling and thereby influence the efficacy of anti-PD-1 immunotherapy.¹³⁵ One *A. muciniphila* strain isolated from a healthy donor produces an LPS with a lipid A

Fig. 3 Integrated overview of key mechanisms that link the gut microbiota, immune cell trafficking, and tumor immunity across landmark studies. These studies examine distinct yet interconnected pathways linking the gut and tumors, including metabolite recirculation (a, b), bacterial translocation (c–e), immune cell trafficking (f–h), and neurotransmitter-mediated stress responses (i). **a** A choline-rich diet enhances microbial production of trimethylamine, which is converted to TMAO.¹⁶⁰ Circulating TMAO activates macrophages through the type I IFN pathway, reprogramming the tumor microenvironment in pancreatic cancer to support effector CD8⁺ T-cell activation and responsiveness to checkpoint inhibitors. Dietary choline supplementation or TMAO treatment reduces tumor burden and restores immunotherapy sensitivity. **b** In hepatocellular carcinoma, upregulated BA synthesis and bile acid–CoA:amino acid *N*-acyltransferase (BAAT)-mediated conjugation drive the accumulation of conjugated primary BAs (e.g., taurochenodeoxycholic acid (TCDCA)) and secondary species (e.g., lithocholic acid (LCA)).²⁴⁰ These metabolites suppress antitumor CD8⁺ T-cell function, with TCDCA causing oxidative stress and LCA inducing endoplasmic reticulum (ER) stress. BAAT inhibition restores T-cell activity and sensitizes tumors to PD-1 blockade. **c** Compromise of the gut vascular barrier (GVB) allows *E. coli* to translocate to the liver, where they establish a premetastatic niche, promoting CRC metastasis.²⁴¹ The endothelial marker PV-1 serves as a biomarker for GVB disruption and predicts metastatic spread. **d** The commensal *Lactobacillus reuteri* can translocate into tumors and metabolize dietary tryptophan into indole-3-aldehyde (I3A).¹ I3A activates the aryl hydrocarbon receptor (AhR) in CD8⁺ T-cells, stimulating IFN- γ -driven cytotoxic immunity and potentiating ICI responses. **e** Acylation pattern of bacterial LPSs determines their immunostimulatory potential. Hexa-acylated LPS from specific gut commensals potently activates the host immune system via TLR4, promoting antitumor CD8⁺ T-cell responses and improving the efficacy of anti-PD-1 therapy.¹³⁵ By contrast, penta-acylated LPS species fail to activate immunity and are correlated with resistance to checkpoint blockade. **f** Kaede photoconvertible mice were used to map intestinal immune cell migration, showing continuous egress of B, T, and innate lymphoid cells from the colon to peripheral organs through a sphingosine-1-phosphate (S1P)-dependent pathway.²⁴² Under eubiosis, sphingosine kinase activity sustains S1P gradients and normal lymphocyte trafficking; dysbiosis or inflammation disrupts this axis, trapping cells in mucosal tissues and altering systemic immune homeostasis. **g** Loss of the lipid-sensing receptor TREM2 (triggering receptor expressed on myeloid cells-2) on intestinal macrophages reprograms them toward a pro-inflammatory state, reducing IL-10 production and expanding *R. gnavus* in the colon.²⁴³ This microbial shift drives TNF-producing CD4⁺ T cells to migrate from the gut to tumors, amplifying local antitumor responses and improving anti-PD-1 efficacy. **h** Antibiotics or dysbiosis downregulate the adhesion molecule MAdCAM-1 in the ileum, allowing $\alpha\beta\gamma\delta$ Treg17 (Tr17) cells to migrate into tumors and suppress immunity.⁴ This process, driven by *Enterocloster* species and BA signaling, undermines anti-PD-1 efficacy. **i** Extraintestinal tumors induce a β -adrenergic-driven stress ileopathy that damages the ileal mucosa, increasing permeability and enabling dysbiosis dominated by *Clostridium* species.¹¹⁴ This fosters tumor progression, whereas β -blockers or vancomycin restore barrier integrity, limit microbial overgrowth, and slow cancer growth. Figure was created using BioRender.com.

moiety that beneficially tunes innate immune signaling and restores intestinal and metabolic homeostasis.¹³⁶ LPS of *A. muciniphila* also lacks the O-antigen (the most variable and accessible portion of LPS¹³⁴), which results in the induction of IL-10 expression and consequent anti-inflammatory effects.¹³⁷ The O-antigen of *Salmonella* shields the lipid A moiety, delaying or reducing TLR4 recognition and consequently weakening host antibacterial responses.¹³⁸

LPS in the blood triggers an innate immune response and leads to the production of LBP and soluble CD14 (sCD14) to bind and neutralize it (Fig. 2). Hence, high concentrations of circulating LBP and sCD14 are frequently used as indirect markers of microbial translocation and have been linked to inflammatory sequelae in cirrhosis, although their utility as definitive surrogates remains under active investigation.¹³⁹ Western-style high-fat diets, which correlate with dysbiosis, are associated with increased gut permeability and higher serum sCD14 levels.¹⁴⁰ In metabolic disorders, elevated LBP and sCD14 frequently accompany insulin resistance, thus supporting the concept of “metabolic endotoxemia” driven by dysbiosis.^{141,142} Notably, these markers have clinical prognostic value: one study has linked high baseline LBP and anti-flagellin IgA levels to increased risk of hepatocellular carcinoma in patients with liver disease,¹⁴³ suggesting that gut-derived circulating endotoxin may promote oncogenesis in the liver.

Low-grade, systemic inflammation is a hallmark of unhealthy gut microbial states. Patients with CRC commonly show elevated serum pro-inflammatory cytokines (such as tumor necrosis factor- α (TNF- α) and IL-6) and C-reactive protein alongside high LBP.⁸⁷ These inflammatory mediators are not specific to the gut, but when found in combination with evidence of microbial translocation, they strengthen the case that dysbiosis is driving systemic inflammation. Importantly, interventions that modulate the microbiome can attenuate these markers. For example, supplementation with *A. muciniphila* has been shown to lower circulating LPS, LBP, and inflammatory cytokines in mouse models.¹⁴⁴ Indeed, *Akkermansia* p2261 is currently being investigated as a live biotherapeutic designed to improve ICI therapy responses (NCT05865730) and has very recently been shown to improve CAR T-cell therapy.¹⁴⁵ Such findings suggest that correcting

dysbiosis not only modifies microbial composition but also dampens systemic inflammatory responses, reinforcing the causal link between gut microbiota and systemic inflammation. Therefore, tracking a panel of LPS-related genes and proteins¹³⁵ and blood cytokines offers a window into the host’s inflammatory response to the gut microbiota.

A variety of small-molecule metabolites produced or modulated by gut microbes serve as indirect markers of dysbiosis in host circulation. SCFAs are again a prominent example and, although mostly assessed in stool, can also be easily measured in serum. Higher systemic butyrate levels generally indicate a fiber-rich diet and healthy microbiota, whereas low SCFAs or an imbalanced SCFA ratio can signal the loss of key beneficial taxa. SCFA levels not only help signal dysbiosis but may actively influence outcomes in diseases like cancer by shaping T-cell responses.¹²⁶ Clinical studies have linked deficient SCFA production to conditions such as graft-versus-host disease and poor responses to cancer immunotherapies, spurring interest in SCFA supplementation or diets to restore microbial balance.^{15,77,88,146,147}

However, mouse models have highlighted inconsistencies in treatment outcomes following supplementation with SCFAs. They can enhance PD-1 blockade when administered orally (drinking water or gavage) at physiologically relevant concentrations.^{148–150} By contrast, supplementing mice with butyrate in their drinking water appears to be detrimental for CTLA-4 blockade,¹⁵¹ suggesting that baseline SCFA levels or their supplementation may differentially affect ICI regimens. Moreover, the route of administration and context matter; systemic butyrate given by intraperitoneal injection improved anti-PD-1 efficacy,¹²⁷ whereas intratumoral butyrate may drive tumor fatty acid oxidation and lead to treatment resistance.¹⁵² Notably, SCFA supplementation without ICI does not improve tumor control or survival.¹²⁷ These results highlight the need for more research in this area before implementing them in clinical practice.

Perhaps the most exciting new serum marker is soluble MAdCAM-1 (sMAdCAM-1). Our lab recently reported that low baseline sMAdCAM-1 levels are strongly associated with gut dysbiosis in cancer patients.^{4,24} In several independent patient cohorts, low sMAdCAM-1 predicted poorer outcomes and was linked to either recent antibiotic use or intrinsic dysbiosis.⁴

Mechanistically, antibiotic-induced dysbiosis leads to the “bloom-ing” of bacteria from the *Enterocloster* genus, which down-regulates MAdCAM-1 expression in the gut vasculature owing to altered BA metabolism (Fig. 3h). This, in turn, allows immunosup-pressive gut-resident $\alpha 4\beta 7^+ \text{FoxP3}^+$ regulatory Th17 (Tr17) cells to exit the gut and migrate to peripheral tumors, where they can blunt local antitumor immunity.⁴ Conversely, high sMAdCAM-1 indicates an intact gut immune recruitment axis and correlates with richer microbial diversity. Thus, sMAdCAM-1 has been proposed as a “gut check” biomarker for dysbiosis in oncology and ICI therapy responses.¹⁵³ Low levels indicate microbiome imbalance and immune impairment, potentially identifying patients who might benefit from MCIs before or during immunotherapy. Indeed, a recent retrospective validation of the negative prognostic value of lower sMAdCAM-1 has been reported in 612 patients in first- and second-line therapies for metastatic renal cell carcinoma (RCC).¹⁵⁴

Other gut-derived metabolites can serve as proxies for dysbiosis. Perturbations in microbiota-dependent tryptophan metabolism have been reported in IBD, metabolic syndrome, and Western lifestyles, all contexts associated with microbial imbalance.¹⁵⁵ Another example is trimethylamine-*N*-oxide (TMAO), a hepatic metabolite generated from gut-derived precursors such as choline and L-carnitine.¹⁵⁶ Elevated TMAO levels have been linked to cardiovascular disease, type II diabetes, and obesity,¹⁵⁷ all of which correlate with dysbiosis. Broad-spectrum antibiotic use reduces TMAO production,¹⁵⁸ further underscoring its microbial origin. However, this effect appears to be context-dependent, as preclinical studies in breast¹⁵⁹ and pancreatic cancer models¹⁶⁰ indicate that TMAO may improve responses to ICIs (Fig. 3a). For instance, TMAO and its microbial precursors reprogrammed tumor-associated macrophages toward a pro-inflammatory, type I interferon (IFN)-dependent phenotype, augmenting CD8⁺ T-cell activity and potentiating anti-PD-1 efficacy in murine pancreatic cancer.¹⁶⁰ These findings demonstrate that microbiota-derived metabolites may either impair or amplify immunotherapy outcomes depend-ing on the metabolic and microbial context.

Taken together, these biomarkers illustrate that dysbiosis leaves measurable systemic and mucosal imprints that can be tracked in oncology cohorts, providing mechanistic links between microbial imbalance, systemic inflammation, and therapy response.

SIMPLIFYING GUT MICROBIOME COMPLEXITY FOR CLINICAL UTILITY

Given the inherent complexity and variability of gut microbiome data, it is necessary to simplify it into an easily interpretable, more clinically relevant metric. Doing so has several advantages. First, “-omics” data are noisy and high-dimensional, and rarely have a clear clinical meaning. By contrast, a standardized index that accurately reflects enteric health allows for bedside interpretability, rapid patient stratification, longitudinal tracking, and cross-study comparisons. Second, broad diversity metrics or ratios fail to capture the nuances of enteric health, whereas more sophisticated algorithms can account for confounding variables and the degree of importance that a particular feature (i.e., taxon) can contribute to the model. Finally, custom scoring can be disease-agnostic, meaning that a single standard health index can potentially flag dysbiosis even for conditions whose bacterial markers are not known.¹⁶¹ Thus, having a robust, rationally designed, and well-validated model could aid caregivers and researchers in finding correlations and causality between the composition and function of the gut microbiome and disease states. In this section, we focus our discussion on efforts to design objective metrics for dysbiosis, either for research purposes or as assays for routine clinical care.

