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Review

Microbiota-derived metabolites: Key modulators of cancer immunotherapies

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SUMMARY

The human gut microbiome shapes local and systemic immune responses and influences cancer immunotherapy outcomes. Microbial metabolites, including short-chain and branched-chain fatty acids, bile acids, tryptophan derivatives, and others, influence anti-tumor immunity by modulating immune cells, tumor growth, and the tumor microenvironment. These metabolites impact the efficacy of immune checkpoint inhibitors, allogeneic stem cell transplantation, chimeric antigen receptor T cell therapies, and immune-related adverse events. However, interindividual microbiome variability, antibiotic exposure, and the context-dependent pro- and anti-inflammatory effects of metabolites present significant challenges for clinical translation. Microbiome-based therapies, including live biotherapeutic products, dietary modifications (such as prebiotics), and synthetic metabolite compounds (postbiotics), are being developed for use in combination with immunotherapy. This review outlines how metabolites influence immunotherapy outcomes and discusses translational approaches to harness them for clinical practice. Future research should focus on validating metabolite-based biomarkers and tailoring metabolite-based interventions to enhance efficacy and reduce toxicity across different immunotherapies.

INTRODUCTION

The human gut microbiome—the entirety of microorganisms inhabiting the human gut including bacteria, archaea, viruses, fungi, and other microbes—their genetic material, and the interactions among these organisms and with their surrounding environment have been associated with cancer initiation, progression, and immunotherapy outcomes. Gut dysbiosis, characterized by a loss of microbial diversity and domination by pathobionts, was associated with resistance to immune checkpoint inhibitors (ICIs) in patients with advanced melanoma and other solid tumors.[1] Reduced intestinal bacterial diversity was correlated with worse overall survival (OS) after allogeneic stem cell transplantation (allo-SCT) and an increased incidence of graft-versus-host disease (GVHD).[2] The loss of even a single species can have devastating effects on GVHD-related mortality, as evident for the protective *Blautia* genus.[3] These insights, combined with the microbiome's ability to promote carcinogenic pathways and interact with established cancer hallmarks, such as inflammation and immune evasion, led to its inclusion as a new "Hallmark of Cancer" in 2022.[4]

Recent preclinical and clinical studies indicate that microbiota-derived immunomodulatory metabolites (IMMs), including short-chain fatty acids (SCFAs), branched-chain fatty acids (BCFAs), bile acids (BAs), and indoles, can promote anti-tumor immune responses, modulate regulatory T (T_{reg}) cells,[5,6] and maintain intestinal homeostasis.[7] They act locally in the gut or cross the intestinal barrier via active transport, passive diffusion, or immune-cell-mediated translocation, influencing dendritic cell (DC) activation, T cell differentiation, and cytokine production, shaping the tumor microenvironment (TME) and affecting tumor growth.[8,9] Specific metabolites enhance the efficacy of ICIs by promoting antigen presentation and T cell infiltration, whereas others contribute to immune evasion by inducing T_{reg} cells or suppressing inflammation. Metabolites can function as substrates in various metabolic reactions and as ligands in signaling pathways.[10] The primary metabolites in each class, along with the key mechanisms by which they contribute to anti-tumor immunity, are summarized in Table 1.

Given the expanding use of ICIs, chimeric antigen receptor T cell (CAR-T cell) therapies, and allo-SCT in oncology, along with the ability of metabolites to modulate immune responses, identifying novel IMMs and understanding how they influence treatment outcomes, has immediate clinical relevance. Insights into the gut microbiome metabolism could inform biomarker-guided patient stratification, reduce immune-related toxicities, and support metabolite-based adjuvant strategies. As fecal and plasma metabolomics enter clinical trials, this research is

Table 1. Classes of microbial metabolites, their sources, and mechanisms

Metabolite	Prebiotics	Properties	Key mechanisms	References
Short-chain fatty acids (e.g., acetic acid, propionic acid, butyric acid)	fiber-rich diet	anti-tumorigenic	HDAC inhibition, GPCR signaling, ID2-mediated IL-12 signaling → $CD8^+$ memory T cell expansion, alternative energy source in TME, T_{reg} induction	Furusawa et al.,[11] Bachem et al.,[12] He et al.[13]
Branched-chain fatty acids (e.g., isobutyric acid, isovaleric acid)	amino acid-rich diet (leucine, isoleucine, valine)	ambivalent	NF-κB inhibition → suppression of inflammation, intestinal epithelial integrity → isovaleric acid: cancer progression in CRC	Rios-Covian et al.,[14] Ezzine et al.,[15] Yachida et al.[16]
Kynurenine	tryptophan	pro-tumorigenic	directly and indirectly (via depletion of essential tryptophan by IDO) → T cell dysfunction	Liu et al.,[17] Siska et al.,[18] Fujiwara et al.[19]
Indoles	tryptophan	anti-inflammatory, anti-tumorigenic	AHR signaling, modulation of the IL-22 and IL-10 axis, type I interferon signaling → improved T_{reg} function and differentiation, organ homeostasis, prevention of autoimmunity and hyperinflammation → indole-3-carboxyaldehyde (ICA): reduction of IDO activity; act via IFN-I signaling to protect and repair the mucosal barrier from damage	Hubbard et al.,[20] Zelante et al.,[21] Swimm et al.,[22] Bek et al.,[23] Heidegger et al.,[24,25] Fong et al.[26]
Bile acids (cholic acid derivates)	cholesterol, primary BA	anti-tumorigenic, anti-inflammatory	FXR signaling, TGR5 signaling → IFN-I and IFN-III expression, activation of IFN-stimulated genes, improved cancer immunosurveillance → RORγt suppression, Foxp3: Th17↓, T_{reg}↑	Wahlstrom et al.,[27] Lindner et al.,[28] Hu et al.,[29] Grau et al.,[30] Winkler et al.,[31] Zitvogel et al.,[32] Hang et al.,[33] Paik et al.,[34] Campbell et al.[35]
Desaminotyrosine (DAT)	flavonoids (e.g., tea, wine, citrus fruits)	anti-tumorigenic	amplification of IFN-I signaling → expansion of antigen-specific T cells, improved anti-tumor and anti-viral activity	Chun et al.,[36] Steed et al.,[37] Joachim et al.[38]

(*Continued on next page*)

Table 1. ***Continued***

Metabolite	Prebiotics	Properties	Key mechanisms	References
Urolithin A	ellagitannin, ellagic acid (e.g., pomegranates, berries, walnuts)	anti-tumorigenic	mitophagy → augmented Wnt signaling → proliferation of T memory stem cells TME modification: reduced M2 macrophages, increased MHC-II expression, infiltration with central memory and naive $CD4^+$ and $CD8^+$ T cells	Cerdá et al.,[39–41] Selma et al.,[42] Tomas-Barberan et al.,[43] Cortes-Martin et al.,[44] Luan et al.,[45] Denk et al.,[46] Verma et al.,[47] Ginefra et al.,[48] Mehra et al.[49]
Trimethylamine *N*-oxide (TMAO)	choline, L-carnitine (e.g., squid, shellfish)	anti-tumorigenic	enhanced NLRP3 inflammasome, potentiation of IFN-I responsive regulators → upregulation of M1 macrophages, promotion of Th1 and Th17 differentiation, shift from T_{reg} to $CD8^+$ effector T cells	Li et al.,[50] Craciun and Balskus,[57] Wang et al.,[58] Lang et al.,[59] al-Waiz et al.,[51] Mirji et al.,[52] Wu et al.[53]
Inosine	NA (microbiome-mediated release from epithelial gut cells)	anti-tumorigenic	A2AR binding → expression of IL12Rβ2 and IFN-γ, alternative energy source for $CD8^+$ T cells, increased tumor antigen presentation	Brown et al.,[54] Mager et al.,[55] Allard et al.,[56] Wang et al.[57]

HDAC, histone deacetylase; GPCR, G-protein-coupled receptor; ID2, inhibitor of DNA-binding 2; TME, tumor microenvironment; IL, interleukin; NF-κB, nuclear factor κB; CRC, colorectal cancer; IDO, indoleamine 2,3-dioxygenase; AHR, aryl hydrocarbon receptor; T_{reg}, regulatory T cell; FXR, farnesoid X receptor; TGR5, G-protein-coupled bile acid receptor 1; RORγt, retinoic acid receptor-related orphan nuclear receptor-γt; Foxp3, forkhead box p3; A2AR, G-protein-coupled adenosine A2A receptor.

poised to shape patient management in the near future, paving the way for personalized immunotherapy.

This review explores the role of microbial metabolites in cancer immunotherapy. It aims to bridge the gap between microbial metabolite research and clinical practice, prioritizing human and clinical data while drawing on preclinical findings when necessary. The review will specifically focus on the impact of microbial metabolites in ICI, allo-SCT, and CAR-T cell therapies.

IMMUNE-MODULATORY MICROBIAL METABOLITES

Metabolites and their site of action

Microbial metabolites are produced as intermediaries or end products of bacterial metabolism in the human gastrointestinal tract, primarily in the colon. Some studies also suggest that intratumoral bacteria can produce metabolites.[58]

Some metabolites exert their effects locally within the intestine and are best quantified in stool. For example, SCFAs and indoles play a local role in maintaining intestinal barrier integrity by promoting mucus production, tight-junction protein expression, and epithelial cell regeneration, as well as modulating local immune responses (e.g., DC maturation and T_{reg} cell induction). Others evade first-pass metabolism in the liver and can reach distant organs via the portal and systemic circulation, exerting systemic effects. For example, SCFAs' systemic effects include hepatic gluconeogenesis, adipose tissue lipolysis, and insulin sensitivity, linking them to host energy metabolism. Indoles, BA derivatives, and other metabolites can modulate systemic immune responses by acting on secondary lymphoid organs or modulating cytokine profiles.[59]

The bioavailability, pharmacokinetics, and context-specific activity of metabolites vary significantly, underscoring the importance of both their site-specific production and receptor-mediated action in host tissues. Indeed, the systemic impact of metabolites on distant cells and tissues remains largely unexplored. Lymphoid organs such as the spleen and liver, which are linked by the portal circulation, may encounter higher metabolite concentrations than those measurable in plasma. Similarly, the roles of secondary metabolism and biotransformation of metabolites in the liver, in healthy or tumor tissues, are unclear. Factors such as the physicochemical properties of metabolites (e.g., molecular size and solubility) and host-specific characteristics (e.g., intestinal permeability and hepatic metabolism) may further determine whether a metabolite acts locally or systemically. These factors warrant further study.

Short-chain fatty acids and branched-chain fatty acids

SCFAs—saturated fatty acids with chains of up to six carbon atoms—are the primary metabolic products of gut microbiota, mainly through anaerobic fermentation of proteins and indigestible dietary fibers such as polysaccharide plant cell walls, resistant starch, and soluble oligosaccharides, as well as endogenous substances like mucin.[60,61] The most abundant SCFAs in the gut are acetic acid, propionic acid, and butyric acid, detectable in stool samples at a ratio of 60:20:20.[62] Other SCFAs, such as formic acid (methanoic acid) and valeric acid (pentanoic acid), occur in smaller quantities. Dietary fiber intake influences intestinal SCFA levels—highest in the cecum and decreasing along the colon—and the composition of the intestinal microbiome.[63] Microbial pathways for the synthesis of acetic and propionic acid include acetyl-coenzyme A (CoA) and succinate pathways, while butyric acid is primarily generated via the butyryl-CoA:acetate CoA-transferase (BCoAT) route by members of clostridial clusters IV and XIVa, including *Faecalibacterium prausnitzii* and *Roseburia* spp.[64]

SCFAs are absorbed via the portal vein and extensively metabolized on the first pass,[65] resulting in distinct concentration gradients from the gut lumen to peripheral tissues. Butyric acid is primarily absorbed by intestinal epithelial cells, serving as a significant energy source.[66] Hepatocytes in the liver take up propionic acid, which is utilized as a substrate for gluconeogenesis.[67] Acetic acid is the most abundant SCFA in the systemic circulation.[68]

SCFAs modulate immune responses by activating specific G-protein-coupled receptors (GPR41, GPR43, and GPR109A), which are differentially expressed on epithelial, adipose, and innate and adaptive immune system cells.[62] This receptor engagement modulates intracellular signaling pathways, suppressing cyclic AMP signaling while activating alternative cascades, such as mammalian target of rapamycin (mTOR), and contributes to diverse immune and metabolic effects, depending on GPR distribution and SCFA specificity. GPR41 is widely expressed in many tissues, whereas GPR43 is confined mainly to immune and lymphoid cells; both receptors are activated by acetic acid, propionic acid, and butyric acid, while GPR109A responds primarily to butyric acid. For instance, GPR43 activation can trigger inflammasome activity in epithelial cells, recruit neutrophils, and promote T_{reg} function.[62]

SCFAs function as histone deacetylase (HDAC) inhibitors. Butyric acid is its most potent inhibitor, followed by propionic and acetic acids.[69] By modulating the balance between histone acetyltransferases (HATs) and HDACs, SCFAs influence the histone acetylation status, thereby altering chromatin structure and regulating gene transcription in immune cells.

SCFAs influence T cell function and differentiation through GPR-mediated signaling and epigenetic regulation. A combination of acetic acid, propionic acid, and butyric acid induces colonic T_{reg} cells and protects against T cell-mediated colitis in mice by activating GPR43.[6] Butyric acid promotes the differentiation of intestinal T_{reg} cells via histone H3 acetylation at the *Foxp3* promoter and non-coding sequence regions, thereby alleviating T cell-induced colitis.[11] Conversely, butyric acid induces interferon-γ (IFN-γ) and granzyme B expression in $CD8^+$ cytotoxic T cells and Tc17 cells via HDAC inhibition[70] and promotes $CD8^+$ T cell long-term survival as memory cells, which is associated with a metabolic shift that disconnects glycolysis from the tricarboxylic acid (TCA) cycle and enables sustained glutaminolysis.[12] SCFAs also serve as an energy source and enhance $CD8^+$ T cell persistence in the TME by improving their ability to compete with tumor cells for glucose.[13]

Beyond SCFAs, the intestinal microbiota can ferment branched-chain amino acids, including leucine, isoleucine, and valine, producing BCFAs such as isobutyric acid, 2-methylbutyric acid, and isovaleric acid. These are found in lower concentrations in stool and serum compared to SCFAs. BCFA levels are diet dependent, with higher concentrations

associated with a protein-rich diet and lower levels linked to a diet rich in fiber intake.[14]

The molecular mechanisms underlying the effects of BCFAs remain largely unclear. BCFAs have been shown to suppress inflammation, promote intestinal epithelial integrity, and inhibit the nuclear factor κB (NF-κB) pathway.[15] Some BCFAs have also been implicated in tumor development and metastasis. Isovaleric acid has been linked to cancer progression in colorectal cancer (CRC).[16] By increasing TPH2 expression, isovaleric acid stimulates 5-hydroxytryptamine (5-HT) production in enteric neurons.[71] 5-HT, in turn, activates Wnt/β-catenin signaling in CRC stem cells, promoting their self-renewal and contributing to CRC tumorigenesis. Serum isobutyric acid has been linked to CRC metastasis,[72] with levels significantly higher in metastatic CRC patients compared to those with non-metastatic disease. Mechanistically, isobutyric acid activates RACK1 in tumor cells, leading to increased AKT and FAK phosphorylation, which in turn results in the transcriptional activation of proteins associated with metastasis.

Tryptophan metabolites and indoles

Tryptophan is an essential yet relatively scarce amino acid, derived almost exclusively through dietary intake. It is the precursor for hormones (such as serotonin) and messenger molecules (such as kynurenine) produced by the host and its commensals.[73] Tryptophan is metabolized via three interconnected routes: the kynurenine, serotonin, and indole pathways.

Kynurenines are produced from tryptophan by the enzyme indoleamine 2,3-dioxygenase (IDO) and exert immunosuppressive and tumor-promoting properties. IDO is overexpressed in the TME, which depletes T cells of essential tryptophan while concurrently overloading them with suppressive kynurenines, leading to T cell dysfunction.[17,18] For this reason, IDO inhibition is considered a target in improving cancer (immuno-)therapy.[19]

Serotonin, synthesized via host tryptophan hydroxylase 2, and its derivatives influence neurocognition and depression[73] as well as immunosuppression, cancer progression, and anti-tumor immunity.[74]

Indoles are the main class of tryptophan-derived microbial metabolites. Indole derivatives can be produced by strictly anaerobic commensals, such as lactobacilli, as well as aerobic or facultative anaerobic pathobionts of the class Proteobacteria, including *Klebsiella* and *E. coli*, which convert tryptophan to indole via the enzyme tryptophanase.[75] The production and biotransformation of indole derivatives is a multi-step process that requires a multitude of different microbes encoding varying biosynthesis enzymes, such as aldehyde dehydrogenases.[20,21] The aryl hydrocarbon receptor (AHR), expressed on epithelial and immune cells, is the central cellular receptor for microbial indoles. The AHR pathway plays a crucial role in maintaining homeostasis in various organs, including the gut and the placenta, and in protecting the host from autoimmunity and hyperinflammation. It modulates the interleukin-22 (IL-22) and IL-10 axis, promoting T_{reg} cell function and differentiation.[20]

However, not all indoles act exclusively as AHR ligands; compounds like indole-3-carboxaldehyde (ICA, IUPAC name indole-3-carbaldehyde) also activate type I IFN (IFN-I) signaling pathways.[22,73] IFN-I activation enhances ICI efficacy across multiple cancers.[23–25]

Bile acids

BAs are cholesterol derivatives involved in essential physiological processes, including lipid digestion, glucose metabolism, and pathogen resistance. During digestion, the gallbladder empties into the duodenum, where BAs support the emulsification of dietary fats. Primary BAs are synthesized in the liver and conjugated with taurine or glycine. In the small intestine, they undergo deconjugation by microbial bile salt hydrolases. Specific bacteria, such as *Clostridium scindens*, express the bile-acid-inducible (bai) operon, a cluster of genes encoding enzymes, including BaiB, BaiCD, BaiE, and BaiF, which catalyze the 7α-dehydroxylation of primary to secondary BAs.[76] While most BAs are reabsorbed in the ileum via enterohepatic circulation, approximately 5% undergo additional bacterial modifications in the colon, generating secondary BAs that reach intestinal concentrations in the micromolar range.

BAs function as signaling molecules that regulate host metabolism via nuclear and membrane-bound receptors, including the farnesoid X receptor (FXR) and G-protein-coupled BA receptor 1 TGR5 (Gpbar1).[27] Gut microbiota-mediated modifications of BAs alter their receptor signaling properties, which influence microbial composition through direct antimicrobial effects and innate immune responses.[28]

BA receptor signaling via TGR5 can potentiate anti-viral and anti-tumoral immune responses by enhancing IFN-I expression. For example, the primary BA chenodeoxycholic acid (CDCA) triggers serum IFN-β levels.[29] Microbiota-modified lithocholic acid (LCA) and deoxycholic acid (DCA) promote type III IFN responses in the small intestine.[30] DCA, derived from *Clostridium scindens*, enhances anti-viral immunity by restoring plasmacytoid DC and MyD88-dependent IFN-I responses.[31] IFNs play a critical role in cancer immunosurveillance by activating IFN-stimulated genes (ISGs) and are essential for the efficacy of various anti-cancer therapies, as high intratumoral IFN-I expression correlates with favorable patient outcomes.[32]

Gut bacteria such as *Eggerthella lenta* convert LCA into 3-oxolithocholic acid (3-oxoLCA) and isolithocholic acid (isoLCA), which suppress T helper 17 (Th17) cell differentiation by inhibiting the transcription factor retinoic acid receptor-related orphan nuclear receptor-γt (RORγt). Administration of these metabolites to mice reduces Th17 and increases the T_{reg} cell population in the intestinal lamina propria.[33] These BAs and their biosynthesis genes are reduced in patients with inflammatory bowel disease, suggesting that metabolites may contribute to immune modulation in inflammatory disorders.[34]

The secondary BA 3β-hydroxydeoxycholic acid (isoDCA) enhances T_{reg} cell differentiation by modulating DC immunostimulatory properties, partially through FXR signaling, suggesting a microbial metabolite-dependent induction of extrathymic T_{reg} cells.[35]

Polyphenol derivatives

DAT

Desaminotyrosine (DAT) is produced by anaerobic gram-positive *Flavonifractor plautii* from the metabolism of flavonoids, a

group of polyphenolic compounds primarily found in tea, wine, and citrus fruits.[36] DAT enhances anti-viral immunity in mice by amplifying IFN-I signaling[37] and boosts ICI efficacy in solid tumor models.[38]