Diversity and richness as health indicators

Diversity-based metrics remain one of the most widely used and accessible proxies for assessing gut microbial health. Indeed, a central distinction between eubiosis and dysbiosis is the level of ecological diversity: eubiotic individuals typically harbor a richly diverse and stable microbial ecosystem, whereas dysbiosis is often marked by reduced diversity and compositional instability.¹⁶² Notably, α -diversity at baseline has been associated with favorable clinical outcomes in ICI therapies.^{5,147} Whereas higher body-mass index, weight, and blood pressure are generally associated with reduced diversity, increased gut microbial richness has been associated with regular physical activity and high intake of fruits and vegetables — factors generally attributed to good health.¹⁶³ Reduced diversity has also been documented across a broad spectrum of diseases, including diarrhea,¹⁶⁴ metabolic syndrome, obesity,¹⁶⁵ and IBD.¹⁶⁶

However, diversity readouts and their correlations with treat-ment outcomes or disease states can vary depending on cohort size and technical artifacts.¹⁶⁷ Moreover, diversity alone cannot distinguish between beneficial and pathogenic communities, nor can it capture functional capacity or ecological dynamics. Thus, although diversity metrics have traditionally served as convenient proxies for gut health, the field is increasingly shifting toward more comprehensive and mechanistically informative approaches.

Enterotypes and community typologies

Enterotypes were an early attempt to simplify gut microbial data by grouping microbial communities on the basis of the predominance of specific taxa. The concept defines clusters typically dominated by genera like *Bacteroides*, *Prevotella*, and *Ruminococcus*, although subsequent research has sometimes expanded or refined these categories.^{168,169} They can help stratify populations on the basis of broad gut microbiota composition, facilitating investigations into microbiota-driven disease mech-anisms and therapeutic interventions. They also simplify the complexity of the gut microbiota into classifiers that can potentially help researchers and clinicians better understand the microbiome’s relationship with diet, metabolism, health, and disease.¹⁷⁰

Several studies have illustrated the practical usefulness of enterotypes. In CRC, distinct signatures within enterotypes suggest potential diagnostic applications and personalized therapeutic strategies.¹⁷¹ They have also proven relevant in metabolic disorders like type 2 diabetes, in which *Bacteroides*-dominant enterotypes are correlated with higher metabolic risk profiles compared to *Prevotella*-dominant types, enabling targeted preventive or therapeutic interventions.^{172,173} In addition, enter-otypes can predict individual responses to probiotics, with metabolic improvements like reductions in blood glucose or triglycerides being enterotype-dependent.¹⁷⁴

However, enterotypes are subject to several controversies and challenges. A central debate revolves around whether gut microbiota form discrete clusters or exist along continuous “enterogadients”.¹⁶⁹ In addition, methodological sensitivity impacts enterotype identification, with variations in clustering techniques, distance metrics, and sequencing methods leading to inconsistent results.^{169,175} Their clinical applicability is further challenged by emerging evidence that enterotypes exhibit variability over time, which is shaped by factors like diet, disease, and environmental changes.¹⁷⁶ Moreover, diet and geography significantly influence enterotype distributions, underscoring the importance of considering these variables when interpreting enterotype-related findings.¹⁶⁹ Finally, the number of enterotypes remains contentious, with different studies reporting 2–4 clus-ters,^{177,178} depending on the methods used and the populations studied. These inconsistencies and the absence of standardized protocols complicate cross-study comparisons and interpretations, limiting the broad applicability of enterotype research.

Microbial guilds and functional groups

Originally an ecological concept, guilds are groups of species that use similar resources in similar ways and contribute to the same ecological function.^{179,180} Microbiologists have adapted this idea by defining a guild as a set of bacteria that rise and fall together in abundance and collaborate to deliver the same ecological function.^{180,181} These co-abundant taxa may originate from diverse phylogenies, but they share overlapping niche requirements and biological capabilities.^{87,146,181–183} This perspective highlights how microbial groups interact within complex ecosystems in the broader community, providing a function-focused alternative to cataloging diverse taxonomies.^{180,181}

Given the complexity of microbial interactions, the identification of guilds requires sophisticated analytical methods. One approach is co-abundance network analysis, which clusters microorganisms on the basis of shared abundance trends.^{146,180–182,184,185} Techniques such as hierarchical clustering and weighted correlation network analysis have already been used to classify different taxa into guilds.^{182,185} These methods have revealed interesting contrasts within the microbiome, like the “two competing guilds” architecture, in which a health-associated foundation guild counterbalances a pathological guild enriched in virulence factors and antibiotic-resistance genes.^{146,182,184,185} The foundation guild typically contains butyrate-producing and mucosa-protective taxa such as *Faecalibacterium* spp., *Akkermansia* spp., and members of the *Lachnospiraceae* and *Oscillospiraceae* families.^{87,146,182,185} Context-specific variants of this beneficial guild have also been documented, such as the “fecal guild” expanding $\gamma\delta$ intra-epithelial lymphocytes and a “small-intestinal guild” dominated by *Lactobacillus*, *Enterococcus*, and *Faecalibaculum*.¹⁸⁶ By contrast, the pathological guild aggregates facultative or obligate anaerobes, such as *Enterocloster* and *Hungatella hathewayi*, pro-inflammatory oral taxa, and enterobacteria, whose outgrowth accompanies chronic inflammation, metabolic syndrome, and cancer progression.^{4,184,187,188} Intriguingly, a third metabolically defined guild composed of facultative anaerobes that exploit respiratory electron acceptors — such as oxygen — often surges during dysbiosis and serves as a broader ecological marker of disturbance.¹⁷ Identifying these guilds can help explain how shifts in collective metabolic capacity, rather than single-taxon perturbations, translate into host-level phenotypes and disease trajectories.

However, we still lack a rigorous and universally accepted framework for defining and quantifying guild units. Some criteria are so broad that they blur ecological meaning, whereas others are so restrictive that they exclude potentially significant taxa.¹⁷⁹ Closing this gap will require systematic mapping of the metabolic pathways that distinguish one guild from another, whether fiber digestion and SCFA biosynthesis in health-promoting consortia or virulence and antibiotic-resistance pathways in pathogenic groupings.¹⁴⁶

Equally vital are well-designed longitudinal studies that track how guilds — and the gut microbiota in general — evolve during ICI therapy. Temporal stability of certain beneficial functions or taxa has emerged as a consistent feature for durable responses.¹⁸⁹ Responders tend to be enriched in *Clostridia*, particularly *Lachnospiraceae*, with SCFA-producing and fiber-degrading capacities, and pathways enriched in polyamine synthesis.^{189,190} Remarkably, one study also identified flagellin-related genes within these stable *Clostridia*, whose derived peptides structurally mimicked tumor-associated antigens and elicited cross-reactive CD8⁺ T-cell responses, providing a mechanistic link between microbial persistence and antitumor immunity.¹⁸⁹ Another study found that postbiotics derived from *Lactobacillus paracasei* could upregulate HLA class I expression on cancer cells by engaging the NLRC5 protein (a major regulator of HLA expression), thus leading to tumor antigen-specific T-cell activation and synergistic anticancer effects with PD-1 blockade therapy.¹⁹¹ Importantly, the

microbial composition of patients receiving anti-PD-1 therapy is not static, and taxa that appear agnostic toward treatment outcomes at baseline may become deleterious at later timepoints.¹⁹⁰ Indeed, ~9–10 months after treatment initiation, non-responders frequently display an expansion of Gram-negative taxa, accompanied by elevated neutrophil-to-lymphocyte ratios and signatures of LPS-driven inflammation.¹³² Collectively, these findings indicate that longitudinal stability, both in microbial presence and immune-relevant function, may reveal more information for predicting ICI efficacy than baseline diversity alone.

Epistatic effects between bacteria have also been shown to influence their function, which may translate into clinical consequences.^{24,192} These interbacterial relationships may redefine our understanding of microbiome-immune-tumor interactions and warrant further investigation to clarify their clinical relevance. Only by pairing precise functional annotation with temporal dynamics can we transform the guild framework from a descriptive shorthand into a predictive tool for personalized interventions.

ML- and multi-omics-driven biomarker discovery

In recent years, the pursuit of reliable biomarkers for gut dysbiosis has shifted markedly toward ML- and multi-omics-driven strategies. Several dysbiosis indices have already been proposed in the context of various conditions, including cirrhosis,¹⁹³ gastrointestinal maladies,^{194–196} gout,¹⁹⁷ heart disease,^{198,199} and cancer.^{200,201} Other researchers have attempted to develop a more generalized approach,^{161,188,202} and at least one study has integrated metagenomics and proteomics to define a functional, rather than compositional, metric for dysbiosis.²⁰³ Wei et al.²⁰⁴ have written the most recent review of the variety of algorithms and formulae being used to calculate dysbiosis metrics, and we encourage readers to consult their article for further insights.

Examples of some early models for calculating a dysbiosis score relied primarily on ratios of bacteria of interest in a disease-specific context. For example, the Microbial Dysbiosis Index was developed in the context of Crohn’s disease (CD) and is calculated as the logarithmic ratio of bacteria that increase in CD patients to those that decrease.²⁰⁵ This model inspired the development of the Microbial Community Polarization index in a study of CRC carcinogenesis.²⁰¹ Another early metric is the GA-map dysbiosis test, which uses a probe-based assay and a proprietary algorithm to identify and rank the degree of dysbiosis in IBD patients against a reference “normobiotic” microbiome.¹⁹⁴

Recently, more sophisticated models validated against hundreds to thousands of individuals have emerged. Jaeyun Sung developed the Gut Microbiome Health Index (GMHI).¹⁶¹ His group examined the metagenomic data from 4347 microbiomes to develop a mathematical formula based on the prevalence and relative abundance of 50 key bacterial species, 43 of which (86%) were labeled as “health scarce” species. This strategy enabled the GMHI to discriminate between healthy and diseased individuals with an accuracy of 73.7%. The GMHI was later upgraded on the basis of a larger data set of 8059 metagenomes from 54 studies and renamed the Gut Microbiome Wellness Index 2 (GMWI2).²⁰² In addition, the GMWI2 used a strategy that weighted the contribution of each taxon on the basis of its strength of association with dysbiosis rather than assuming that all taxa contribute equally. These differences enabled GMWI2 to outperform GMHI and achieve a higher dysbiosis classification accuracy of up to 90%.²⁰²

The HACK (Health-Associated Core Keystone) index takes a different approach. Rather than determining which microbial species are associated with disease, Goel et al.¹⁸⁸ aimed to identify the hallmarks of a eubiotic gut and concluded that subjects with deviations from this compositional benchmark are dysbiotic. They analyzed 45,424 adult metagenomes from 42 countries and identified 201 key taxa using three key parameters: their association with non-diseased subjects; stability over time (i.e.,

how prevalent they remained within longitudinal samples); and correlation with host health. The authors could then use the 17 top taxa in their ranked list to identify which individuals are dysbiotic, an approach that demonstrated similar or better performance than the GMW12 or another published metric called the “dysbiotic score”.¹⁹⁵ Notably, all 17 bacteria on this list — including *F. prausnitzii*, *Roseburia* spp., and *Alistipes shahii* — are obligate anaerobes and known butyrate producers.²⁰⁶

ML is crucial for analyzing such high-dimensional data. Unsupervised clustering and supervised models sift through thousands of microbial genes, metabolites, and proteins to find optimal marker panels. For example, large cancer microbiome consortia (such as the EU ONCOBIOME project²⁰⁷) have applied ML to define composite dysbiosis scores predictive of therapy response. The TOPOSCORE — one example developed by our lab (discussed below)²⁰⁰ — is designed to guide interventions and identify patients who might benefit from MCIs such as dietary changes, probiotics, live therapeutics, postbiotics, or FMT before or alongside immunotherapy.²⁰⁸ Although it requires prospective validation, the TOPOSCORE demonstrates how ML can integrate complex microbial signatures into a practical biomarker tool for clinical decision making.

ML-based models have demonstrated that dysbiosis in cancer resembles dysbiosis in the context of other chronic inflammatory conditions. This suggests that dysbiosis scoring may be broadly applicable across disease states, potentially eliminating the need for disease-specific models. In addition, instead of examining each biomarker individually, researchers are integrating metagenomics, metabolomics, proteomics, and immunological data to uncover multi-dimensional signatures of dysbiosis.

One compelling example is the metagenome-informed metaproteomics pipeline.²⁰³ This approach uses shotgun metagenomic sequencing to inform high-resolution mass spectrometry-based proteomics, enabling nearly complete identification of proteins in stool and distinguishing endogenous, microbial, and dietary proteins.²⁰³ Applying this pipeline to IBD patients led to the discovery of dozens of novel proteins (both host- and microbe-derived) that were altered in dysbiosis. Remarkably, certain combinations of these proteins could discriminate disease subtype and severity, outperforming fecal calprotectin (the current gold standard) in diagnostic accuracy. The analysis also revealed previously unrecognized human anti-microbial peptides upregulated in dysbiotic guts.²⁰³ These peptides and other host- and bacteria-derived proteins, such as haptoglobin, ppdK (a glycolytic enzyme), and type 1 glyceraldehyde-3-phosphate dehydrogenase, form a rich new class of biomarker candidates. By profiling the bacterial proteome, metaproteomics delineates the functional activities that are preserved or impaired in dysbiosis. This kind of multi-omic atlas of the gut ecosystem is revolutionizing biomarker discovery: rather than relying on hypothesis-driven markers, it enables data-driven identification of signature molecules that track gut health with high specificity.

These methods are only a few examples of the many models presented in the literature for determining whether a patient is dysbiotic. Nevertheless, fundamental questions remain. Are classical approaches based on relative abundance variations the most informative means of studying gut microbial composition? Given the numerous confounding factors in microbiome research and the challenges of linking compositional data to quantitative pathophysiological parameters,^{209,210} complementary strategies should be considered. These include quantifying bacterial loads or absolute species counts²¹¹ through methods such as 16S rRNA amplicon sequencing, flow cytometry, and ML-based analyses.^{209,210,212,213} Are deviations of the gut microbiota repertoire the cause or the consequence of the morbid state with its comorbidities? Finally, what are the underlying factors driving dysbiosis?

KEY FACTORS CONTRIBUTING TO CANCER-ASSOCIATED DYSBIOSIS

To delve deeper into these pivotal questions, we used our innovative TOPOSCORE metric.²⁰⁰ This ecology-centric approach identifies gut microbial species that are correlated with survival in cancer immunotherapy based on ICIs alone or in combination with chemotherapy in metastatic patients. TOPOSCORE is an individualized metric of dysbiosis, whereas guilds, alpha and beta diversity, and the other methods described above require population-level datasets to yield meaningful information. The TOPOSCORE demonstrates performance comparable to those of other models while still retaining clinical practicality, enabling longitudinal assessment of the impact of disease evolution or treatment and translating into a rapid, bedside-compatible qPCR test. Thus, it offers a user-friendly approach that can rapidly determine whether a patient has gut dysbiosis at any time during their treatment regimen.²⁰⁰

Using shotgun metagenomics and co-abundance network analysis based on the MetaPhlAn 4 (ChocoPhlAn 2021) pipeline,²¹⁴ our lab identified two distinct and antagonistic microbial constellations, referred to as species interaction groups (SIGs), which provide valuable insights into the dynamics driving dysbiosis.

The SIG1 group is composed of 37 bacterial species frequently associated with worse overall survival following immunotherapy. It consists of two smaller clusters that were identified when the pre-constructed co-abundance network was partitioned by Ward-linkage hierarchical clustering. One cluster is composed of taxa resident primarily in the oral cavity, like *Veillonella* spp., *Streptococcus* spp., and *Bifidobacterium dentium*. The other cluster contains gut-resident obligate anaerobes known for their BA production, such as *Clostridium* spp., *Enterocloster* spp., and *H. hathewayi*. Functionally, these organisms converge on BA metabolism and pro-inflammatory signaling, with oral taxa introducing aerotolerant, inflammation-prone communities and *Enterocloster* and *Clostridium* spp. disrupting mucosal immune homing. This is notable because (as discussed above) we have previously demonstrated that species from the *Enterocloster* genus can bloom following cessation of antibiotics, leading to altered BA metabolism, reduced MAdCAM-1 expression, and exodus of enteric Tr17 cells to distant tumors.⁴ SIG1 bacteria also display functions related to fatty acid oxidation, succinate fermentation to butanoate, degradation of L-histidine and L-phenylalanine, and the biosynthesis of L-serine, L-lysine, and L-glycine.²⁰⁰

By contrast, the SIG2 group contains 45 bacterial species that are often associated with a better treatment prognosis (Fig. 4). All bacteria in this group are gut-resident obligate anaerobes and butyrate producers that stabilize barrier function and promote T-cell tolerance, such as *Faecalibacterium* spp.,²¹⁵ *E. rectale*,²¹⁶ *Coprococcus comes*,²¹⁷ *Roseburia inulinivorans*,²¹⁸ and *Oscillibacter* sp. ER4.²¹⁹ Butyrate modulates peripheral immune tolerance,²²⁰ acts as a key resource for colonocytes,¹²⁶ improves gut barrier integrity,¹²⁹ and helps maintain the O₂-deprived environment in the gut lumen necessary for the growth of anaerobic bacteria.^{130,221} The SIG2 bacterial consortium also has functions associated with tRNA processing, peptidoglycan biosynthesis, purine nucleoside and mannan–stachyose degradation, the GABA shunt, L-glutamate and L-glutamine biosynthesis, the polyamine pathway (including L-ornithine and L-arginine biosynthesis), and sulfur oxidation.²⁰⁰

The fecal prevalence of these species (evaluated using MetaPhlAn 4, ChocoPhlAn 2021²¹⁴) is used to calculate and assign an individual SIG score (*S*). This value is defined as:

$$S = \frac{1}{2} \left(\frac{N_{SIG2}}{45} - \frac{N_{SIG1}}{37} + 1 \right)$$

Where N_{SIG1} and N_{SIG2} are the numbers of unique SIG1 and SIG2 taxa detectable in a fecal sample, respectively. Patients who are evaluated as $S \leq 0.535$ are SIG1+ (dysbiotic), whereas those with a

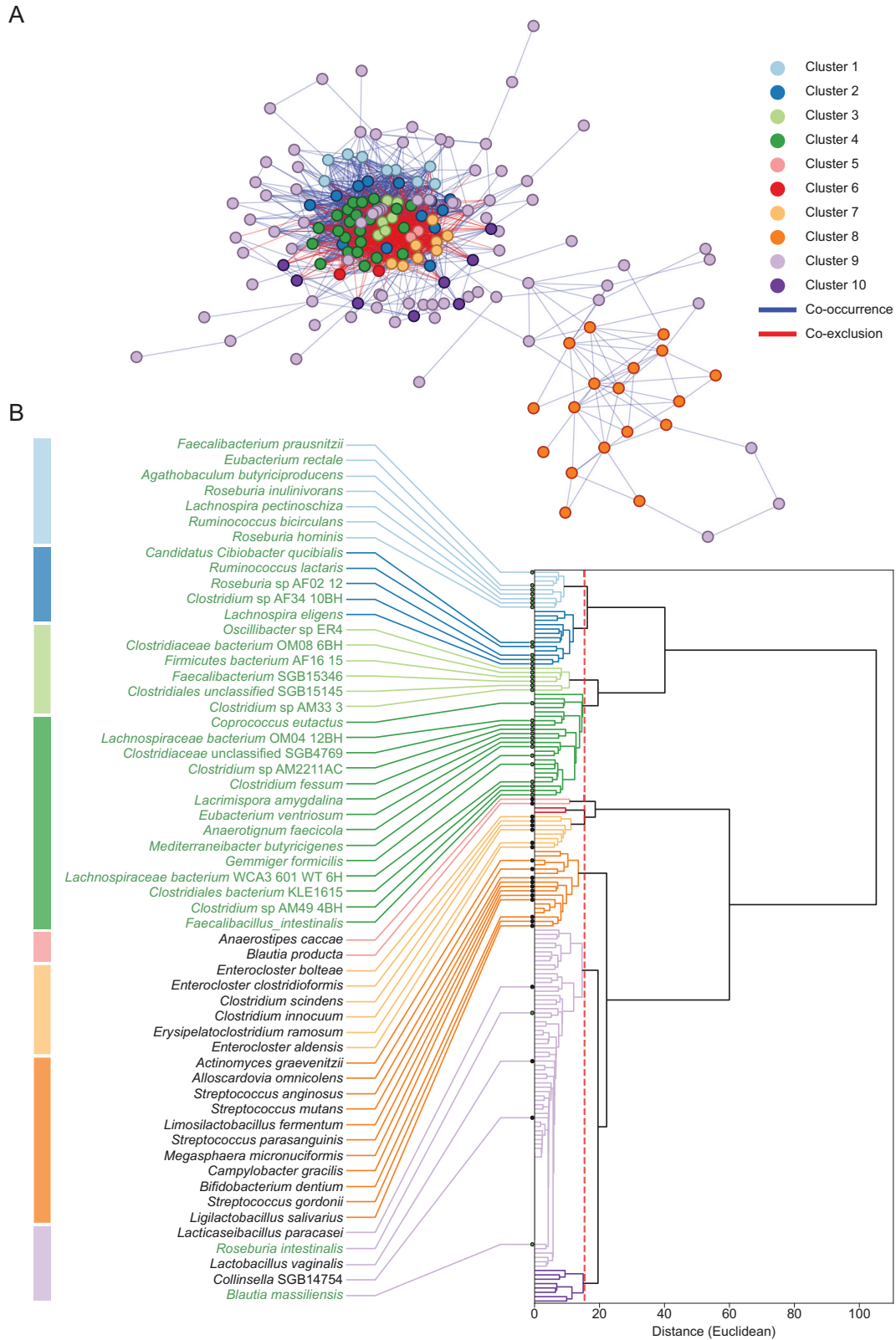


Fig. 4 Expanded gut microbiome network and hierarchical clustering in a pan-cancer cohort. **a** Co-abundance network reconstructed with the workflow presented by Derosa et al.^{24,201} but applied to the microbiomes of all 955 cancer patients in our dataset (NSCLC, $n = 556$; RCC, $n = 82$; UC, $n = 133$; CRC, $n = 182$). Nodes (individual metagenomic species) are colored by cluster. Blue edges denote positive co-occurrence and red edges negative co-exclusion. **b** Dendrogram of the same species, with colored bars beside the dendrogram corresponding to the network clusters shown in **a**. Species labeled in green are immunotherapy-responsive SIG2 bacteria, whereas those in black are resistance-associated SIG1 bacteria. The dashed red line marks the distance threshold that segregates the 10 major clusters.

score $S \geq 0.791$ are SIG2+ (eubiotic). “Gray zone” patients — i.e., those not immediately assigned to SIG1+ or SIG2+ — are further stratified on the basis of the prevalence and relative abundance of *A. muciniphila* SGB9226. Our lab and others have demonstrated the antitumor effects of *A. muciniphila*,^{13,222,223} but we have also shown that there is a non-linear, trichotomic relationship between the relative abundance of *A. muciniphila* SGB9226 and patient overall survival.³ Gray zone patients who lack or have an overabundance ($> 4.799\%$) of *A. muciniphila* are considered SIG1+, whereas others with “normal” levels are considered SIG2+. Our TOPOSCORE formula has been validated across hundreds of patients and multiple malignancies, demonstrating a predictive accuracy of 69.8%.²⁰⁰ In addition, a distilled variant incorporating 21 bacterial taxa — five SIG1 and 17 SIG2 — can better predict the survival of CRC and melanoma patients. The TOPOSCORE algorithm is implemented in R and freely available on GitHub (<http://github.com/valerioebba/TOPOSCORE>), with an interactive Shiny application accessible via the ONCOBIOME Atlas (<https://oncobiome-atlas.shinyapps.io/TOPOSCORE/>).