Urolithin A

The natural polyphenols ellagitannin and ellagic acid, contained in pomegranates, berries, and walnuts, are microbially converted to urolithin A (UA).[39–41] This step depends on gut microbiota, where the role of commensal *Gordonibacter*, a gram-positive, strictly anaerobic bacterium within the Coriobacteria class, has been postulated,[42] but the species required for UA synthesis have yet to be identified.[43,44] UA promotes mitophagy, a mechanism that eliminates dysfunctional mitochondria.[45,46] This augments Wnt signaling and leads to the proliferation of T memory stem cells,[46] which are associated with enhanced tumor-suppressive properties.[47] Additionally, UA enhances $CD8^+$ T cell infiltration into tumors and improves the efficacy of adoptive T cell transfer.[46] Besides acting on mitochondria, orally administered UA resulted in the proliferation of naive $CD8^+$ T cells, differentiation into a memory phenotype, and enhancement of cytokine secretion via FOXO1.[48] In a pancreatic ductal adenocarcinoma (PDAC) model, UA treatment reduced M2-polarized macrophages and increased major histocompatibility complex II (MHC-II) expression as well as central memory and naive $CD4^+$ and $CD8^+$ T cells.[49]

TMAO

Trimethylamine *N*-oxide (TMAO) is derived from dietary nutrients such as choline and L-carnitine and is abundant in seafood, including squid and shellfish.[50] Its synthesis requires two steps: first, enzymatic conversion by choline trimethylamine lyase[77] by genera in the Clostridia class (e.g., *Blautia*, *Ruminococcus*, and *Roseburia*)[78] and second, hepatic oxidation by flavin monooxygenase.[79] Its production depends on intestinal bacteria[51] and can be upregulated by a choline-rich diet[78] but is impaired following antibiotic treatment with metronidazole.[52] TMAO exhibits direct pro-inflammatory properties through the NLRP3 inflammasome and the potentiation of ISGs. Both pathways induce an immunostimulatory, M1-polarized macrophage phenotype characterized by proinflammatory cytokines (IL-1β and IL-12) and co-stimulatory molecules (CD40), thus promoting Th1 and Th17 cell differentiation.[52,53] In addition, TMAO can shift the T cell transcriptional profile from a regulatory to a boosted $CD8^+$ effector phenotype by rebalancing opposing regulators (e.g., RICTOR and FOXP3).[52] In a triple-negative breast cancer (TNBC) model, TMAO induced pyroptosis of tumor cells through the endoplasmic reticulum stress kinase PERK and enhanced cytotoxic T cell activity as evidenced by increased IFN-γ and tumor necrosis factor α (TNF-α) secretion.[78]

Inosine

Inosine is a bioactive, purine nucleoside released from intestinal epithelial cells utilizing bacterial ADP-ribosyltransferase Bxa[54] or produced directly by bacterial species, including *Bifidobacterium pseudolongum*.[55] Mechanistically, the binding of inosine to the G-protein-coupled adenosine A2A receptor (A2AR) results in an increase of IL-12Rβ2 and the transcription of IFN-γ.[55] In addition, inosine has been shown to enhance tumor immunogenicity by increasing the presentation of tumor antigens.[56] It represents an alternative carbon source for $CD8^+$ T cells deprived of glucose, thereby boosting their proliferation and function.[57]

MICROBIAL METABOLITES AS MODULATORS OF CANCER IMMUNOTHERAPY

Metabolites and immune checkpoint inhibition

Immune checkpoint inhibition has driven substantial advancements in cancer treatment.[1] By targeting immune checkpoints, such as PD-1, PD-L1, and CTLA-4, ICI therapies block pathways that tumors exploit to evade immune surveillance, thereby enabling immune cells to recognize and attack tumor cells.[2] However, the effectiveness of ICI therapies can vary widely among individuals.[80] Recent research has identified the composition of the human intestinal microbiome and microbial metabolites as master regulators influencing the efficacy of ICIs. Please refer to Figure 1 for a graphical abstract.

SCFAs

In n = 52 patients with solid tumors receiving anti-PD-1 ICIs, increased stool levels of acetic (hazard ratio [HR], 0.29; 95% confidence interval [CI], 0.15–0.54), propionic (HR, 0.08; 95% CI, 0.03–0.20), butyric (HR, 0.31; 95% CI, 0.16–0.60), and valeric (HR, 0.53; 95% CI, 0.29–0.98) (as well as plasma concentrations of isovaleric acid [HR, 0.38; 95% CI, 0.14–0.99]) acids were associated with better PFS. The authors concluded that SCFA levels reflect the microbiome's metabolic output and may serve as a biomarker for predicting ICI responsiveness.[81] These findings are consistent with a study in n = 11 non-small cell lung cancer (NSCLC) patients treated with the anti-PD-1 antibody nivolumab. Stool levels of propionic acid were significantly increased in NSCLC patients who responded to therapy.[82] The positive association between stool SCFAs and response to ICIs aligns with serum metabolomics in NSCLC patients, where concentrations of acetic, butyric, and propionic acids were positively correlated with response to anti-PD-1 treatment and with PD-1 expression on $CD8^+$ T cells.[83] *Roseburia intestinalis* and its metabolite, butyric acid, enhanced ICIs by increasing $CD8^+$ T cell infiltration and activation in mouse models of CRC, suppressing tumor growth in microsatellite instability (MSI)-high CRC, and synergizing with anti-PD-1 therapy in microsatellite stable (MSS) CRC to overcome resistance to immunotherapy.[84]

In contrast, a study examining anti-CTLA-4 (ipilimumab) in n = 40 patients with metastatic melanoma found that serum levels of butyric and propionic acids were inversely correlated with PFS and OS. Preclinical mouse models demonstrated that oral administration of butyric acid reduced the efficacy of anti-CTLA-4 ICIs and restrained the upregulation of ICOS on T cells and the accumulation of tumor-specific T cells, suggesting that SCFAs limited anti-CTLA-4 activity.[85]

The combination of ICIs and oral gavage of the BCFA isobutyric acid in mice challenged with CT26 colon cancer enhanced the efficacy of immunotherapy. This synergistic effect was accompanied by elevated intratumoral PD-1 and IFN-γ and a higher infiltration of the tumor tissue by $CD3^+$ T cells.[86]

The seemingly contradictory effects of SCFAs on ICI efficacy—where some studies associate higher SCFA levels with improved PFS/OS, while others report the opposite—are likely

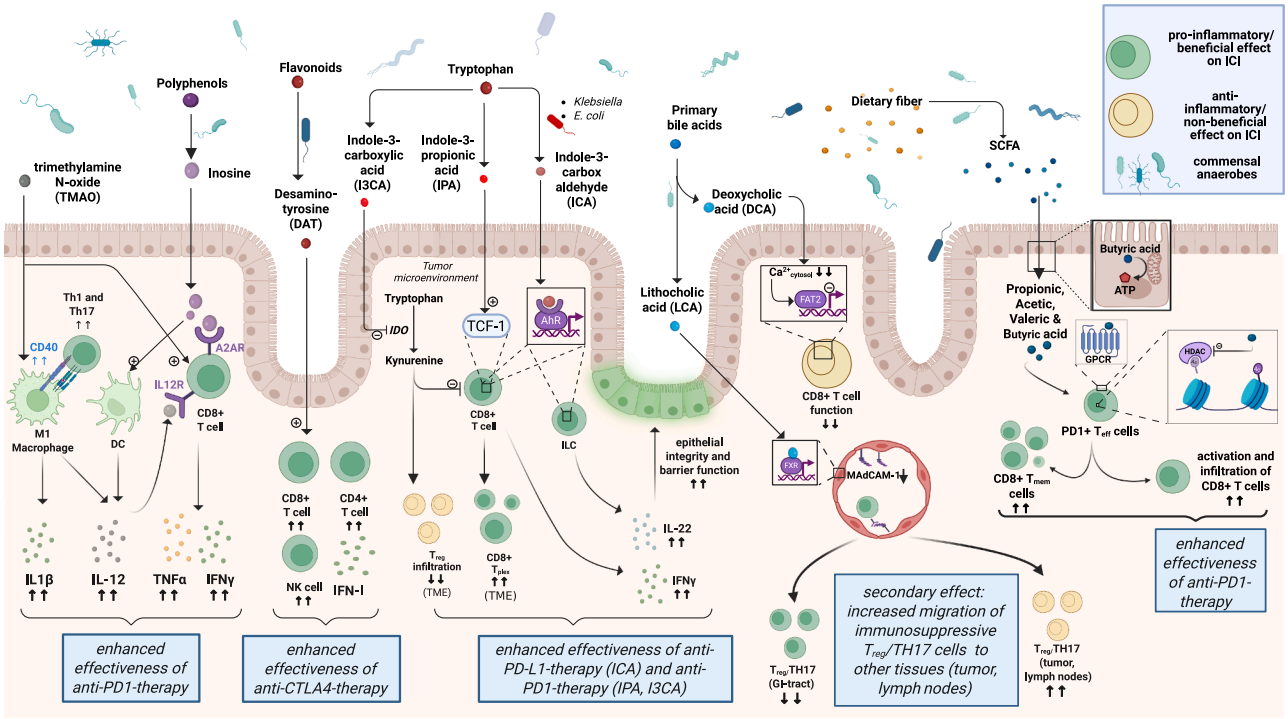

Figure 1. Microbial metabolites modulate immune responses, epithelial integrity, and metabolism during immune checkpoint blockade

Schematic overview of key microbial metabolites and their mechanisms of action in the context of immune checkpoint inhibition (ICI). Figure created with BioRender.

rooted in multiple context-dependent variables. First, the type of ICI targeted is critical: butyric acid may enhance anti-PD-1/PD-L1 efficacy by promoting $CD8^+$ T cell activation yet may impair anti-CTLA-4 responses by expanding T_{reg} cells. Second, tumor type matters: studies supporting SCFA-driven benefit involved NSCLC or CRC, while those reporting negative associations were in melanoma, each with distinct immunological microenvironments. Moreover, the source of SCFA measurement—stool vs. serum—adds complexity to the analysis. While fecal SCFA levels reflect luminal microbial activity with local immunoregulatory effects (e.g., on epithelial cells and mucosal immunity), serum levels may better capture systemic bioavailability and host absorption, potentially influencing peripheral immune responses (i.e., in peripheral blood, secondary lymphoid organs, or the TME). Variability in sampling methods, analytical platforms (e.g., gas chromatography-mass spectrometry vs. liquid chromatography-mass spectrometry), and data normalization further complicates cross-study comparisons, as do the small sample sizes across studies. Differences in gut microbial composition across cohorts, diet, and antibiotic exposure likely influence outcomes.

While promising as predictive biomarkers, SCFAs likely exert bidirectional effects depending on ICI class, host microbiota, and tumor-immune context.