To better understand the factors driving dysbiosis, as defined by the TOPOSCORE, we retrospectively analyzed metagenomics data from 5346 healthy volunteers obtained from publicly available sources²²⁴ and 955 cancer patients²⁴ diagnosed with non-small cell lung cancer (NSCLC, $n = 556$), RCC ($n = 82$), and urothelial carcinoma (UC, $n = 133$), along with CRC patients ($n = 182$) from the AtezoTRIBE study (NCT03721653).^{225–227} In healthy individuals, most stool samples fell into the eubiotic SIG2 category (~68%), whereas only ~4% mapped to the adverse SIG1 group (Fig. 5a, left plot). Cancer patients showed the opposite pattern: 34% of subjects were SIG1, whereas ~23% were SIG2. The mirror-histogram plots on the right further highlight a clear increase in the number of SIG1 taxa per metagenomic sample among the cancer patients, indicating a significant re-structuring of the microbiomes. We can also see that there is a clear consequence when we examine correlations between the number of prevalent SIG1 bacteria in cancer patients and overall survival (Fig. 5b). Each dot represents an individual microbiome at baseline, with CRC, NSCLC, RCC, or UC patients represented in blue, orange, green, and brown, respectively. Overall survival decreases as the SIG1 count increases (Simpson $R = -0.19$, $P < 0.0001$), whereas the analogous analysis for SIG2 shows no significant correlation ($R = 0.06$, $P = 0.066$). Thus, it is the accumulation of SIG1 organisms, rather than the prevalence of SIG2, that predicts poor treatment outcomes following immunostimulation with ICI.

Moreover, clear patterns emerge when centered log-ratio-normalized abundances are plotted as a heatmap (Supplementary information, Figs. S1, S2). Both healthy individuals and cancer patients classified as SIG2+ demonstrate a diverse representation of SIG2 bacteria with very low levels of SIG1 bacteria. Notably, some of the SIG1 bacteria are not completely absent but rather appear to maintain homeostasis at low levels. Individuals classified as SIG1+ appear to fall into at least two distinct groups based on the identity of the SIG1 bacteria that dominate, which we herein define as Type 1 and Type 2 compositional dysbiosis. Type 1 dysbiosis is characterized as a modest loss of SIG2 bacteria with evidence of oral species spillover, as exemplified by the presence of aerotolerant genera like *Veillonella* and *Streptococcus*. Type 2 dysbiosis presents as a near complete collapse of the SIG2 consortium with concomitant over-representation of gut-resident, obligate anaerobes in the SIG1 group (e.g., *Enterocloster* and *Clostridium* genera). These two dysbiosis trajectories highlight distinct ecological failures: Type I reflects a loss of colonization resistance against aerotolerant invaders, whereas Type II reflects the collapse of fermentative consortia critical for SCFA production and barrier integrity.

We then examined how increased numbers of SIG1 bacteria impact patient survival (Fig. 5c). Using the median value of nine SIG1 taxa as a threshold, we showed that patients with a higher

SIG1 burden (black curves) experienced markedly worse overall and progression-free survival; median time to progression was reduced by almost 2 years compared with that of patients harboring nine or fewer SIG1 bacteria at baseline (green curves). We next investigated which microbes drive the signal (Fig. 5d). The volcano plot of cancer samples identifies *Clostridium innocuum*, *Enterocloster clostridioformis*, and *Erysipelatoclostridium ramosum* as among the taxa most strongly enriched in high-SIG1 carriers, whereas Lachnospiraceae bacterium WCA3 or *Faecalibacterium* SGB15346 predominate in the low-SIG1 subset. Notably, a similar result was found among SIG1+ healthy volunteers, who also had higher abundances of species from the *Enterocloster* and *Clostridium* genera. Taken together, these results position SIG1 enrichment as a hallmark of cancer-associated dysbiosis, tie it to shorter survival, and pinpoint candidate organisms responsible for the compositional shift.

ONCOBIOME NETWORK: ESTABLISHING AN INTERNATIONAL CLINICAL FRAMEWORK FOR MCIS

The concept of “gut health” as a clinical biomarker is rapidly gaining traction in oncology, transitioning from a theoretical construct to an actionable tool for patient stratification and treatment guidance. We are now beginning to recognize that eubiosis, rather than being defined by a fixed catalog of taxa, depends on gut microbial resistance (the ability to withstand insult) and resilience (the capacity to recover after disturbance).^{189,228} Dysbiosis can be framed as a disruption of these dynamics rather than a simple loss of diversity, as exemplified by longitudinal studies of patients receiving anti-PD-1 therapy.¹⁸⁹ Stable taxa and functions are enriched in therapy responders across multiple cohorts, further supporting the idea that a resilient microbiome underpins effective ICI therapies.^{190,229} In this context, metabolic independence — the capacity to synthesize essential cofactors, amino acids, and nucleotides — has emerged as a key ecological trait that promotes enteric health.^{228,230}

Clinical evidence from FMT trials, which are being investigated to correct dysbiosis, appears to illustrate this ecological perspective. These studies demonstrated that recipients’ gut microbiota diverged from baseline after FMT and matched those of their respective donors.^{229,231,232} Moreover, their compositions remained stably divergent from baseline over the 7–65-day follow-up, demonstrating that donor-specific engraftment can persist for weeks, providing there is no influence from external factors.²³¹ Indeed, one study reported that a patient who received antibiotics 11 weeks after their first FMT required a second FMT almost a year later to restore donor-like microbiota, thus highlighting the fragility of engraftment under external pressures.²³² The persistence of engraftment was supported by follow-up studies showing recurrence of dysbiosis and clinical relapse after antibiotic use, which was also subsequently corrected with a second FMT.²³³

Together, these studies indicate that FMT is not a static transfer of taxa but a dynamic intervention whose success depends on the recipient’s resistance and resilience, the donor’s microbial repertoire, and external factors. However, the duration of these effects is still not understood. The longest follow-up among FMT trials for cancer patients has been 3 months.^{229,231,232,234} Evidently, this short timeframe has been sufficient to reset the holosystem and prime it for improved responses to ICI (mouse models have demonstrated that FMT can restore the expression of ileal MADCAM-1⁴), but long-term effects still need to be determined. Furthermore, there is an assumption that successful engraftment following FMT is required for clinical success. This has yet to be conclusively demonstrated. In fact, a metagenomics analysis of 316 FMTs indicated that clinical success does not rely on successful colonization, microbial displacement, or bacterial reinstatement.²³⁰ Building on these ecological and translational

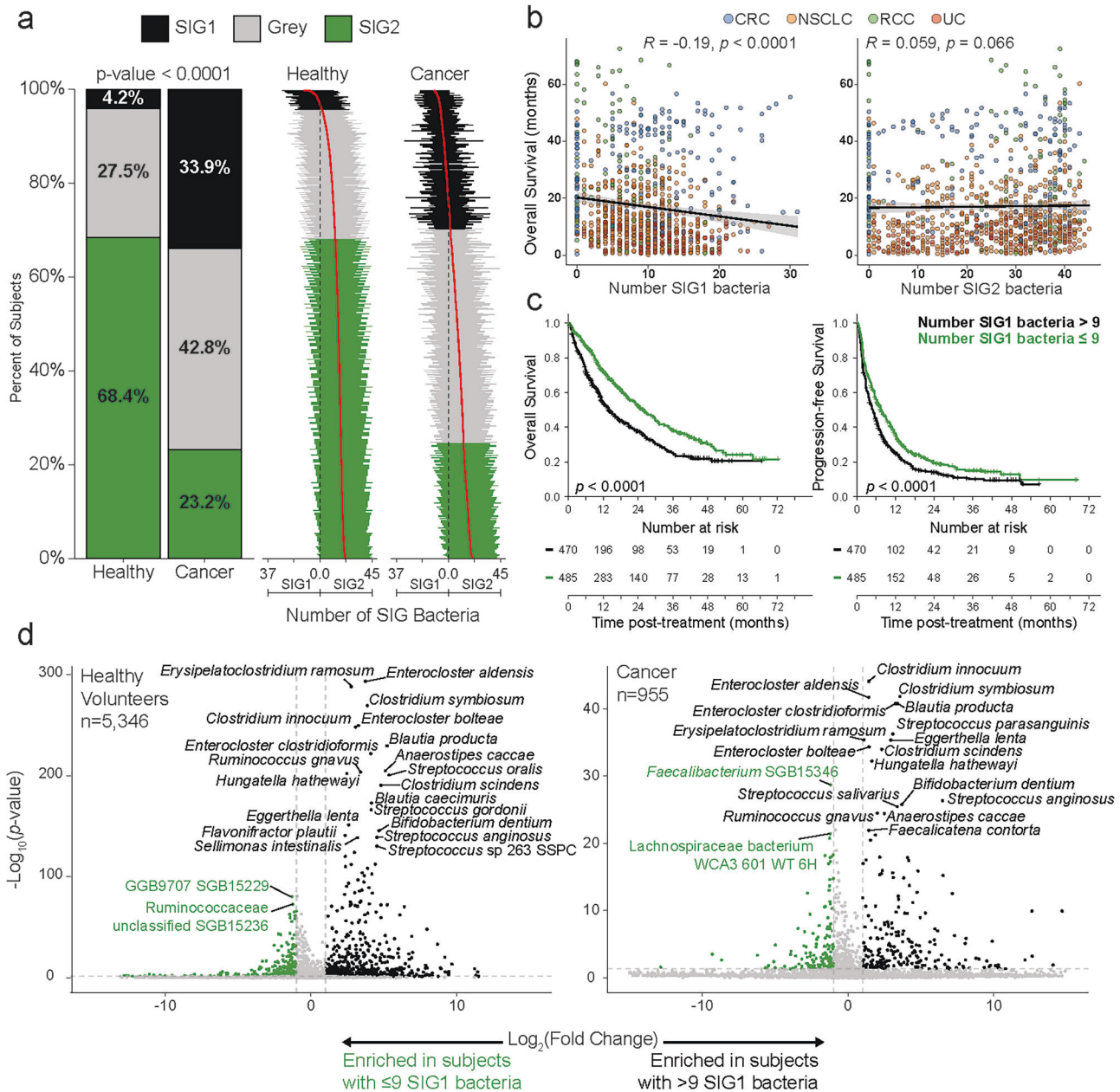


Fig. 5 SIG1 enrichment is a microbiome signal of dysbiosis and poor clinical outcome across cancers. TOPOSCORE profiling of 5346 healthy volunteers and 955 cancer patients confirmed that an excess of SIG1 taxa — bacteria linked to ICI resistance — marks a shift from eubiosis to dysbiosis and carries systemic consequences. **a** Cancer patients are significantly more likely than healthy subjects to have a dysbiotic (SIG1) gut or be classified as Gray (Fisher's exact test, $P < 0.0001$). The right panel shows that in healthy individuals, the balance is typically skewed toward having more SIG2 bacteria, whereas in cancer patients, the balance shifts toward a dominance of SIG1 bacteria, highlighting an ecological re-organization. **b** Across CRC, NSCLC, RCC, and UC patients, the number of SIG1 species is negatively correlated with overall survival (Spearman, $R = -0.19, P < 0.0001$). By contrast, SIG2 counts show no meaningful association with survival ($R = 0.059, P = 0.066$). **c** Using a median threshold of $n = 9$ SIG1 species, patients above the cut-off experience markedly shorter overall and progression-free survival (log-rank $P < 0.0001$), further underscoring the clinical relevance of SIG1. **d** Volcano plots reveal that SIG1-high healthy volunteers (left) and cancer patients (right) are consistently enriched for pro-inflammatory (*E. ramosum*), immunosuppressive (*Enterocloster* spp., *H. hathewayi*, *Clostridium* spp.), or oral taxa, suggesting that these genera are the principal ecological drivers of dysbiosis. SIG, Species interacting group.

insights, large-scale initiatives are now seeking to operationalize this knowledge within a clinical framework.