Tryptophan metabolites

In a cohort of $n = 23$ NSCLC patients receiving anti-PD-1 therapy, plasma IDO activity was measured before treatment initiation and at the first follow-up. Patients with progressive disease had significantly increased IDO activity, while those responding to treatment showed stable activity. Supporting these findings, patients with progressive disease exhibited higher plasma tryptophan levels, consistent with the role of IDO in initiating tryptophan breakdown.[87]

ICA, produced by the probiotic *Lactobacillus*, improved anti-tumor immunity in a melanoma mouse model. Combining ICA via oral gavage alongside anti-PD-L1 therapy significantly reduced tumor growth compared to anti-PD-L1 treatment alone. Mechanistically, ICA activated the AHR pathway, stimulating IFN-γ production in $CD8^+$ T cells, thereby enhancing the effectiveness of ICIs. Moreover, a tryptophan-rich diet, serving as a prebiotic to provide precursors for ICA biosynthesis by bacteria, amplified the efficacy of anti-PD-L1 treatment compared to a low-tryptophan diet in mice, suggesting that tryptophan-metabolizing microbes play a role in enhancing anti-tumor responses. These results were validated in $n = 42$ melanoma patients treated with anti-PD-1 and IFN-α, where serum baseline ICA levels were significantly higher in patients responding to therapy.[58]

The tryptophan metabolite indole-3-propionic acid (IPA), derived from *Lactobacillus johnsonii*, enhanced the effectiveness of anti-PD-1 treatment in a mouse model of CRC by promoting the differentiation of $CD8^+$ T cells into T_{pex} cells, a subset characterized by high transcription factor 1 (TCF-1) expression and responsiveness to anti-PD-1 therapy. This led to an increase in $CD8^+$ effector T cells within the TME. This was confirmed in patient-derived CRC organoids and across different cancer types (murine B16-F10 melanoma and 4T1 breast cancer models).[88]

The tryptophan-derived metabolite indole-3-carboxylic acid (I-3-CA, not to be confused with the carbaldehyde ICA), secreted by the probiotic *Lactobacillus gallinarum*, enhanced the response to anti-PD-1 therapy in microsatellite-unstable MC38 and microsatellite-stable CT26 CRC mouse models.[26] I-3-CA competes with kynurenine for binding to the AHR. In $CD4^+$ T cells, *in vitro* treatment with I-3-CA counteracts kynurenine-induced AHR activation and the subsequent differentiation into T_{reg} cells. Additionally, I-3-CA reduces IDO1 expression in the TME, resulting in decreased kynurenine production, lower T_{reg} cell infiltration, and enhanced $CD8^+$ T cell function. This highlights the interplay between the indole and IDO/kynurenine pathways.[26]

Tryptophan metabolite treatment was shown to modulate immune-related adverse events (irAEs). In a mouse model of ICI-associated colitis, combining anti-CTLA-4 treatment with dextran sulfate sodium (DSS), orally administered ICA improved epithelial barrier integrity and immune homeostasis through the AHR/IL-22 pathway, thereby protecting against ICI-associated colitis without compromising anti-tumor effects in a B16 melanoma model. Notably, ICA supplementation altered the gut microbiome composition to favor SCFA production, as indicated by increased levels of propionic, butyric, valeric, and isobutyric acids in fecal samples.[89]

This body of work highlights the therapeutic potential of tryptophan-derived microbial metabolites in enhancing the efficacy of anti-PD-1/PD-L1 treatments across preclinical and clinical models. Metabolites such as ICA, IPA, and I-3-CA enhance $CD8^+$ T cell function, reduce immunosuppressive T_{reg} cells, and modulate the TME through the AHR and IDO/kynurenine pathways. The findings are well supported by murine data and human correlative studies, although small cohort sizes and tumor heterogeneity warrant cautious interpretation. Significantly, therapeutic ICA also mitigated ICI-associated colitis, suggesting that these metabolites may serve dual roles as immunotherapy enhancers and protectants against irAEs.

Bile acids

A recent study reported that antibiotic treatment created an environment conducive to the overgrowth of *Enterocloster* species, which are linked to immune suppression in cancer patients.[90] Post-antibiotic recolonization by *Enterocloster* led to changes in BA metabolism, mainly through increased levels of secondary BAs, such as LCA. LCA and its derivatives can act as FXR ligands, which suppress the expression of MAdCAM-1 in the ileal vasculature. This downregulation of MAdCAM-1 weakened the retention of immunosuppressive $T_{reg}17$ cells in the gut, allowing them to migrate to tumors and lymph nodes, where they promote resistance to ICIs.[90]

In contrast, elevated DCA levels in CRC patients suppressed $CD8^+$ T cell effector function by inhibiting Ca^{2+}-NFAT2 signaling through plasma membrane Ca^{2+} ATPase. The presence of bacteria harboring DCA biosynthetic genes was correlated with reduced $CD8^+$ T cell activity and increased tumor growth, an effect reversed by disrupting BA metabolism via chelation, genetic deletion, or bacteriophage targeting of DCA-producing *Clostridium scindens*.[91]

Two recent studies on hepatocellular carcinoma (HCC) highlight diverging associations between BAs and ICIs. In HCC patients treated with ICIs, ursodeoxycholic acid (UDCA) and ursocholic acid were enriched in the stool samples of responder patients.[92] In a translational study, primary and secondary BAs accumulated in liver HCC samples. High BA levels correlated with reduced tumor-specific T cell responses and resistance to anti-PD-1 therapy, while inhibiting the BA-conjugating enzyme BA-CoA:amino acid *N*-acyltransferase enhanced immunotherapy efficacy.[93]

BAs exhibit dual effects: Some secondary BAs promote ICI resistance by facilitating T_{reg} cell migration, while others enhance anti-tumor immunity. BAs' context-dependent effects may rely on their structural class, site of action, tumor context, and patient-individual factors such as antibiotics or microbial shifts.

Other metabolites

In preclinical models, oral DAT administration combined with anti-CTLA-4 ICIs in melanoma and anti-PD-1 therapy in PDAC-bearing mice significantly enhanced tumor control and increased survival. The combination of DAT and anti-CTLA-4 ICIs resulted in a higher activation of $CD4^+$ and $CD8^+$ T cells and a higher abundance of IFN-γ-producing natural killer cells within the TME. Interestingly, oral DAT supplementation could rescue the detrimental effects of broad-spectrum antibiotic treatment on the efficacy of anti-CTLA-4 therapy.[38]

Inosine, produced by *Bifidobacterium pseudolongum*, can leak out of the gut and into the systemic circulation following intestinal barrier defects, enhancing ICIs with anti-CTLA4 and anti-PDL1 in various cancer models (CRC, bladder, and melanoma). This phenomenon was attributed to shifting T cell fate toward an anti-tumorigenic Th1 phenotype.[55]

TMAO has been implicated in enhancing ICI therapy. In a preclinical PDCA model, combining TMAO with ICI therapy led to superior tumor control and survival compared to ICI therapy alone.[52] Mechanistically, TMAO activated the IFN-I pathway, promoting an immunostimulatory phenotype in macrophages. Clinically, the abundance of bacterial taxa capable of producing trimethylamine (TMA), a precursor of TMAO, was associated with a better response to anti-PD-1 treatment in PDAC patients. Furthermore, CutC-encoding bacteria, which drive TMA production, have been linked to improved OS in patients treated with anti-PD-1 therapy.[52]

In $n = 360$ patients with TNBC, higher plasma levels of TMAO correlated with an activated immune microenvironment and a better response to immunotherapy.[78] The *CutC*-encoding commensal *Clostridiales*, which colonize the mammary gland, were identified as a source of TMAO and its precursors. Mechanistically, TMAO induced pyroptosis through the endoplasmic reticulum stress kinase PERK, thereby enhancing tumor-directed $CD8^+$ T cells.

Future directions and clinical implications

Eleven bacterial strains, comprising rare and low-abundance components of the human microbiome, were isolated from human fecal samples and orally administered to germ-free mice. Colonization with the 11-strain bacterial consortium enhanced the efficacy of anti-PD-1 therapy in a syngeneic subcutaneous MC38 CRC model. Mechanistically, the consortium induced IFN-γ-producing $CD8^+$ T cells in the intestine, a process dependent on $CD103^+$ DCs and MHC-Ia molecules.[94] Notably, these $CD8^+$ T cells were also enriched in other organs, suggesting a

potential systemic effect mediated by bacterial metabolites, as metabolomic profiling revealed increased levels of mevalonate and dimethylglycine in the mice's cecal contents and sera. A recent study found that high plasma mevalonate levels correlated with better responses to anti-PD-1 therapy, enhanced PD-L1 expression, and T cell function, as well as improved OS, in NSCLC patients.[95]

A contemporary analysis employed integrative multi-omics approaches to analyze the fecal microbiomes and metabolomes in patients undergoing anti-PD-1/PD-L1 therapy, uncovering specific microbial (response-associated enterotypes) and metabolic signatures (the phenylalanine metabolite phenylacetylglutamine) associated with treatment response.[96]

In summary, metabolites play a crucial role in modulating ICI therapy by influencing immune cell activation, tumor metabolism, and therapeutic resistance. SCFAs have been linked to improved ICI efficacy by promoting $CD8^+$ T cell infiltration and activation. However, their effects vary depending on the specific immune checkpoint targeted. Additional microbial metabolites, such as inosine, TMAO, and DAT demonstrate potential in enhancing ICI efficacy through diverse mechanisms, including IFN-I activation and immune cell reprogramming. Only inosine has been tested in a clinical trial (see "pre- and postbiotics" below). Integrative multi-omics presents a promising avenue for identifying novel metabolite combinations that can serve as biomarkers and therapeutic compounds.

Metabolites and chemo-immunotherapy

Tumors exhibiting an inflamed phenotype[97,98]—characterized by $CD8^+$ tumor-infiltrating lymphocytes (TILs), robust antigen presentation, and elevated levels of pro-inflammatory cytokines (e.g., IFN-γ and IL-2)—tend to respond better to ICIs.[99] In contrast, tumors with an immune-cell-deprived microenvironment show minimal responsiveness, particularly to monotherapy.[100] Combining ICIs with chemotherapy can enhance immune responses by modulating the TME and converting an immune-excluded phenotype into a more immune-permissive one.[101] Increasing evidence suggests that microbial metabolites play a role in improving the efficacy of such chemo-immunotherapies.

For example, oxaliplatin is considered an immunogenic agent due to its ability to promote an adaptive immune response by releasing damage-associated molecular patterns (DAMPs) from dying tumor cells (reviewed in Galluzzi et al.[102]). Additionally, it induces tumor T cell infiltration[103] and immunogenic cell death.[104] In mouse models of CRC (including Mc38 and colitis-associated CRC), supplementation with butyric acid improved oxaliplatin efficacy, particularly when the microbiome was disrupted by antibiotic treatment.[13] The authors proposed a mechanism in which butyric acid enhances $CD8^+$ T cell responses by upregulating ID2 (inhibitor of DNA-binding 2)-mediated IL-12 signaling.