ONCOBIOME is a large-scale international initiative that seeks to manipulate the gut microbiota to improve outcomes in cancer immunotherapy.²⁰⁷ It was launched in 2019 and brings together academic institutions, research centers, and private industry partners across eight countries. ONCOBIOME views dysbiosis as a treatable condition, not just a biomarker. Thus, it is built around

two central objectives: first, to define Gut OncoMicrobiome Signatures (GOMs)⁴³ and translate them into diagnostics and clinically actionable interventions; and second, to translate this knowledge into innovative and effective MCIs. To achieve these goals, ONCOBIOME emphasizes broad multidisciplinary collaboration to standardize metagenomic protocols, integrate microbiome, immune, and clinical datasets, and apply GOMs to guide precision immunotherapy. The TOPOSCORE is one deliverable that was

developed and validated by this project.^{200,207} Through these efforts, ONCOBIOME has laid the scientific and infrastructural groundwork for microbiome-informed precision oncology.

One initiative within the broader ONCOBIOME framework is IMMUNOLIFE2 (NCT07001618), a randomized Phase II trial of FMT to restore ICI sensitivity in cancer patients who have become resistant owing to antibiotics. Pioneering studies in mice and melanoma patients have already demonstrated its safety and efficacy in improving anti-PD1 therapy,^{13,229,231,232,235} and additional studies and meta-analyses are investigating its mechanism of action.^{230,234} IMMUNOLIFE2 targets advanced lung cancer patients who previously received antibiotics and subsequently demonstrated primary resistance to PD-1 blockade therapy. The goal is to administer MaaT033 — an oral formulation of standardized, pooled-donor FMT capsules — in combination with anti-PD-1 therapy and compare outcomes against a standard-of-care control arm to determine whether FMT can restore immunotherapy responsiveness in patients who would otherwise have poor outcomes following antibiotic-induced resistance. The success of these studies could support the implementation of antibiotic stewardship recommendations²³⁶ and pave the way for routine microbiota restoration (via FMT or next-generation MCIs) before re-challenging cancer patients with immunotherapy.

Launched in 2025, the Seerave Global OncoBiome Atlas expands the ONCOBIOME initiative from a European-based research consortium into a globally integrated clinical and translational platform (<https://oncobiomeatlas.org>). The Atlas is designed to be an interactive, longitudinal resource that links microbiome profiles, comedication records, and immunotherapy outcomes across international cancer cohorts. Its core mission is to systematically track the use of microbiome-modulating drugs to better understand their real-world effects on the gut ecosystem and treatment efficacy. Thus, whereas ONCOBIOME focuses on defining GOMs and developing MCIs, the Seerave Atlas provides the global infrastructure necessary to validate, refine, and implement those interventions in routine oncology care.

Together, ONCOBIOME and the Seerave Global OncoBiome Atlas create a platform for the prospective validation of microbiome-based tools, like the TOPOSCORE, which will soon be hosted on the Seerave website. The free accessibility of this tool enables clinicians and researchers to investigate the clinical relevance of gut microbial profiles, particularly the number and identity of SIG1 bacteria, for predicting immunotherapy outcomes (Fig. 5). MCIs are increasingly being evaluated in clinical trials worldwide.²³⁶ Now, this partnership will enable investigators to apply tools like the TOPOSCORE prospectively across multiple cancer types to validate their efficacy, better stratify patients, and refine therapeutic strategies.

CONCLUSION

The gut microbiome is a central determinant of immunotherapy efficacy, particularly in patients treated with ICIs. With this understanding, more nuanced models for predicting whether a patient is dysbiotic — and thus less likely to respond to treatment — are incorporating fecal, serological, and taxonomic biomarkers and are continually being developed. Exact definitions for dysbiosis and eubiosis remain elusive. Nonetheless, translational efforts aim to operationalize these findings through dedicated diagnostics, longitudinal monitoring, and targeted interventions, and ongoing clinical trials are directly testing whether MCIs can restore ICI sensitivity in patients.^{88,236} Importantly, these efforts can be translated to other immunotherapies. For example, CAR T-cells,^{237,238} hematopoietic stem cell transplantation,²³⁹ and vaccines²⁴⁴ are all affected by gut microbial composition. Although individual taxa vary across cohorts, three ecological themes recur: preservation of SCFA-producing commensals, containment of oral and BA-modulating pathobionts,

and maintenance of barrier integrity. Framing dysbiosis through these shared functions provides a mechanistic bridge across otherwise disparate taxonomic associations. Moving forward, the integration of microbiome metrics into precision oncology represents a logical and necessary extension of current treatment plans.

REFERENCES

- Bender, M. J. et al. Dietary tryptophan metabolite released by intratumoral *Lactobacillus reuteri* facilitates immune checkpoint inhibitor treatment. *Cell* **186**, 1846–1862.e26 (2023).
- Derosa, L., Routy, B., Kroemer, G. & Zitvogel, L. The intestinal microbiota determines the clinical efficacy of immune checkpoint blockers targeting PD-1/PD-L1. *Oncoimmunology* **7**, e1434468 (2018).
- Derosa, L. et al. Intestinal *Akkermansia muciniphila* predicts clinical response to PD-1 blockade in patients with advanced non-small-cell lung cancer. *Nat. Med.* **28**, 315–324 (2022).
- Fidelle, M. et al. A microbiota-modulated checkpoint directs immunosuppressive intestinal T cells into cancers. *Science* **380**, eabo2296 (2023).
- Gopalakrishnan, V. et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **359**, 97–103 (2018).
- Hakozaki, T. et al. The gut microbiome associates with immune checkpoint inhibition outcomes in patients with advanced non-small cell lung cancer. *Cancer Immunol. Res.* **8**, 1243–1250 (2020).
- Matson, V. et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* **359**, 104–108 (2018).
- Messaoudene, M. et al. The DAV132 colon-targeted adsorbent does not interfere with plasma concentrations of antibiotics but prevents antibiotic-related dysbiosis: a randomized phase I trial in healthy volunteers. *Nat. Commun.* **15**, 8083 (2024).
- Park, J. S. et al. Targeting PD-L2-RGMB overcomes microbiome-related immunotherapy resistance. *Nature* **617**, 377–385 (2023).
- Phelps, C. M. et al. Exercise-induced microbiota metabolite enhances CD8 T cell anti-tumor immunity promoting immunotherapy efficacy. *Cell* **188**, 5680–5700.e28 (2025).
- Pinato, D. J. et al. Antibiotic therapy and outcome from immune-checkpoint inhibitors. *J. Immunother. Cancer* **7**, 287 (2019).
- Routy, B. et al. The gut microbiota influences anticancer immunosurveillance and general health. *Nat. Rev. Clin. Oncol.* **15**, 382–396 (2018).
- Routy, B. et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **359**, 91–97 (2018).
- Shatila, M. et al. Gastrointestinal infection before immune checkpoint inhibition hinders treatment efficacy and increases the risk of colitis. *Cancer Med.* **14**, e71123 (2025).
- Spencer, C. N. et al. Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. *Science* **374**, 1632–1640 (2021).
- Derosa, L. et al. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. *Ann. Oncol.* **29**, 1437–1444 (2018).
- Winter, S. E. & Bäuml, A. J. Gut dysbiosis: Ecological causes and causative effects on human disease. *Proc. Natl. Acad. Sci. USA* **120**, e2316579120 (2023).
- The Human Microbiome Project Consortium Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
- The Integrative HMP (iHMP) Research Network Consortium The integrative human microbiome project. *Nature* **569**, 641–648 (2019).
- Hooks, K. B. & O'Malley, M. A. Dysbiosis and its discontents. *mBio* **8**, e01492–17 (2017).
- Olesen, S. W. & Alm, E. J. Dysbiosis is not an answer. *Nat. Microbiol.* **1**, 16228 (2016).
- Yong, E. There is no 'healthy' microbiome. *The New York Times* (2 November 2014).
- Ponziani, F. R. et al. Gut dysbiosis and fecal calprotectin predict response to immune checkpoint inhibitors in patients with hepatocellular carcinoma. *Hepatol. Commun.* **6**, 1492–1501 (2022).
- Birebent, R. et al. Surrogate markers of intestinal dysfunction associated with survival in advanced cancers. *Oncoimmunology* **14**, 2484880 (2025).
- World Health Organization. *Constitution of the World Health Organization* 1–18 (World Health Organization, 1946).
- López-Otín, C. & Kroemer, G. Hallmarks of health. *Cell* **184**, 33–63 (2021).
- Martino, C. et al. Microbiota succession throughout life from the cradle to the grave. *Nat. Rev. Microbiol.* **20**, 707–720 (2022).
- Gomez de Agüero, M. et al. The maternal microbiota drives early postnatal innate immune development. *Science* **351**, 1296–1302 (2016).
- Thorburn, A. N. et al. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat. Commun.* **6**, 7320 (2015).

30. Rasmussen, M. A. et al. Ecological succession in the vaginal microbiota during pregnancy and birth. *ISME J.* **14**, 2325–2335 (2020).
31. Ardic, C., Usta, O., Omar, E., Yildiz, C. & Memis, E. Caesarean delivery increases the risk of overweight or obesity in 2-year-old children. *J. Obstet. Gynaecol.* **41**, 374–379 (2021).
32. Shao, Y. et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature* **574**, 117–121 (2019).
33. Stokholm, J. et al. Maturation of the gut microbiome and risk of asthma in childhood. *Nat. Commun.* **9**, 141 (2018).
34. Munblit, D., Verhasselt, V. & Warner, J. O. Editorial: human milk composition and health outcomes in children. *Front. Pediatr.* **7**, 319 (2019).
35. Thaïss, C. A. et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* **159**, 514–529 (2014).
36. Thaïss, C. A. et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* **167**, 1495–1510.e12 (2016).
37. Bailey, M. T. et al. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav. Immun.* **25**, 397–407 (2011).
38. Zhao, S. et al. Adaptive evolution within gut microbiomes of healthy people. *Cell Host Microbe* **25**, 656–667.e8 (2019).
39. Biagi, E. et al. Gut microbiota and extreme longevity. *Curr. Biol.* **26**, 1480–1485 (2016).
40. Ghosh, T. S., Shanahan, F. & O'Toole, P. W. The gut microbiome as a modulator of healthy ageing. *Nat. Rev. Gastroenterol. Hepatol.* **19**, 565–584 (2022).
41. Grajeda-Iglesias, C. et al. Oral administration of *Akkermansia muciniphila* elevates systemic antiaging and anticancer metabolites. *Ageing* **13**, 6375–6405 (2021).
42. Pang, S. et al. Longevity of centenarians is reflected by the gut microbiome with youth-associated signatures. *Nat. Aging* **3**, 436–449 (2023).
43. Thomas, A. M. et al. Gut OncoMicrobiome Signatures (GOMS) as next-generation biomarkers for cancer immunotherapy. *Nat. Rev. Clin. Oncol.* **20**, 583–603 (2023).
44. Sinha, T. et al. Analysis of 1135 gut metagenomes identifies sex-specific resistome profiles. *Gut Microbes* **10**, 358–366 (2019).
45. Zhang, X. et al. Sex- and age-related trajectories of the adult human gut microbiota shared across populations of different ethnicities. *Nat. Aging* **1**, 87–100 (2021).
46. Zhernakova, A. et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* **352**, 565–569 (2016).
47. d’Afflitto, M., Upadhyaya, A., Green, A. & Peiris, M. Association between sex hormone levels and gut microbiota composition and diversity—a systematic review. *J. Clin. Gastroenterol.* **56**, 384–392 (2022).
48. Peters, B. A. et al. Menopause is associated with an altered gut microbiome and estrobolome, with implications for adverse cardiometabolic risk in the hispanic community health study/study of Latinos. *mSystems* **7**, e0027322 (2022).
49. Santos-Marcos, J. A. et al. Influence of gender and menopausal status on gut microbiota. *Maturitas* **116**, 43–53 (2018).
50. Zhao, H. et al. Compositional and functional features of the female premenopausal and postmenopausal gut microbiota. *FEBS Lett.* **593**, 2655–2664 (2019).
51. Conforti, F. et al. Sex-based heterogeneity in response to lung cancer immunotherapy: a systematic review and meta-analysis. *J. Natl. Cancer Inst.* **111**, 772–781 (2019).
52. Conforti, F. et al. Cancer immunotherapy efficacy and patients’ sex: a systematic review and meta-analysis. *Lancet Oncol.* **19**, 737–746 (2018).
53. Graham, J., Abdel-Rahman, O., Choueiri, T. K. & Heng, D. Y. C. Re: Fabio Conforti, Laura Pala, Vincenzo Bagnardi, et al. Cancer immunotherapy efficacy and patients’ sex: A systematic review and meta-analysis. *Lancet Oncol* 2018; 19:737–46: Outcomes of metastatic renal cell carcinoma by gender: Contrasting results from the International mRCC Database Consortium. *Eur. Urol.* **74**, e139–e140 (2018).
54. Conforti, F. et al. Reply to Jeffrey Graham, Omar Abdel-Rahman, Toni K. Choueiri, and Daniel Y. C. Heng’s Letter to the Editor re: Fabio Conforti, Laura Pala, Vincenzo Bagnardi, et al. Cancer immunotherapy efficacy and patients’ sex: a systematic review and meta-analysis. *Lancet Oncol* 2018;19:737–746: Outcomes of Metastatic Renal Cell Carcinoma by Gender: Contrasting Results from the International mRCC Database Consortium. *Eur. Urol.* **75**, e34–e35 (2019).
55. Haupt, S., Caramia, F., Klein, S. L., Rubin, J. B. & Haupt, Y. Sex disparities matter in cancer development and therapy. *Nat. Rev. Cancer* **21**, 393–407 (2021).
56. Ye, Y. et al. Sex-associated molecular differences for cancer immunotherapy. *Nat. Commun.* **11**, 1779 (2020).
57. Wallis, C. J. D. et al. Association of patient sex with efficacy of immune checkpoint inhibitors and overall survival in advanced cancers. *JAMA Oncol.* **5**, 529–536 (2019).
58. Gacesa, R. et al. Environmental factors shaping the gut microbiome in a Dutch population. *Nature* **604**, 732–739 (2022).
59. Rothschild, D. et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature* **555**, 210–215 (2018).
60. Turpin, W. et al. Association of host genome with intestinal microbial composition in a large healthy cohort. *Nat. Genet.* **48**, 1413–1417 (2016).
61. Lopera-Maya, E. A. et al. Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project. *Nat. Genet.* **54**, 143–151 (2022).
62. Qin, Y. et al. Combined effects of host genetics and diet on human gut microbiota and incident disease in a single population cohort. *Nat. Genet.* **54**, 134–142 (2022).
63. Kurilshikov, A. et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat. Genet.* **53**, 156–165 (2021).
64. Zhernakova, D. V. et al. Host genetic regulation of human gut microbial structural variation. *Nature* **625**, 813–821 (2024).
65. Goodrich, J. K. et al. Human genetics shape the gut microbiome. *Cell* **159**, 789–799 (2014).
66. Goodrich, J. K. et al. Genetic determinants of the gut microbiome in UK twins. *Cell Host Microbe* **19**, 731–743 (2016).
67. De Filippo, C. et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. USA* **107**, 14691–14696 (2010).
68. Yatsunenko, T. et al. Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227 (2012).
69. Deschasaux, M. et al. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nat. Med.* **24**, 1526–1531 (2018).
70. Falony, G. et al. Population-level analysis of gut microbiome variation. *Science* **352**, 560–564 (2016).
71. He, Y. et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nat. Med.* **24**, 1532–1535 (2018).
72. Mueller, S. et al. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Appl. Environ. Microbiol.* **72**, 1027–1033 (2006).
73. Monda, V. et al. Exercise modifies the gut microbiota with positive health effects. *Oxid. Med. Cell. Longev.* **2017**, 3831972 (2017).
74. Sohail, M. U., Yassine, H. M., Sohail, A. & Thani, A. A. A. Impact of physical exercise on gut microbiome, inflammation, and the pathobiology of metabolic disorders. *Rev. Diabet. Stud.* **15**, 35–48 (2019).
75. Varghese, S., Rao, S., Khattak, A., Zamir, F. & Chauri, A. Physical exercise and the gut microbiome: A bidirectional relationship influencing health and performance. *Nutrients* **16**, 3663 (2024).
76. GBD 2017 Diet Collaborators Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **393**, 1958–1972 (2019).
77. David, L. A. et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2014).
78. Do, M. H., Lee, E., Oh, M. J., Kim, Y. & Park, H. Y. High-glucose or -fructose diet cause changes of the gut microbiota and metabolic disorders in mice without body weight change. *Nutrients* **10**, 761 (2018).
79. Fackelmann, G. et al. Gut microbiome signatures of vegan, vegetarian and omnivore diets and associated health outcomes across 21,561 individuals. *Nat. Microbiol.* **10**, 41–52 (2025).
80. Fajstova, A. et al. Diet rich in simple sugars promotes pro-inflammatory response via gut microbiota alteration and TLR4 signaling. *Cells* **9**, 2701 (2020).
81. de Vos, W. M., Tilg, H., Van Hul, M. & Cani, P. D. Gut microbiome and health: mechanistic insights. *Gut* **71**, 1020–1032 (2022).
82. Oliver, A. et al. High-fiber, whole-food dietary intervention alters the human gut microbiome but not fecal short-chain fatty acids. *mSystems* **6**, e00115–e00121 (2021).
83. Siddiqui, M. T. & Cresci, G. A. M. The immunomodulatory functions of butyrate. *J. Inflamm. Res.* **14**, 6025–6041 (2021).
84. Ferrere, G. et al. Ketogenic diet and ketone bodies enhance the anticancer effects of PD-1 blockade. *JCI Insight* **6**, e145207 (2021).
85. Sofi, F., Cesari, F., Abbate, R., Gensini, G. F. & Casini, A. Adherence to Mediterranean diet and health status: meta-analysis. *BMJ* **337**, a1344 (2008).
86. Wang, D. D. et al. The gut microbiome modulates the protective association between a Mediterranean diet and cardiometabolic disease risk. *Nat. Med.* **27**, 333–343 (2021).
87. Alves Costa Silva, C., Fidelle, M., Almonte, A. A., Derosa, L. & Zitvogel, L. Gut microbiota-related biomarkers in immuno-oncology. *Annu. Rev. Pharm. Toxicol.* **65**, 333–354 (2025).
88. Almonte, A. A., Thomas, S. & Zitvogel, L. Microbiota-centered interventions to boost immune checkpoint blockade therapies. *J. Exp. Med.* **222**, e20250378 (2025).
89. Rousseau, A. et al. Concomitant comedications and survival with first-line pembrolizumab in advanced non-small-cell lung cancer. *JAMA Netw. Open* **8**, e2529225 (2025).
90. Montégut, L. et al. Benzodiazepines interfere with the efficacy of pembrolizumab-based cancer immunotherapy. Results of a nationwide cohort