5-Fluorouracil (5-FU) induces antigen uptake and IL-12 secretion by DCs, leading to the generation of highly cytotoxic T cells[105] and the selective eradication of myeloid-derived suppressor cells (MDSCs)[106] in the TME, thereby promoting a pro-inflammatory milieu. The polyphenol derivative UA has demonstrated sensitizing properties in 5-FU-resistant colon cancer cells *in vitro* and *in vivo* by downregulating drug transporters that enable efflux through FOXO3-FOXM1 rebalancing.[107] Therefore, UA likely also amplifies the pro-immunogenic effects of 5-FU when combined with ICIs.

The impact of microbial metabolites on chemotherapy has been extensively reviewed elsewhere.[108] However, these examples highlight that microbial metabolites not only directly influence the efficacy and outcomes of ICI therapy but also exert indirect effects when combined with chemotherapy.

Metabolites and allo-SCT

SCFA

Severe gastrointestinal (GI) GVHD has been associated with reduced bacterial biomass, lower alpha diversity, and distinct microbiota alterations, particularly a decrease in anaerobic bacteria from the Lachnospiraceae (including *Blautia*) and Ruminococcaceae families.[2,3,109] Patients with severe GVHD exhibited a marked reduction in fecal SCFA levels, including acetic, propionic, and butyric acids, whereas mild acute GVHD cases retained propionic acid-producing *Blautia* species.[110] Butyric acid levels were consistently decreased across all stages of GI GVHD.

In children undergoing allo-SCT, significant and progressive reductions in fecal butyric acid and other SCFAs were observed within the first 14 days post transplant, particularly in those receiving antibiotics targeting anaerobes. Lower butyric and propionic acid levels at day +14 were associated with subsequent GVHD development.[111]

Another study investigated the relationship between the microbiome and chronic GVHD by analyzing plasma samples from allo-SCT patients at day +100. At this time point, lower circulating levels of propionic acid and butyric acid were observed in patients who later developed chronic GVHD compared to those who did not.[112]

Butyrogenic bacteria are strongly suppressed during the peri-engraftment period, and low butyric acid-producing capacity correlates with reduced microbial diversity and increased GI GVHD severity.[113] BCoAT is a key enzyme in microbial butyric acid synthesis, converting butyryl-CoA to butyric acid using acetic acid. Broad-spectrum antibiotic use before transplantation was an independent factor associated with a diminished abundance of butyrogenic bacteria. Low BCoAT copy numbers at GVHD onset and day +30 were linked to higher GVHD-associated and transplantation-related mortality rates.[114]

In summary, there is a consistent link between reduced levels of SCFAs, particularly butyric acid, and the development and severity of GI GVHD following allo-SCT, highlighting the critical role of conditioning, antibiotics, and altered nutrition-induced depletion of anaerobic microbes, especially butyrogenic bacteria, in GVHD onset. Despite these insights, clinical translation faces challenges including the inevitability of broad-spectrum antibiotic administration, the impracticality of measuring fecal rather than blood metabolites as reliable biomarkers, and the limited number of controlled clinical studies aimed at restoring or preserving SCFA production during the peri-engraftment period (discussed in "pre- and postbiotics" below).

Bile acids

A recent study investigated how BAs influenced mouse GVHD models. It found that T cell-driven inflammation reduced

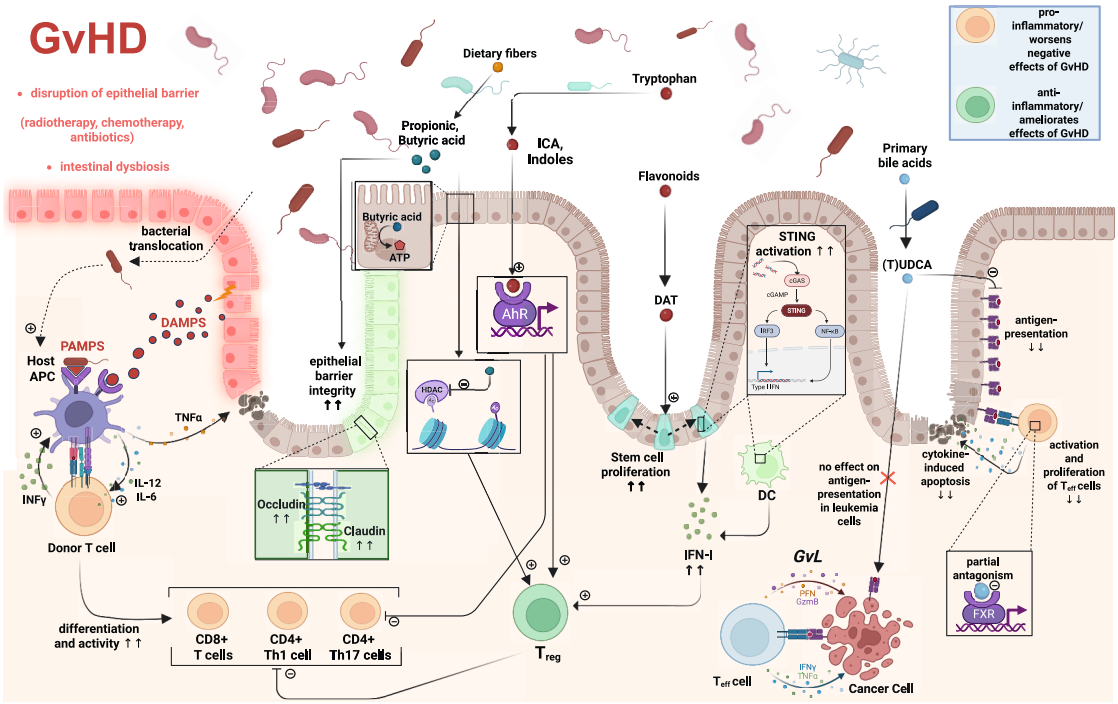

Figure 2. Microbial metabolites influence immune regulation, barrier function, and metabolic pathways in allogeneic stem cell transplantation (allo-SCT)

Schematic overview of microbial metabolites and their immunological, epithelial, and metabolic effects relevant to allo-SCT. Figure created with BioRender.

microbiome-encoded bile salt hydrolase (BSH) genes, resulting in decreased cecal content and plasma levels of immunomodulatory BAs, such as 3-oxoLCA and isoLCA, enhanced FXR activation, and worse disease outcomes. In patients receiving allo-SCT, dysbiosis and loss of microbiota-derived BAs were associated with GVHD. UDCA, an FXR antagonist, reduced T cell proliferation *in vitro* and was associated with a lower risk of GVHD-related mortality in patients.[28]

In murine allo-BMT, tauroursodeoxycholic acid (TUDCA) protected intestinal epithelial cells from cytokine-induced cell death. Systemic administration of TUDCA decreased the severity of GVHD by reducing antigen presentation and preventing epithelial apoptosis. It also maintained the graft-versus-leukemia (GVL) effect without altering gut microbiota composition.[115]

Tryptophan metabolites

Indole treatment in murine allo-BMT models, via tryptophanase-positive or -negative strains of *E. coli* or exogenous administration of ICA, reduced GVHD severity by limiting gut inflammation and epithelial damage while preserving GVL responses. ICA upregulated IFN-I-associated genes, but its protective effects were lost in mice lacking IFN-I signaling.[22]

Metabolomic analysis of HLA-matched allo-SCT donor-recipient pairs revealed significant differences in metabolite profiles, particularly at the onset of acute GVHD: a decreased production of AHR ligands such as 3-indoxyl sulfate, indole acetate, indole acetylglutamine, and indole propionic acid may limit tolerogenic IDO induction and affect allogeneic T cell reactivity.[116]

Other metabolites

DAT was associated with improved survival and reduced relapse in allo-SCT patients by protecting the intestinal barrier and promoting regeneration. Preclinical models show that DAT prevents GVHD, enhances GVL responses, and remains an effective therapeutic in antibiotic-induced dysbiosis. Mechanistically, DAT activates mTORC1-dependent intestinal stem cell proliferation and engages STING to maintain stem cell function while modulating T cell responses.[117] For a graphical abstract, please refer to Figure 2.

Bacteria from the *Enterococcus* genus are associated with a greater incidence of GVHD and mortality, and lactose provides a substrate for their growth.[118] Researchers investigated whether enterococcal expansion in allo-SCT patients is linked to lactose tolerance by genotyping n = 602 patients for the rs4988235 single-nucleotide polymorphism (SNP), which regulates lactase expression. Domination by *Enterococcus* persisted longer in lactose malabsorbers after antibiotic cessation, suggesting that lactose availability in the gut influences the recovery of the microbiota.

Combination of metabolites

Recently, we reported the 2-year follow-up of a prospective, observational, longitudinal, two-center study of n = 78 patients receiving allo-SCT. The study integrated amplicon sequencing, viral metagenomics, and targeted stool metabolomics using multi-omics factor analysis (MOFA), a computational framework designed to detect shared patterns across complex datasets[119] and associate them with clinical outcomes. Following

transplantation, we observed a marked and progressive decline in bacterial and viral diversity as well as in IMMs. MOFA identified a functional microbiome signature of a consortium of bacteria within the families Lachnospiraceae and Oscillospiraceae associated with specific IMMs. These metabolites—butyric acid, propionic acid, isobutyric acid, ICA, and DAT—formed the basis of the Immunomodulatory Metabolite Risk Index (IMM-RI). Clinical validation of the IMM-RI was based on time-to-event analyses, which showed that patients with a low-risk IMM-RI score had significantly improved OS, reduced rates of acute GI GVHD, and lower relapse incidence.[120] Exposure to broad-spectrum antibiotics, affecting over 95% of patients, was linked to near-total depletion of these metabolites, reinforcing the clinical relevance of maintaining microbial metabolite production during the peri-engraftment period.

Human data and preclinical models support experimental validation of the IMM-RI metabolite DAT as a therapeutic. These models demonstrate that DAT protects intestinal integrity, limiting GVHD while concomitantly supporting GVL effects, even under antibiotic-induced dysbiosis.[117]

Future directions and clinical implications

Metabolites are crucial in modulating immune responses and maintaining epithelial barrier integrity, thereby limiting excessive immune activation during allo-SCT. SCFAs, particularly butyric acid and propionic acid, are consistently reduced in patients with GVHD and are linked to enhanced T_{reg} cell induction and epithelial protection. Similarly, BAs such as UDCA and TUDCA mitigate GVHD severity by modulating T cell responses and preserving epithelial homeostasis. Indeed, the BA UDCA is already widely used in allo-SCT centers to prevent transplant-related complications. Indole derivatives promote tolerogenic pathways via AHR signaling, while the microbial metabolite DAT supports intestinal stem cell regeneration. Given their different cellular and molecular targets, a combined metabolite risk index such as the IMM-RI, which integrates key immunomodulatory metabolites independently associated with distinct clinical factors (e.g., toxicity and relapse), may provide a robust biomarker to predict outcomes more reliably. However, clinical translation is hindered by antibiotic-driven depletion of anaerobic microbes, limited systemic detectability of key metabolites, and a scarcity of interventional trials aimed at preserving or restoring their production during the vulnerable peri-engraftment period. Current studies rely on dietary substrates to improve metabolite bioavailability (please refer to "pre- and postbiotics" below).