- study including over 50,000 participants with advanced lung cancer. *Oncoimmunology* **14**, 2528955 (2025).
91. Vich Vila, A. et al. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat. Commun.* **11**, 362 (2020).
 92. Bavishi, C. & Dupont, H. L. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. *Aliment. Pharm. Ther.* **34**, 1269–1281 (2011).
 93. Clooney, A. G. et al. A comparison of the gut microbiome between long-term users and non-users of proton pump inhibitors. *Aliment. Pharm. Ther.* **43**, 974–984 (2016).
 94. Jackson, M. A. et al. Proton pump inhibitors alter the composition of the gut microbiota. *Gut* **65**, 749–756 (2016).
 95. Lin, Y. T. et al. Anti-acid drug treatment induces changes in the gut microbiome composition of hemodialysis patients. *Microorganisms* **9**, 286 (2021).
 96. Seto, C. T., Jeraldo, P., Orenstein, R., Chia, N. & DiBaise, J. K. Prolonged use of a proton pump inhibitor reduces microbial diversity: implications for *Clostridium difficile* susceptibility. *Microbiome* **2**, 42 (2014).
 97. Singh, G. et al. The effect of gastric acid suppression on probiotic colonization in a double blinded randomized clinical trial. *Clin. Nutr. ESPEN* **47**, 70–77 (2022).
 98. Palleja, A. et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nat. Microbiol.* **3**, 1255–1265 (2018).
 99. Aasmets, O. et al. A hidden confounder for microbiome studies: medications used years before sample collection. *mSystems* **10**, e00541–25 (2025).
 100. Ricaurte, D. et al. High-throughput transcriptomics of 409 bacteria-drug pairs reveals drivers of gut microbiota perturbation. *Nat. Microbiol.* **9**, 561–575 (2024).
 101. Algavi, Y. M. & Borenstein, E. A data-driven approach for predicting the impact of drugs on the human microbiome. *Nat. Commun.* **14**, 3614 (2023).
 102. Nagata, N. et al. Population-level metagenomics uncovers distinct effects of multiple medications on the human gut microbiome. *Gastroenterology* **163**, 1038–1052 (2022).
 103. Ticinesi, A. et al. Gut microbiota composition is associated with polypharmacy in elderly hospitalized patients. *Sci. Rep.* **7**, 11102 (2017).
 104. Elkrief, A. et al. Antibiotics are associated with worse outcomes in lung cancer patients treated with chemotherapy and immunotherapy. *NPJ Precis. Oncol.* **8**, 143 (2024).
 105. Lopes, S. et al. Do proton pump inhibitors alter the response to immune checkpoint inhibitors in cancer patients? A meta-analysis. *Front. Immunol.* **14**, 1070076 (2023).
 106. Montégut, L. et al. Benzodiazepines compromise the outcome of cancer immunotherapy. *Oncoimmunology* **13**, 2413719 (2024).
 107. Ley, R. E., Turnbaugh, P. J., Klein, S. & Gordon, J. I. Microbial ecology: human gut microbes associated with obesity. *Nature* **444**, 1022–1023 (2006).
 108. Hsu, C. L. & Schnabl, B. The gut-liver axis and gut microbiota in health and liver disease. *Nat. Rev. Microbiol.* **21**, 719–733 (2023).
 109. Crudele, L., Gadaleta, R. M., Cariello, M. & Moschetta, A. Gut microbiota in the pathogenesis and therapeutic approaches of diabetes. *EBioMedicine* **97**, 104821 (2023).
 110. Iliev, I. D., Ananthakrishnan, A. N. & Guo, C. J. Microbiota in inflammatory bowel disease: mechanisms of disease and therapeutic opportunities. *Nat. Rev. Microbiol.* **23**, 509–524 (2025).
 111. Masenga, S. K. et al. Recent advances in modulation of cardiovascular diseases by the gut microbiota. *J. Hum. Hypertens.* **36**, 952–959 (2022).
 112. Bindels, L. B. et al. Increased gut permeability in cancer cachexia: mechanisms and clinical relevance. *Oncotarget* **9**, 18224–18238 (2018).
 113. Pötgens, S. A. et al. *Klebsiella oxytoca* expands in cancer cachexia and acts as a gut pathobiont contributing to intestinal dysfunction. *Sci. Rep.* **8**, 12321 (2018).
 114. Yonekura, S. et al. Cancer induces a stress ileopathy depending on β -adrenergic receptors and promoting dysbiosis that contributes to carcinogenesis. *Cancer Discov.* **12**, 1128–1151 (2022).
 115. Kim, H. C. et al. Gut microbiota dysbiosis induced by brain tumors modulates the efficacy of immunotherapy. *Cell Rep.* **44**, 115825 (2025).
 116. Valles-Colomer, M. et al. Neuroblastoma is associated with alterations in gut microbiome composition subsequent to maternal microbial seeding. *EBioMedicine* **99**, 104917 (2024).
 117. Kokolus, K. M. et al. Beta blocker use correlates with better overall survival in metastatic melanoma patients and improves the efficacy of immunotherapies in mice. *Oncoimmunology* **7**, e1405205 (2018).
 118. Wangel, A. G. & Deller, D. J. Malabsorption syndrome associated with carcinoma of the bronchus. *Gut* **6**, 73–76 (1965).
 119. Jukic, A., Bakiri, L., Wagner, E. F., Tilg, H. & Adolph, T. E. Calprotectin: from biomarker to biological function. *Gut* **70**, 1978–1988 (2021).
 120. Lanas, Á et al. Fecal occult blood and calprotectin testing to prioritize primary care patients for colonoscopy referral: the advantage study. *United Eur. Gastroenterol. J.* **11**, 692–699 (2023).
 121. Bromke, M. A., Neubauer, K., Kempniński, R. & Krzystek-Korpacka, M. Faecal calprotectin in assessment of mucosal healing in adults with inflammatory bowel disease: a meta-analysis. *J. Clin. Med.* **10**, 2203 (2021).
 122. Heinzl, S. et al. Elevated fecal calprotectin is associated with gut microbial dysbiosis, altered serum markers and clinical outcomes in older individuals. *Sci. Rep.* **14**, 13513 (2024).
 123. Rocha, I. M. G. D. et al. Pro-inflammatory diet is correlated with high *Veillonella rogosae*, gut inflammation and clinical relapse of inflammatory bowel disease. *Nutrients* **15**, 4148 (2023).
 124. Lu, F., Inoue, K., Kato, J., Minamishima, S. & Morisaki, H. Functions and regulation of lipocalin-2 in gut-origin sepsis: a narrative review. *Crit. Care* **23**, 269 (2019).
 125. Sugi, K., Saitoh, O., Hirata, I. & Katsu, K. Fecal lactoferrin as a marker for disease activity in inflammatory bowel disease: comparison with other neutrophil-derived proteins. *Am. J. Gastroenterol.* **91**, 927–934 (1996).
 126. Bachem, A. et al. Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated CD8+ T cells. *Immunity* **51**, 285–297.e5 (2019).
 127. Zhu, X. et al. Microbial metabolite butyrate promotes anti-PD-1 antitumor efficacy by modulating T cell receptor signaling of cytotoxic CD8 T cell. *Gut Microbes* **15**, 2249143 (2023).
 128. Alvandi, E., Wong, W. K. M., Joglekar, M. V., Spring, K. J. & Hardikar, A. A. Short-chain fatty acid concentrations in the incidence and risk-stratification of colorectal cancer: a systematic review and meta-analysis. *BMC Med.* **20**, 323 (2022).
 129. Parada Venegas, D. et al. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front. Immunol.* **10**, 277 (2019).
 130. Lee, M. H. et al. How bile acids and the microbiota interact to shape host immunity. *Nat. Rev. Immunol.* **24**, 798–809 (2024).
 131. Casado-Bedmar, M. & Viennois, E. MicroRNA and gut microbiota: Tiny but mighty—novel insights into their cross-talk in inflammatory bowel disease pathogenesis and therapeutics. *J. Crohns Colitis* **16**, 992–1005 (2021).
 132. McCulloch, J. A. et al. Intestinal microbiota signatures of clinical response and immune-related adverse events in melanoma patients treated with anti-PD-1. *Nat. Med.* **28**, 545–556 (2022).
 133. Marino, M. et al. Serum zonulin and colorectal cancer risk. *Sci. Rep.* **14**, 28171 (2024).
 134. Fux, A. C. et al. Heterogeneity of lipopolysaccharide as source of variability in bioassays and LPS-binding proteins as remedy. *Int. J. Mol. Sci.* **24**, 8395 (2023).
 135. Sardar, P. et al. Gut microbiota-derived hexa-acylated lipopolysaccharides enhance cancer immunotherapy responses. *Nat. Microbiol.* **10**, 795–807 (2025).
 136. Sun, L. et al. *Akkermansia muciniphila*-derived hypoacylated rough-type lipopolysaccharides alleviate diet-induced obesity via activation of TLR4-IL-23-IL-22 immune axis. *Imeta* **4**, e70066 (2025).
 137. Garcia-Vello, P. et al. The lipooligosaccharide of the gut symbiont *Akkermansia muciniphila* exhibits a remarkable structure and TLR signaling capacity. *Nat. Commun.* **15**, 8411 (2024).
 138. Duerr, C. U. et al. O-antigen delays lipopolysaccharide recognition and impairs antibacterial host defense in murine intestinal epithelial cells. *PLoS Pathog.* **5**, e1000567 (2009).
 139. Koutsounas, I., Kaltsa, G., Siakavellas, S. I. & Bamias, G. Markers of bacterial translocation in end-stage liver disease. *World J. Hepatol.* **7**, 2264–2273 (2015).
 140. Tabung, F. K. et al. Influence of dietary patterns on plasma soluble CD14, a surrogate marker of gut barrier dysfunction. *Curr. Dev. Nutr.* **1**, e001396 (2017).
 141. Moreno-Navarrete, J. M. et al. Circulating lipopolysaccharide-binding protein (LBP) as a marker of obesity-related insulin resistance. *Int. J. Obes.* **36**, 1442–1449 (2012).
 142. Liang, H., Hussey, S. E., Sanchez-Avila, A., Tantiwong, P. & Musi, N. Effect of lipopolysaccharide on inflammation and insulin action in human muscle. *PLoS One* **8**, e63983 (2013).
 143. Fedirko, V. et al. Exposure to bacterial products lipopolysaccharide and flagellin and hepatocellular carcinoma: a nested case-control study. *BMC Med.* **15**, 72 (2017).
 144. Zhao, S. et al. *Akkermansia muciniphila* improves metabolic profiles by reducing inflammation in chow diet-fed mice. *J. Mol. Endocrinol.* **58**, 1–14 (2017).
 145. Marcos-Kovandzic, L. et al. Gut microbiota modulation through *Akkermansia* spp. supplementation increases CAR T-cell potency. *Cancer Discov.* **15**, 1905–1926 (2025).
 146. Wu, G. et al. A core microbiome signature as an indicator of health. *Cell* **187**, 6550–6565.e11 (2024).
 147. Gamrath, L. et al. Role of the microbiome and diet for response to cancer checkpoint immunotherapy: a narrative review of clinical trials. *Curr. Oncol. Rep.* **27**, 45–58 (2025).
 148. Yang, Y. et al. Gut microbiota and SCFAs improve the treatment efficacy of chemotherapy and immunotherapy in NSCLC. *NPJ Biofilms Microbiomes* **11**, 146 (2025).
 149. Murayama, M. et al. Isobutyric acid enhances the anti-tumor effect of anti-PD-1 antibody. *Sci. Rep.* **14**, 11325 (2024).

150. Kang, X. et al. Roseburia intestinalis generated butyrate boosts anti-PD-1 efficacy in colorectal cancer by activating cytotoxic CD8+ T cells. *Gut* **72**, 2112–2122 (2023).
151. Coutzac, C. et al. Systemic short chain fatty acids limit antitumor effect of CTLA-4 blockade in hosts with cancer. *Nat. Commun.* **11**, 2168 (2020).
152. Zhu, R., Gu, S., Tao, Y. & Zhang, Y. Butyrate confers colorectal cancer cell resistance to anti-PD-1 therapy by promoting CPT1A-mediated fatty acid oxidation. *Discov. Oncol.* **16**, 935 (2025).
153. Chelvanambi, M. & Wargo, J. A. MAdCAM-1: a newly identified microbial “gut check” for T cells. *Trends Immunol.* **44**, 568–570 (2023).
154. Saliby, R. M. et al. Gut-associated checkpoint as a prognostic biomarker in metastatic renal cell carcinoma (mRCC): results from a randomized first-line clinical trial. *J. Clin. Oncol.* **43**, 4512 (2025).
155. Agus, A., Planchais, J. & Sokol, H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* **23**, 716–724 (2018).
156. Janeiro, M. H., Ramírez, M. J., Milagro, F. I., Martínez, J. A. & Solas, M. Implication of trimethylamine N-oxide (TMAO) in disease: Potential biomarker or new therapeutic target. *Nutrients* **10**, 1398 (2018).
157. Tang, W. H. W. & Hazen, S. L. Microbiome, trimethylamine N-oxide, and cardiometabolic disease. *Transl. Res.* **179**, 108–115 (2017).
158. Lee, J. et al. Antibiotic-induced intestinal microbiota depletion can attenuate the acute kidney injury to chronic kidney disease transition via NADPH oxidase 2 and trimethylamine-N-oxide inhibition. *Kidney Int.* **105**, 1239–1253 (2024).
159. Wang, H. et al. The microbial metabolite trimethylamine N-oxide promotes anti-tumor immunity in triple-negative breast cancer. *Cell Metab.* **34**, 581–594.e8 (2022).
160. Mirji, G. et al. The microbiome-derived metabolite TMAO drives immune activation and boosts responses to immune checkpoint blockade in pancreatic cancer. *Sci. Immunol.* **7**, eabn0704 (2022).
161. Gupta, V. K. et al. A predictive index for health status using species-level gut microbiome profiling. *Nat. Commun.* **11**, 4635 (2020).
162. Zitvogel, L. & Kroemer, G. Cancer and the metaorganism. *Cancer Discov.* **14**, 658–662 (2024).
163. Manor, O. et al. Health and disease markers correlate with gut microbiome composition across thousands of people. *Nat. Commun.* **11**, 5206 (2020).
164. Duvall, C., Gibbons, S. M., Gurry, T., Irizarry, R. A. & Alm, E. J. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. *Nat. Commun.* **8**, 1784 (2017).
165. Turnbaugh, P. J. et al. A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484 (2009).
166. Matsuoka, K. & Kanai, T. The gut microbiota and inflammatory bowel disease. *Semin Immunopathol.* **37**, 47–55 (2015).
167. Walters, W. A., Xu, Z. & Knight, R. Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Lett.* **588**, 4223–4233 (2014).
168. Arumugam, M. et al. Enterotypes of the human gut microbiome. *Nature* **473**, 174–180 (2011).
169. Cai, J. A. et al. Gut microbiota enterotypes mediate the effects of dietary patterns on colorectal neoplasm risk in a Chinese population. *Nutrients* **15**, 2940 (2023).
170. Kovatcheva-Datchary, P. et al. Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of *Prevotella*. *Cell Metab.* **22**, 971–982 (2015).
171. Thomas, A. M. et al. Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. *Nat. Med.* **25**, 667–678 (2019).
172. Park, S., Zhang, T. & Kang, S. Fecal microbiota composition, their interactions, and metagenome function in US adults with type 2 diabetes according to enterotypes. *Int. J. Mol. Sci.* **24**, 9533 (2023).
173. Wang, J. et al. Enterotype bacteroides is associated with a high risk in patients with diabetes: a pilot study. *J. Diabetes Res.* **2020**, 6047145 (2020).
174. Lee, S. et al. Different reactions in each enterotype depending on the intake of probiotic yogurt powder. *Microorganisms* **9**, 1277 (2021).
175. Heintz-Buschart, A. et al. Integrated multi-omics of the human gut microbiome in a case study of familial type 1 diabetes. *Nat. Microbiol.* **2**, 16180 (2016).
176. Xiao, L., Wang, J., Zheng, J., Li, X. & Zhao, F. Deterministic transition of enterotypes shapes the infant gut microbiome at an early age. *Genome Biol.* **22**, 243 (2021).
177. Costea, P. I. et al. Enterotypes in the landscape of gut microbial community composition. *Nat. Microbiol.* **3**, 8–16 (2018).
178. Koren, O. et al. A guide to enterotypes across the human body: meta-analysis of microbial community structures in human microbiome datasets. *PLoS Comput. Biol.* **9**, e1002863 (2013).
179. Rivas-Santisteban, J. et al. Quantifying microbial guilds. *ISME Commun.* **4**, ycae042 (2024).
180. Wu, G., Zhao, N., Zhang, C., Lam, Y. Y. & Zhao, L. Guild-based analysis for understanding gut microbiome in human health and diseases. *Genome Med.* **13**, 22 (2021).
181. Zhao, L., Wu, G. & Zhao, N. Guild-based approach for mitigating information loss and distortion issues in microbiome analysis. *J. Clin. Invest.* **134**, e185395 (2024).
182. Guo, M. et al. Guild-level microbiome signature associated with COVID-19 severity and prognosis. *mBio* **14**, e0351922 (2023).
183. Zhao, L. et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science* **359**, 1151–1156 (2018).
184. Clavijo-Salomon, M. A. & Trinchieri, G. Unlocking the power of the microbiome for successful cancer immunotherapy. *J. Immunother. Cancer* **13**, e011281 (2025).
185. Tang, S. et al. Guild-level signature of gut microbiome for diabetic kidney disease. *mBio* **15**, e0073524 (2024).
186. Jia, L. et al. A transmissible $\gamma\delta$ intraepithelial lymphocyte hyperproliferative phenotype is associated with the intestinal microbiota and confers protection against acute infection. *Muc. Immunol.* **15**, 772–782 (2022).
187. Derosa, L. et al. Microbiota-centered interventions: the next breakthrough in immuno-oncology? *Cancer Discov.* **11**, 2396–2412 (2021).
188. Goel, A. et al. Toward a health-associated core keystone index for the human gut microbiome. *Cell Rep.* **44**, 115378 (2025).
189. Macandog, A. D. G. et al. Longitudinal analysis of the gut microbiota during anti-PD-1 therapy reveals stable microbial features of response in melanoma patients. *Cell Host Microbe* **32**, 2004–2018.e9 (2024).
190. Björk, J. R. et al. Longitudinal gut microbiome changes in immune checkpoint blockade-treated advanced melanoma. *Nat. Med.* **30**, 785–796 (2024).
191. Ferrari, V. et al. Sensitizing cancer cells to immune checkpoint inhibitors by microbiota-mediated upregulation of HLA class I. *Cancer Cell* **41**, 1717–1730.e4 (2023).
192. Rice, T. A. et al. Interspecies commensal interactions have nonlinear impacts on host immunity. *Cell Host Microbe* **30**, 988–1002.e6 (2022).
193. Bajaj, J. S. et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J. Hepatol.* **60**, 940–947 (2014).
194. Casén, C. et al. Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. *Aliment Pharm. Ther.* **42**, 71–83 (2015).
195. Lloyd-Price, J. et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* **569**, 655–662 (2019).
196. Saffouri, G. B. et al. Small intestinal microbial dysbiosis underlies symptoms associated with functional gastrointestinal disorders. *Nat. Commun.* **10**, 2012 (2019).
197. Guo, Z. et al. Intestinal microbiota distinguish gout patients from healthy humans. *Sci. Rep.* **6**, 20602 (2016).
198. Mayerhofer, C. C. K. et al. Low fibre intake is associated with gut microbiota alterations in chronic heart failure. *ESC Heart Fail.* **7**, 456–466 (2020).
199. Xia, G. H. et al. Stroke Dysbiosis Index (SDI) in gut microbiome are associated with brain injury and prognosis of stroke. *Front. Neurol.* **10**, 397 (2019).
200. Derosa, L. et al. Custom scoring based on ecological topology of gut microbiota associated with cancer immunotherapy outcome. *Cell* **187**, 3373–3389.e16 (2024).
201. Nakatsu, G. et al. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat. Commun.* **6**, 8727 (2015).
202. Chang, D. et al. Gut Microbiome Wellness Index 2 enhances health status prediction from gut microbiome taxonomic profiles. *Nat. Commun.* **15**, 7447 (2024).
203. Valdés-Mas, R. et al. Metagenome-informed metaproteomics of the human gut microbiome, host, and dietary exposome uncovers signatures of health and inflammatory bowel disease. *Cell* **188**, 1062–1083.e36 (2025).
204. Wei, S., Bahl, M. I., Baunwall, S. M. D., Hvas, C. L. & Licht, T. R. Determining gut microbial dysbiosis: a review of applied indexes for assessment of intestinal microbiota imbalances. *Appl. Environ. Microbiol.* **87**, e00395–21 (2021).
205. Gevers, D. et al. The treatment-naïve microbiome in new-onset Crohn’s disease. *Cell Host Microbe* **15**, 382–392 (2014).
206. Duncan, S. H., Barcenilla, A., Stewart, C. S., Pryde, S. E. & Flint, H. J. Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Appl. Environ. Microbiol.* **68**, 5186–5190 (2002).
207. Zitvogel, L. et al. Impact of the ONCOBIOME network in cancer microbiome research. *Nat. Med.* **31**, 1085–1098 (2025).
208. Elkrief, A. et al. The gut microbiome as a target in cancer immunotherapy: opportunities and challenges for drug development. *Nat. Rev. Drug Discov.* **24**, 685–704 (2025).
209. Vandeputte, D. et al. Quantitative microbiome profiling links gut community variation to microbial load. *Nature* **551**, 507–511 (2017).
210. Tito, R. Y. et al. Microbiome confounders and quantitative profiling challenge predicted microbial targets in colorectal cancer development. *Nat. Med.* **30**, 1339–1348 (2024).
211. Vieira-Silva, S. et al. Quantitative microbiome profiling disentangles inflammation- and bile duct obstruction-associated microbiota alterations across PSC/IBD diagnoses. *Nat. Microbiol.* **4**, 1826–1831 (2019).