Metabolites and CAR-T cell therapies

Recent observational studies have focused on the detrimental effect of exposure to antibiotics before anti-CD19 CAR-T cell therapy in patients with large B cell lymphoma (LBCL). In these studies, exposure to broad-spectrum antibiotics (of the PIM group [piperacillin/tazobactam, imipenem, and meropenem]) within 4 weeks of CAR-T cell infusion were linked to poor outcomes (HR [95% CI] 3.05 [1.96–4.75][121] and 2.71 [1.76–4.16],[122] respectively), irAEs such as immune effector cell-associated neurotoxicity syndrome (ICANS) and cytokine release syndrome (CRS), as well as disease progression independent of confounders such as tumor burden and the line of therapy.[121,122] Importantly, PIM antibiotics target commensal anaerobes,[123,124] such as those from the genera *Akkermansia*, *Bacteroides*, and *Ruminococcus*, which are linked to favorable therapy outcomes in patients receiving CAR-T cells[121,122] as well as in other T cell-based therapies.[125] Preclinical models of meropenem-induced intestinal dysbiosis (ID)[126] and clinical studies in allo-SCT patients[120] have confirmed that exposure to broad-spectrum antibiotics is linked to the loss of beneficial metabolites and their predominantly anaerobic bacterial producers.[126,127]

Recently, we and others have demonstrated that serum and stool metabolites assayed shortly before CAR-T cell infusion predict CAR-T cell outcomes in LBCL and multiple myeloma (MM) patients.[126,128] Serum indoxyl sulfate, previously described as a surrogate marker for bacterial diversity,[129] and TMAO were associated with better OS in a cohort of n = 40 B cell non-Hodgkin lymphoma patients.[126] As with ICIs, TMAO may prime tumor-associated macrophages and enhance the immunogenicity of cancer cells[52]; however, this remains to be demonstrated preclinically for CAR-T cells.

SCFAs have also been linked to improved outcomes following CAR-T cell therapy.[126,128] Valeric acid exhibited the strongest association with PFS (HR 6.9 for low valeric acid[128]) compared to acetic, butyric, or isovaleric acid.[128] Mechanistically, valeric acid modulates CAR-T cell gene expression through HDAC inhibition and metabolism via enhanced TCA activity, boosting their effector molecule expression and metabolic fitness. Thus, valeric acid was applied toward *ex vivo* CAR-T cell engineering in various preclinical CAR-T cell models of solid and hematological tumors, where it could enhance CAR-T cells' cytolytic properties and persistence *in vivo*.[128,130] Interestingly, host-intrinsic metabolites, such as the ketone body β-hydroxybutyrate, which is produced as an energy source during lipid metabolism following ketogenic or low-calorie diet, demonstrated similar effects, influencing histone acetylation and enhancing TCA activity in a preclinical model of $CD19^+$ LBCL.[131] Please refer to Figure 3 for a graphical abstract.

A recent study linked oncometabolites belonging to the class of acetylated polyamines (resulting from tumoral biotransformation of polyamines) and reduced levels of lysosphingolipids (potentially resulting from increased cancer lipid metabolism) to adverse CAR-T cell outcomes (HR 3.65 [95% CI 1.38–9.67]).[132] High levels of acetylated polyamines and low levels of lysosphingolipids are associated with immune dysregulation, including T cell dysfunction, tumor immune evasion, and increased MDSCs.[133] However, this has not yet been tested in CAR-T cells. Inosine has been linked to increased CAR-T cell functionality, particularly by enhancing stemness and cytolytic function through its use as an alternative energy source. Furthermore, inosine can alter CAR-T cell metabolic programming by reducing glycolysis and upregulating glutaminolysis and polyamine synthesis.[134]

Future directions and clinical implications

Microbial and non-microbial metabolites shape CAR-T cell therapies. In contrast to other immunotherapies, the metabolic state and available energy sources (e.g., glutaminolysis and anaerobic glycolysis) are key in modulating CAR-T cell responses. Improved respiration and histone acetylation (mediated by SCFAs and the ketone body β-hydroxybutyrate), increased glutaminolysis, and decreased glycolysis (inhibited by inosine) are

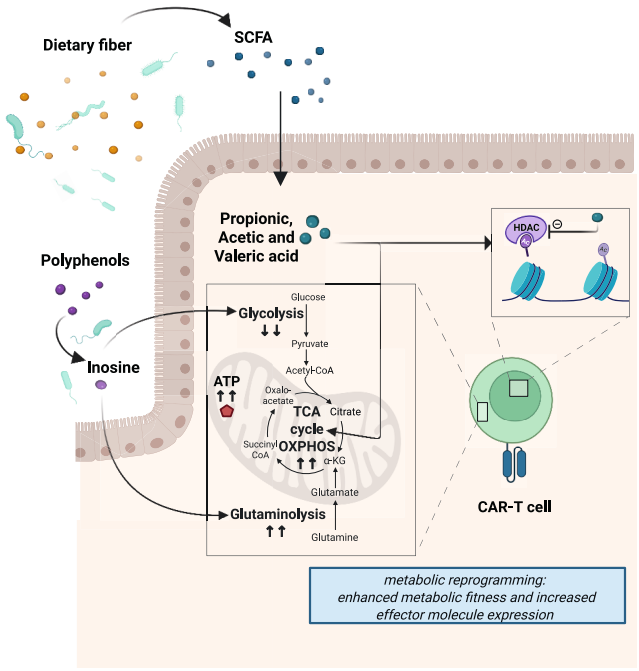

Figure 3. Microbial metabolites affect immune cell function and metabolic signaling in CAR-T cell therapy

Schematic overview of microbial metabolites and their proposed mode of action in CAR-T cell therapy. Figure created with BioRender.

emerging as key regulators of CAR-T cell cytolytic potential, long-term persistence, and metabolic fitness. Unlike allo-SCT or ICIs, metabolites primarily influence CAR-T cell-intrinsic properties and longevity, as the reproductive capacity of a CAR-T cell is limited (vs. the self-renewing, transplanted immune system in allo-SCT), and the product is typically only infused once (vs. multiple cycles of ICIs). This creates an opportunity for optimizing cell persistence and function through metabolically tailored CAR-T cell products.

To date, no studies have been registered that combine metabolites with CAR-T cell therapy. The MD Anderson Cancer Center recently initiated a study to ameliorate antibiotic-induced dysbiosis by combining fecal microbiota transplantation (FMT) with anti-CD19 CAR-T cell therapy (NCT06218602). The study aims to analyze changes in metabolites as a secondary endpoint.

MICROBIAL METABOLITE-BASED THERAPIES

Moving beyond taxonomy-based approaches

Previous meta-analyses of metagenomic studies have highlighted the limited reproducibility of microbial taxa as predictive biomarkers for ICI response.[135,136] While methodological and geographic variations between studies may partially explain this inconsistency, species-level taxonomic profiling may lack the resolution to capture the specific microbial functions influencing ICI outcomes. Strain-level variation within key commensals, such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, underscores the limitations of taxonomic classification alone, as functionally distinct strains within the same species may have opposing immunomodulatory effects. Given this challenge, microbial metabolites offer a promising alternative as functional biomarkers, quantifiable down to the micro- and nanomolar range and providing a direct readout of microbial output.

Metabolite-based biomarkers

Future research should focus on validating metabolite-based biomarkers through multi-center clinical trials to establish their predictive value for immunotherapy outcomes. This involves conducting large-scale studies across multiple centers with harmonized microbiome research frameworks to ensure that any identified metabolite signature correlates with treatment response. Standardization of metabolite profiling methodologies is essential, including protocols for sample collection, processing, and analytical techniques to ensure reproducibility across laboratories. This will require developing consensus protocols, such as streamlined mass spectrometry workflows, along with robust quality controls. Researchers should also integrate longitudinal sampling of various biospecimens, including stool (to capture fecal metabolites), blood or serum (for systemic metabolites), and tumor tissue (for tissue metabolites) at baseline and during therapy to track metabolite dynamics throughout treatment.

Translating metabolite biomarkers into routine clinical practice will require addressing several key considerations. First, the testing platform must provide rapid results, ideally within a few days, to enable clinicians to make informed treatment decisions quickly. For example, complex microbiome biomarker scores have been successfully converted into a qPCR-based assay with a 48-h turnaround time, facilitating their use in routine practice.[137] Second, the metabolite biomarker must demonstrate robustness against common confounding factors such as concurrent medications, antibiotics, or recent dietary intake. Future studies should account for these variables by collecting detailed metadata on patient medication use and diet and ensure the biomarker either withstands such variation or can be interpreted in light of it. Lastly, the biomarker must offer clear evidence of clinical utility, such as improving patient stratification (e.g., identifying responders vs. non-responders) or guiding interventions that enhance patient outcomes. To facilitate adoption in clinical settings, new frameworks for developing microbiome-based diagnostics are needed. This could involve "kitted" assays with validated cutoffs to guide clinical decision making based on metabolite profiles, such as proceeding with immunotherapy, adding microbiome-based interventions to correct dysbiosis, or considering alternative treatments.

Building on this functional perspective of metabolites as microbial output, the development of live biotherapeutic products (LBPs or probiotics), dietary compounds as substrates for metabolite production (prebiotics), and synthetic metabolite and metabolite combination drugs (postbiotics) to harness the therapeutic potential of microbial metabolites is under way. Figure 4 highlights the challenges and opportunities of these microbial metabolite-based interventions, while Table 2 outlines ongoing and completed human clinical trials investigating metabolites in various immunotherapy modalities.

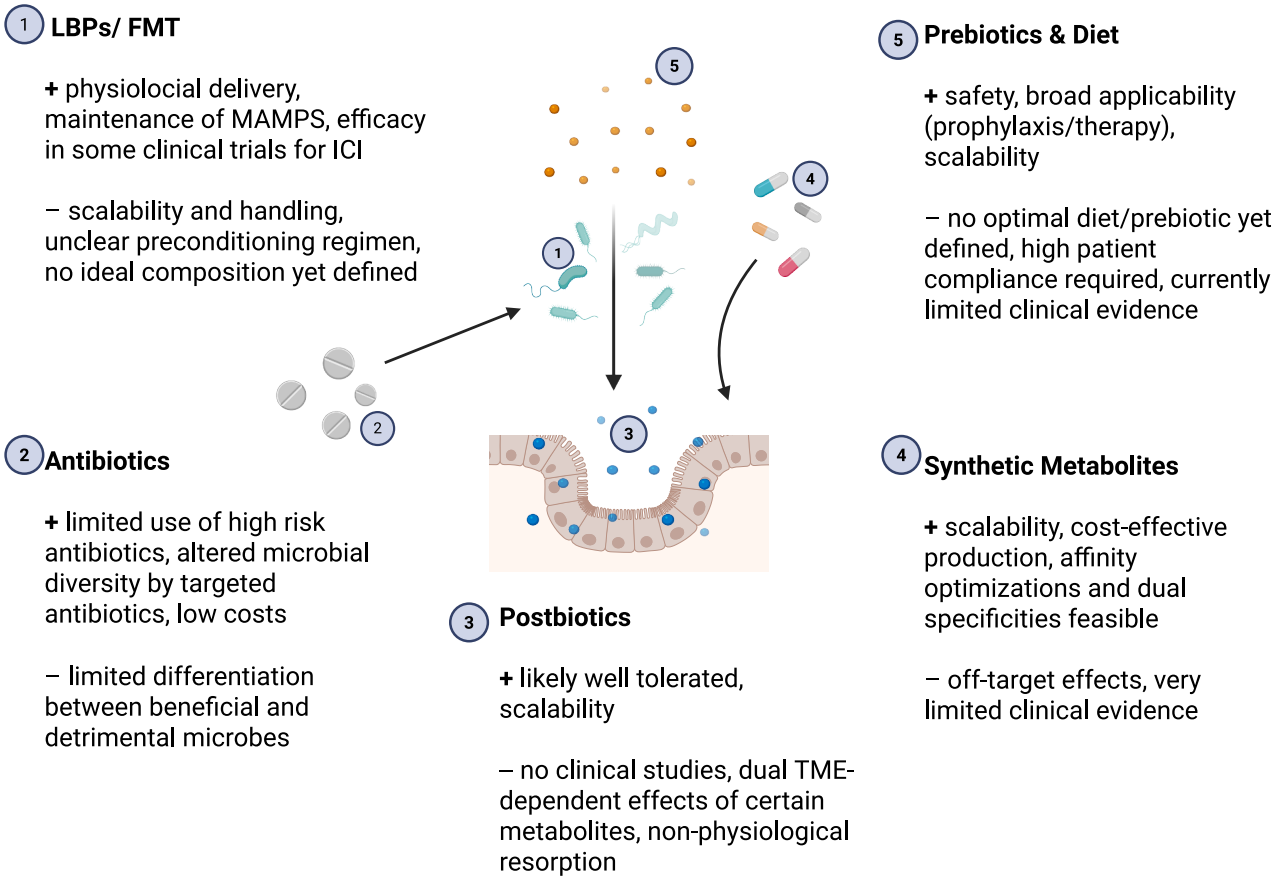

Figure 4. Paths to modify microbial metabolites

Metabolite levels can be influenced utilizing, singly or in combination, live biotherapeutic products (LBPs) or fecal microbiota transplant (FMT) (1), antibiotics (2), metabolites as such as postbiotics (3), synthetic metabolites acting on single or multiple receptors (4), or defined starting substrates (prebiotics) and dietary interventions (5). The figure was created with BioRender.

Metabolite-producing live biotherapeutic products

LBPs are emerging as adjuvants to cancer immunotherapy, aiming to enhance immune responses and improve treatment outcomes. Several early-phase clinical trials have explored the potential of microbiota-based interventions, particularly those involving butyric acid-producing bacteria and defined microbial consortia.

Two independent randomized phase 1 studies in metastatic renal cell carcinoma investigated the potential of CBM588, a live bacterial product containing the butyric acid-producing anaerobe *Clostridium butyricum*. The study arms differed only in the administration of CBM588, with patients in the intervention groups receiving 80 mg of CBM588 orally twice daily; neither trial included a placebo group. CBM588 significantly prolonged PFS in patients receiving nivolumab and ipilimumab (12.7 vs. 2.5 months; HR 0.15, 95% CI 0.05–0.47, $p < 0.001$) (NCT03829111)[140] and improved the objective response rate (ORR) in patients treated with a combination of cabozantinib (anti-VEGFR) and nivolumab (74% [14 of 19] vs. 20% [2 of 10]) (NCT05122546).[141] Importantly, no differences in toxicity between CBM588-treated and control groups were observed in either study.

A phase 1 trial (NCT03817125) in ICI-naive melanoma patients tested SER-401, a preparation of *Firmicutes* bacterial spores extracted and purified from human stool. Unexpectedly, the trial showed that despite SER-401's safety, the treatment group had a lower ORR than placebo (25.0% for the SER-401 group [95% CI 3.2–65.1] vs. 66.7% for the placebo group [95% CI 22.3–95.7]).[142] Subsequent analysis revealed that vancomycin preconditioning in the SER-401 group altered the gut microbiome, impairing butyric acid biosynthesis and promoting pathways linked to ICI resistance and systemic inflammation.

The Microbial Ecosystem Therapeutic 4 (MET4) is another orally administered FMT product for co-administration with ICIs.[143] The consortium comprises 30 species associated with treatment response (e.g., *Enterococcus* and *Bifidobacterium*). The early-phase clinical trial MET4-IO has reached the safety endpoints, achieving desirable relative abundances of the MET4 taxa. However, the planned phase 2 trial in advanced head and neck squamous cell carcinoma (PROMOTE-HN) has been withdrawn (NCT05743777). Indeed, many LBPs in cancer research have been discontinued mainly due to limited efficacy. Evelo Biosciences halted the development of EDP1503 for immuno-oncology,

Table 2. Ongoing and completed clinical trials investigating microbial metabolites

	Trial phase	Disease/condition	Study regimen	Composition	Key finding of intervention group
Live biotherapeutic products (LBPs, "probiotics")					
NCT03829111	phase 1	mRCC	nivolumab + ipilimumab ± CBM588	CBM588 = butyric acid-producing anaerobe *Clostridium butyricum*	prolonged PFS, no increased toxicity
NCT05122546	phase 1	mRCC	nivolumab + cabozantinib ± CBM588		improved ORR, no increased toxicity
NCT03817125	phase 1	unresectable/metastatic melanoma	nivolumab + SER-401 vs. nivolumab + placebo	SER-401 = *Firmicutes* bacterial spores	reduced ORR, vancomycin preconditioning impaired butyric acid biosynthesis and promoted ICI resistance note: development discontinued
NCT05743777	early phase	advanced solid tumors	ICI (any approved anti-PD-1/PD-L1 SOC) + MET-4	MET-4 = microbial ecosystem therapeutic 4; 30 species associated with responsiveness in immunotherapy (e.g., *Enterococcus*, *Bifidobacterium*)	safety endpoints reached, increased relative abundance of the MET4 taxa note: phase 2 trial PROMOTE-HN withdrawn (lack of financial support)
NCT06218602	phase 2	B cell lymphoma; high-risk AB treatment <180 days prior to CAR-T cell therapy	Axi-cel (CD19 CAR-T cell) ± FMT	FMT (colonoscopy FMT procedure and capsules)	not yet published primary objectives: toxicity, response secondary objectives: PFS, OS, changes in gut microbiome, changes in serum and stool metabolites
CONSORTIUM-IO	phase 1	refractory or metastatic melanoma, gastric/GEJ carcinoma, CRC-MSS	nivolumab + VE800	VE800 = 11-strain bacterial consortium (healthy-donor-derived)	low efficacy in all cohorts, increase frequencies of IFN-γ and TNF-α-producing $CD8^+$ T cells in melanoma: better engraftment associated with longer PFS note: development discontinued
NCT03775850	phase 1/2	metastatic TNBC	2 weeks EDP1503 twice daily → pembrolizumab + EDP1503 (every 3 weeks)	EDP1503 = single strain of *Bifidobacterium animalis lactis*	PR 2/12 patients, no SAEs note: development discontinued

(*Continued on next page*)

Table 2. *Continued*

	Trial phase	Disease/condition	Study regimen	Composition	Key finding of intervention group
Prebiotics					
NCT02763033	phase 2	allo-SCT	allo-SCT + RPS from day −7 to day +100	resistant potato starch (RPS)	not yet published feasibility study: significantly higher fecal butyrate levels, more stable dominant plasma metabolites
UMIN000027563	phase 2	allo-SCT	allo-SCT + RS and GFO from conditioning onset to day +28	resistant starch (RS) + commercially available GFO mixture (glutamine, fiber, oligosaccharides)	mitigation of mucosal injury, maintenance of microbial diversity, preservation of/increase in butyrate-producing bacteria, reduced incidence of aGVHD (overall and grade II–IV)
Spencer et al.[138]	observational	(metastatic) melanoma	ICI + assessment of dietary habits and probiotic supplements		improvement of PFS on fiber-rich diet; probiotic intake without PFS benefit
Postbiotics					
Ruutu et al.[139]	randomized open-label multi-center	allo-SCT	allo-SCT + UDCA from conditioning onset to day +90	ursodeoxycholic acid (UDCA) 6 mg/kg BW twice daily	reduced hepatic complications, lower incidence of aGVHD grade III–IV, improve 1-year OS
NCT05809336	phase 2	solid tumors	ICI (any approved anti-PD-1/PD-L1 SOC) ± chemotherapy ± targeted therapy ± inosine	inosine 0.2 g, three times daily	longer median PFS, lower risk of disease progression, longer median OS

mRCC, metastatic renal cell carcinoma; PFS, progression-free survival; ORR, objective response rate; ICI, immune checkpoint inhibitor; SOC, standard of care; AB treatment, antibiotic treatment; FMT, fecal microbiota transplant; OS, overall survival; GEJ, gastro-esophageal junction; CRC-MSS, microsatellite stable colorectal cancer; TNBC, triple-negative breast cancer; PR, partial remission; SAE, severe adverse event; RPS, resistant potato starch; RS, resistant starch; GFO, glutamine, fiber, oligosaccharides; aGVHD, acute graft-versus-host disease; UDCA, ursodeoxycholic acid; BW, body weight.

while Seres Therapeutics discontinued SER-401 for metastatic melanoma. Vedanta Biosciences removed VE800 from its pipeline after phase 1b results showed low efficacy and response rates that did not meet prespecified criteria.[144]

The development of LBPs will hinge on designing immune-stimulatory consortia that can activate anti-tumor immunity without increasing toxicity toward normal tissues. Given that species associated with treatment response or resistance vary widely across cohorts, limiting the reproducibility of taxonomic biomarkers, we propose shifting the focus toward microbial functional profiles, particularly their metabolic outputs, rather than relying solely on the presence, absence, or enrichment of beneficial taxa in responder patients or deleterious taxa in non-responder patients, respectively.