212. Wagner, S. et al. Absolute abundance calculation enhances the significance of microbiome data in antibiotic treatment studies. *Front. Microbiol.* **16**, 1481197 (2025).
213. Nishijima, S. et al. Fecal microbial load is a major determinant of gut microbiome variation and a confounder for disease associations. *Cell* **188**, 222–236.e15 (2025).
214. Blanco-Míguez, A. et al. Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlan 4. *Nat. Biotechnol.* **41**, 1633–1644 (2023).
215. Martín, R. et al. Faecalibacterium: a bacterial genus with promising human health applications. *FEMS Microbiol. Rev.* **47**, fuad039 (2023).
216. Lu, H. et al. Butyrate-producing *Eubacterium rectale* suppresses lymphomagenesis by alleviating the TNF-induced TLR4/MyD88/NF- κ B axis. *Cell Host Microbe* **30**, 1139–1150.e7 (2022).
217. Notting, F., Pirovano, W., Sybesma, W. & Kort, R. The butyrate-producing and spore-forming bacterial genus Coprococcus as a potential biomarker for neurological disorders. *Gut Microbiome* **4**, e16 (2023).
218. Scott, K. P. et al. Substrate-driven gene expression in *Roseburia inulinivorans*: Importance of inducible enzymes in the utilization of inulin and starch. *Proc. Natl. Acad. Sci. USA* **108**, 4672–4679 (2011).
219. Vital, M., Karch, A. & Pieper, D. H. Colonic butyrate-producing communities in humans: an overview using omics data. *mSystems* **2**, e00130–17 (2017).
220. Kim, C. H. Complex regulatory effects of gut microbial short-chain fatty acids on immune tolerance and autoimmunity. *Cell Mol. Immunol.* **20**, 341–350 (2023).
221. Litvak, Y., Byndloss, M. X. & Bäuml, A. J. Colonocyte metabolism shapes the gut microbiota. *Science* **362**, eaat9076 (2018).
222. Zhu, Z. et al. Microbiome and spatially resolved metabolomics analysis reveal the anticancer role of gut *Akkermansia muciniphila* by crosstalk with intratumoral microbiota and reprogramming tumoral metabolism in mice. *Gut Microbes* **15**, 2166700 (2023).
223. Zhu, Z. et al. Landscape of tumoral ecosystem for enhanced anti-PD-1 immunotherapy by gut *Akkermansia muciniphila*. *Cell Rep.* **43**, 114306 (2024).
224. Pasolli, E. et al. Accessible, curated metagenomic data through ExperimentHub. *Nat. Methods* **14**, 1023–1024 (2017).
225. Antoniotti, C. et al. AtezotRIBE: a randomised phase II study of FOLFOXIRI plus bevacizumab alone or in combination with atezolizumab as initial therapy for patients with unresectable metastatic colorectal cancer. *BMC Cancer* **20**, 683 (2020).
226. Antoniotti, C. et al. Upfront FOLFOXIRI plus bevacizumab with or without atezolizumab in the treatment of patients with metastatic colorectal cancer (AtezotRIBE): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet Oncol.* **23**, 876–887 (2022).
227. Antoniotti, C. et al. Upfront fluorouracil, leucovorin, oxaliplatin, and irinotecan plus bevacizumab with or without atezolizumab for patients with metastatic colorectal cancer: Updated and overall survival results of the ATEZOTRIBE study. *J. Clin. Oncol.* **42**, 2637–2644 (2024).
228. Watson, A. R. et al. Metabolic independence drives gut microbial colonization and resilience in health and disease. *Genome Biol.* **24**, 78 (2023).
229. Routy, B. et al. Fecal microbiota transplantation plus anti-PD-1 immunotherapy in advanced melanoma: a phase I trial. *Nat. Med.* **29**, 2121–2132 (2023).
230. Schmidt, T. S. B. et al. Drivers and determinants of strain dynamics following fecal microbiota transplantation. *Nat. Med.* **28**, 1902–1912 (2022).
231. Baruch, E. N. et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* **371**, 602–609 (2021).
232. Davar, D. et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* **371**, 595–602 (2021).
233. Elkrief, A. et al. Immune-related colitis is associated with fecal microbial dysbiosis and can be mitigated by fecal microbiota transplantation. *Cancer Immunol. Res.* **12**, 308–321 (2024).
234. Ianiro, G. et al. Variability of strain engraftment and predictability of microbiome composition after fecal microbiota transplantation across different diseases. *Nat. Med.* **28**, 1913–1923 (2022).
235. Elkrief, A. & Routy, B. First clinical proof-of-concept that FMT can overcome resistance to ICIs. *Nat. Rev. Clin. Oncol.* **18**, 325–326 (2021).
236. Elkrief, A. et al. Gut microbiota in immuno-oncology: a practical guide for medical oncologists with a focus on antibiotics stewardship. *Am. Soc. Clin. Oncol. Educ. Book* **45**, e472902 (2025).
237. Stein-Thoeringer, C. K. et al. A non-antibiotic-disrupted gut microbiome is associated with clinical responses to CD19-CAR-T cell cancer immunotherapy. *Nat. Med.* **29**, 906–916 (2023).
238. Smith, M. et al. Gut microbiome correlates of response and toxicity following anti-CD19 CAR T cell therapy. *Nat. Med.* **28**, 713–723 (2022).
239. Chang, C.-C., Hayase, E. & Jenq, R. R. The role of microbiota in allogeneic hematopoietic stem cell transplantation. *Expert Opin. Biol. Ther.* **21**, 1121–1131 (2021).
240. Varanasi, S. K. et al. Bile acid synthesis impedes tumor-specific T cell responses during liver cancer. *Science* **387**, 192–201 (2025).
241. Bertocchi, A. et al. Gut vascular barrier impairment leads to intestinal bacteria dissemination and colorectal cancer metastasis to liver. *Cancer Cell* **39**, 708–724.e11 (2021).
242. Galván-Peña, S., Zhu, Y., Hanna, B. S., Mathis, D. & Benoist, C. A dynamic atlas of immunocyte migration from the gut. *Sci. Immunol.* **9**, eadi0672 (2024).
243. Di Luccia, B. et al. TREM2 deficiency reprograms intestinal macrophages and microbiota to enhance anti-PD-1 tumor immunotherapy. *Sci. Immunol.* **9**, eadi5374 (2024).
244. Carrier, A. et al. Gut microbiota: a new factor modulating the immunizing potential of viral and cancer vaccines. *Res. Sq.* <https://doi.org/10.21203/rs.3.rs-4294379/v1> (2025).

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AUTHOR CONTRIBUTIONS

A.A.A. and S.T. conducted the literature research and prepared the initial draft. A.A.A. wrote the final manuscript, performed the analyses for Fig. 5 and Supplementary information, Figs. S1, S2, and generated Figs. 1–3, 5, S1, S2. V.I. performed the analyses for Fig. 4. G.K. provided substantive revisions and critical feedback on the initial draft. L.D. and L.Z. jointly supervised the project and corrected the manuscript.

COMPETING INTERESTS

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