Pre- and postbiotics

Microbial metabolites can be increased by direct supplementation (as postbiotics) or by promoting their endogenous production through prebiotic interventions. Prebiotics are indigestible nutritional compounds, such as resistant starch, inulin, or arabinoxylans, fermentable by gut microbiota in the colon, thereby enhancing SCFA production. At the same time, specific fibers support the growth of Ruminococcaceae and *Faecalibacterium*, key butyric acid producers.[138] Dietary interventions rich in polyphenols and amino acids can also boost beneficial metabolites, such as flavonoid derivatives and BCFAs.

Clinical trials investigating the use of prebiotics in allo-SCT are ongoing. A prospective single-arm study evaluated the impact of resistant starch (RS) on mucosal integrity and gut microbiota in allo-SCT recipients, showing a reduction in acute GVHD incidence. Prebiotic intake preserved microbial diversity, maintained butyric acid-producing bacteria, and stabilized post-transplant fecal butyric acid levels.[145] Similarly, a randomized, placebo-controlled clinical study investigated the impact of resistant potato starch (RPS) on gut microbiota and metabolites in patients receiving allo-SCT, showing that fecal butyric acid levels were significantly increased with RPS compared to placebo. A phase 2 trial is under way to assess RPS's effect on GVHD (NCT02763033).[146]

The impact of dietary interventions on treatment outcomes is also being investigated in ICIs. An observational cohort of n = 123 melanoma patients, most of whom received anti-PD-1 ICIs, demonstrated that a fiber-rich diet with no probiotic supplementation significantly improved PFS.[138] In contrast, probiotic intake was not associated with a benefit in PFS.

To date, a limited number of clinical trials have explored the combination of metabolites as postbiotics and immunotherapy.

In a randomized multi-center trial with n = 242 patients, treatment with UDCA after allo-SCT reduced hepatic complications, was associated with a significantly lower incidence of severe (grade III–IV) acute GVHD, and improved 1-year OS.[139] UDCA is used to prevent transplant-related complications and is considered the standard of care for prophylaxis of hepatic sinusoidal obstructive syndrome.

A phase 2 single-center, prospective, randomized, open-label study (NCT05809336) was conducted in n = 172 solid tumor patients to evaluate the effect of inosine in combination with anti-PD-1/PD-L1 inhibitors.[147] Patients in the intervention group received inosine tablets containing 0.2 g of inosine and excipients three times daily along with anti-PD-1/PD-L1 ICIs. In contrast, the control group received only anti-PD-1/PD-L1 with or without targeted therapy and chemotherapy. The inosine group exhibited a 2.6-month longer median PFS (7 vs. 4.4 months; HR 0.63, 95% CI 0.44–0.90, p = 0.011) and a 37% lower risk of disease progression than the non-inosine group. The median OS was not reached in the inosine group, whereas the non-inosine group had a median OS of 29.7 months. The limitations of this study included the small cohort size and the inclusion of multiple cancer types.

Various preclinical models and clinical trials[148] have explored IDO inhibition. However, a phase 3 trial combining the IDO1 inhibitor epacadostat with pembrolizumab failed to show clinical benefits.[149] Additional clinical trials investigating IDO inhibition alone or combined with other immunomodulatory strategies are ongoing.

With ongoing development, microbial metabolites may offer several advantages over LBPs, including ease of synthesis and administration (i.e., via encapsulated delivery) and a potentially favorable safety profile (given their source from dietary components). Metabolites could be employed in deeply immunocompromised patients without incurring a significant risk of infection. Given their scalability (small molecules amenable to large-scale synthesis), chemical stability, and broad applicability as prophylactic or therapeutic compounds, microbial metabolites hold considerable promise for enhancing anti-tumor immunity.

However, the pharmacological development of metabolite-based drugs presents several challenges. Oral supplementation often leads to substantial absorption in the small intestine, whereas the target bacteria primarily reside in the ileum and colon, creating formulation challenges to ensure that metabolites reach their intended site of action. Micro- or nanocarrier systems enabling site-specific delivery may help circumvent this limitation.[150,151] Nanoparticle encapsulation of metabolites must account for complex nano-bio interactions, including the formation of a biomolecular corona that can alter biodistribution and bioactivity. Machine-learning approaches may aid in predicting and optimizing these interactions to ensure targeted delivery while minimizing systemic toxicity.[152] Despite being naturally derived, metabolites are not exempt from toxicity and dosing studies conducted under good clinical practice (GCP) standards. Indeed, the limited patentability of natural compounds adds a financial burden to advancing clinical research in this area.

Conclusion

A recent update to the "danger theory of immunity" proposes that immune activation is driven by signals of cellular distress rather than by distinguishing self from non-self, with systemic danger signals shaping immune responses beyond localized tissue damage. This framework suggests that infectious pathogens and cancer cells, both under evolutionary pressure, actively induce intestinal dysbiosis to subvert immune detection and evade immunosurveillance. Consequently, the gut microbiota plays a central role in modulating immune responses through microbial-associated molecular patterns (MAMPs), metabolites, and BA metabolism, which influence anti-viral and anti-cancer immunity.[8]

The clinical translation of microbial metabolites for cancer immunotherapy presents significant opportunities. Their ability to modulate systemic immunity, enhance cancer immunotherapies, and regulate tumor growth via metabolic and epigenetic pathways makes them valuable therapeutic candidates.[128,130] To date, several interventions have been shown to increase beneficial metabolites, including dietary modifications (e.g., high-fiber prebiotics to boost SCFAs), probiotic supplementation with metabolite-producing LBPs (e.g., *Akkermansia muciniphila* or *Faecalibacterium prausnitzii*), and FMT, which has been shown to transfer microbial profiles from ICI responders to non-responders.[153,154]

Exploring metabolite combinations offers a promising strategy for fine-tuning anti-tumor immunity, given their distinct mechanisms of action. Established in allo-SCT cohorts, the IMM-RI integrates five key metabolites associated with favorable immune outcomes.[120] Each metabolite modulates immunity via specific pathways: butyric acid via T_{reg} cell differentiation through HDAC inhibition,[11] propionic acid via enhanced $CD8^+$ T cell persistence in the TME,[12] isobutyric acid via inhibition of cancer cell survival,[86] ICA by protecting and repairing the mucosal barrier, and DAT via IFN-I amplification.[37] Future research should focus on defining precise metabolite combinations tailored to the specific type of immunotherapy being modulated, whether ICIs, allo-SCT, or CAR-T cell therapy, to ensure the appropriate balance of immune-enhancing and tolerogenic effects.

While promising, the clinical application of microbial metabolites is accompanied by several unresolved challenges. Interindividual variability in gut microbiota composition may lead to inconsistent metabolite production and clinical responses. Exposure to broad-spectrum antibiotics can deplete key metabolites, exacerbating these effects. Some microbial metabolites, such as butyric acid and certain BAs, can exhibit dual effects, either enhancing or suppressing tumor immunity, depending on the immunotherapy modality, tumor, and TME, making precise therapeutic targeting challenging. The exogenous administration of metabolites may result in off-target effects, such as immune dysregulation and adverse metabolic consequences, and may alter the community composition of a patient's microbiota.[155] Additionally, enterohepatic circulation may pose unforeseen risks, including potential hepatotoxicity, as elevated hepatic levels of BCFAs have been associated with non-alcoholic fatty liver disease.[156] Variability in metabolite stability, bioavailability, and host metabolism presents additional barriers to achieving consistent therapeutic outcomes.

Establishing a mechanistic understanding that links specific microbial strains or metabolites to immune functionality remains challenging. Causal relationships require validation using human-relevant systems such as gnotobiotic mice, organoids, and co-culture systems to elucidate how microbial factors modulate immune pathways in the TME or systemically. Combining single-cell immune profiling with spatial metabolomics and multi-omics approaches will enable mapping of how specific metabolites interact with the intestinal epithelium and immune cell subsets in mucosa, lymphoid organs, and tumors. Given the numerous influencing variables, artificial intelligence models, particularly deep-learning-based approaches, may help disentangle the complex high-dimensional interactions between microbial taxa, metabolite levels, immune phenotypes, tumor histologies, and clinical outcomes, thereby facilitating biomarker discovery. For example, a recent study applied microbial community-scale metabolic modeling to predict individual-specific SCFA production profiles from metagenomic data, which could be leveraged to design personalized dietary, prebiotic, or probiotic interventions to optimize SCFA production.[157]

Another challenge is the absence of standardization across sample collection, storage, sequencing platforms, and bioinformatics pipelines. Indeed, a reanalysis of data from a large-scale microbiome study of 33 cancer types revealed fundamental errors, underscoring the need for established analytical tools in microbiome analysis.[158] These inconsistencies introduce methodological bias and hinder cross-study comparisons.

Precision microbiome-based strategies are being explored to address these challenges, for example, prebiotics that selectively enhance the growth of desirable metabolite-producing consortia; engineered bacterial strains designed to metabolize toxic biomolecules or produce and release bioactive molecules, including specific metabolites[159]; and bacteriophages that encode auxiliary metabolic genes (AMGs), which can alter the metabolism of their bacterial hosts, potentially influencing the production of specific metabolites.[120] As interindividual variability remains a significant obstacle, a high degree of personalization will be required, such as customized LBPs or synthetic metabolite cocktails adapted to a patient's microbiome and immune status.

Clinical translation will depend on randomized controlled trials to evaluate the feasibility, safety, and efficacy of microbiota-based interventions across diverse patient populations and immunotherapy platforms. Adaptive trial designs incorporating real-time profiling for microbiome-based allocation or stratification will help manage variability. Addressing regulatory hurdles, defining the optimal type of intervention (factoring in metabolite delivery, preparative regimens, and timing), and carefully selecting patients (clinically driven, such as diet or antibiotics, or biomarker-driven, such as intestinal dysbiosis) will be key to advancing microbial metabolites as precision therapeutics in immuno-oncology.

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DECLARATION OF INTERESTS

H.P.: honoraria: Novartis, Gilead, Abbvie, BMS, Pfizer, Servier, J&J; travel: Gilead, J&J, Novartis, Abbvie, Jazz, Amgen; research: BMS. E.T.O.: honoraria:

BeiGene, AstraZeneca; travel: BeiGene, J&J, Lilly. M.A.F.: honoraria: Novartis, Sanofi; travel: Sanofi.

The metabolite DAT is part of a patent application by E.T.O. and H.P.

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

While preparing this work, the authors used ChatGPT-4 to review spelling and grammar. After using this tool, the authors reviewed and edited the content as needed, taking full responsibility for the publication's content.

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